An Investigation into Long-Chain Polyunsaturated Essential Fatty Acids, Event Related Potential Assessments of Brain Function and Behavioural Measures in Children and Adolescents with and without Attention Deficit Hyperactivity Disorder

Gow, Rachel

Awarding institution:
King's College London

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Author: Rachel Gow

An Investigation into Long-Chain Polyunsaturated Essential Fatty Acids, Event Related Potential Assessments of Brain Function and Behavioural Measures in Children and Adolescents with and without Attention Deficit Hyperactivity Disorder

By

Rachel V. Gow

Department of Child and Adolescent Psychiatry

Institute of Psychiatry

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Abstract

This PhD research project[1] investigated differences in long-chain polyunsaturated fatty acids (LC-PUFA) between male adolescents with and without ADHD, and the relationship between these and performance and event related potential (ERP) assessments of brain function and clinical behavioural measures. ERPs were also investigated in a randomised, double-blind, placebo-controlled clinical trial of 12 weeks PUFA supplementation in ADHD. Comparison of LC-PUFA measures between two groups of ADHD showed no differences in the first group, but in the second group, omega-3/6 indices were lower in ADHD compared to controls. No relationships were observed between LC-PUFA and clinical behaviour measures except between specific omega-3 indices which were inversely related to callous and unemotional traits in the ADHD group only, suggesting a specific relationship with socio-emotional behaviour. Despite performance differences there were no ERP differences between ADHD and controls during the sustained attention task and specific P3 reduction in ADHD for the Go process of the Go/NoGo task. The performance was correlated with LC-PUFA in controls only. Associations were observed between reduced P3 and LC-PUFA in both groups, possibly suggesting enhanced neuronal efficiency. For the emotion processing task, patients showed reduced N2 and N4 amplitudes relative to controls. Omega-3 was associated with more normal N4 function in ADHD for happy faces. The 12 week supplementation trial demonstrated only enhanced P2 to happy faces in the active group relative to placebo at follow up. Overall, the findings demonstrate reduced PUFA blood levels in some groups of ADHD children but limited and very specific associations between fatty acids, clinical measures and brain function in ADHD. The behavioural, biochemistry and neurophysiological associations appear specific to

[1] This PhD project has arisen from and is linked to previous research; the Maudsley ADHD Adolescent Fatty Acid trial which I worked on for 2 years with Professor Eric Taylor. This was a randomised, placebo-controlled double blind clinical trial which recruited 76 participants and randomly allocated them into groups to receive fatty acid supplementation or placebo for 12 weeks. During this trial, I was responsible for both recruiting, screening and assisting with data collection. A small amount of data (n =20) was used for my MSc thesis. Following this, I was invited to include some of the data from this study in my doctorate project. In addition, to collecting other data related to this area during my degree.

socio-emotional behavioural traits and the processing of positive emotions suggesting that PUFA may be more relevant to socio-emotional processes rather than ADHD per se.

Ethical Approval

NHS ethical approval for this PhD project was granted by the Camden & Islington Community Local Research Ethics Committee at The Royal Free Hospital, NW3 2QG (reference numbers: 08/H0722/88). During the course of the study, 2 substantial amendments were further granted. Both parents and adolescents were asked to sign a consent form.

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Table of Contents

List of Tables

List of Figures

Glossary

ALA	Alpha Linolenic Acid (c18:3n-3)
ADDISS	Attention Deficit Disorder Information and Support Service
ADHD	Attention Deficit Hyperactivity Disorder
AA	Arachidonic Acid, (c20:4n6)
APSD	Antisocial Process Screening Device
CHIPS	Children's Interview for Psychiatric Syndromes
CD	Conduct Disorder
CPRS	Conners' Parent Rating Scale
CTRS	Conners' Teacher Rating Scale
DASS	Depression Anxiety Stress Scale
DHA	Docosahexaenoic Acid (c22:6n-3)
DPA	Docosapentaenoic Acid (c22:5n6)
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders-IV
EBD	Emotional and Behavioural Difficulties
EEG	Electroencephalography
ERP	Event Related Potential
EFA	Essential Fatty Acid
EPA	Eicosapentaenoic Acid (c20:5n-3)
ERPs	Event Related Potentials
GLA	Gamma Linoleic Acid
IoP	Institute of Psychiatry
K-BIT	Kaufman Brief Intelligent Test
LC-PUFA	Long-Chain Polyunsaturated Fatty Acids
LA	Linoleic Acid
MAAFA Trial	Maudsley Adolescent ADHD Fatty Acid Trial
MCT	Medium Chain Triglyceride
n-3	Omega-3 Fatty Acid
n-6	Omega-6 Fatty Acid
ODD	Oppositional Defiant Disorder
RBC	Red Blood Cells / Erythrocytes
RCTs	Randomised Controlled Trials
SDQ	Strength and Difficulties Questionnaire

Chapter One: Introduction to Attention Deficit Hyperactivity Disorder

Introduction

Attention Deficit Hyperactivity Disorder (ADHD) is defined by the Diagnostic and Statistic Manual of Mental Disorders (DSM-IV) fourth edition by developmentally inappropriate levels of inattention, hyperactivity and impulsivity (DSM-IV, APA). It is one of the most common psychiatric disorders, existing cross-culturally with a reported global prevalence of approximately 3% -12% (Biederman & Faraone, 2005; Burd, Klug, Coumbe, & Kerbeshian, 2003; Faraone, Sergeant, Gillberg, & Biederman, 2003). ADHD is not a homogenous condition but a complex, multi-faceted, behavioural syndrome with over-lapping symptoms. Its onset is in early childhood and for a diagnosis it should be present before the age of 7 years, although it is often present by age 5 and before the age of 2 (Taylor et al., 2004). It frequently persists into adolescence and adulthood and places those affected at risk for a variety of irregularities in personality development (Taylor, Dopfner, et al., 2004).

Diagnosis of ADHD

ADHD is defined and classified into 3 subtypes by the DSM-IV and similarly by the ICD-10 with the term "hyperkinetic disorder" being reserved for more severe cases (First, Frances, & Pincus, 2004). There are three recognised subtypes of the disorder: (1) *hyperactive-impulsive,* (2) *inattentive* and (3) *combined* (First, et al., 2004). The two distinct behavioural dimensions, inattention and hyperactive-impulsive have been recognised to exist across cultures in a variety of ethnic groups (Barkley, 2002, 2003). The *hyperactive-impulsive* subtype is multidimensional and characterised by deficits in inhibition. The *inattentive* component impairs an individual's ability to pay attention and subsequently ADHD children often struggle to follow instructions or abide by rules (Barkley, 2003).

For a diagnosis to be met, symptoms of hyperactivity/impulsivity and/or inattention must meet the guidelines as set out in the Diagnostic and Statistical Manual for Mental Disorders Volume-IV (DSM-IV) or the International Classification for Diseases Volume-10 (ICD-10) (First, et al., 2004). The severity of symptoms should be such that an individual suffers impairments socially, psychologically, educationally and/or occupationally, all of which are assessed by a clinician via interview or direct observation in multiple settings, for example, at

home and at school (Hill, 2008). The symptoms must also be pervasive and manifest in two or more environments including home, school, social or educational (First, et al., 2004). Most clinicians will collect data using the various scales such as the Conners' rating scales for parents (CPRS) and teachers (CTRS), the Strengths and Difficulties Questionnaire (SDQ) and/or the Child Behaviour Checklist when assessing a child suspecting of having ADHD (Hill, 2008). This is in addition to in-depth parent and child interviews and review of school-reports. The diagnostic process is in essence clinical and computerised assessments of attention are seldom used (Hill, 2008). The clinician will focus on the proportion of inattentive, restless, impulsive and oppositional behaviour which can be attributed to ADHD as opposed to conduct disorder and also weigh up the amount of anger or inattention which is accountable to other disorders (Hill, 2008). Psychiatrists will also out-rule the possibility of an attachment disorder, anxiety or use of illegal drug use as potential reasons for over-activity and inattention (Hill, 2008). The personal (e.g., pregnancy/birth difficulties, substance dependence or abuse, term of pregnancy, delivery complications, admission to neonatal intensive care), medical (e.g., chronic illnesses, trauma, sleep issues), developmental (e.g., social, motor or language difficulties), and social (e.g., specific stressors, income/resources, family structure), history is also obtained to rule out other possible diagnosis, for example, epilepsy, iron deficiency, lead screening (Ryan-Krause, 2010a).

In 2008, The National Institute for Health and Clinical Excellence (NICE) published guidelines for the diagnosis and management of children, young people and adults[2]. The publication of the NICE report is especially relevant as it is estimated that only 50% of the most severe ADHD cases are referred and taken up by health-care teams (Hill, 2008). This is due for a number of reasons including poor detection by schools and general practitioners. The latter group is reported to be inadequately informed about ADHD and typically only make referrals to experts when there is parental pressure (Hill, 2008; Sayal, Taylor, Beecham, & Byrne, 2002). More often than not, there are widely held assumptions that ADHD occurs as a result of inadequate parenting practices, and prejudices against the use of stimulant medication (Hill, 2008). The presentation of ADHD and consequences of undiagnosed or poorly managed ADHD can differ according to the age at which the diagnosis is considered (Ryan-Krause, 2010a). However, it is

[2] http://www.nice.org.uk/CG072

pertinent to note that undiagnosed hyperactivity behaviour is a predisposition for a mental disorder a decade later (Taylor, Chadwick, Heptinstall, & Danckaerts, 1996a).

Risk Factors for ADHD

It is vital, therefore, that a correct diagnosis is made as early and swiftly as possible as many aspects of a child's life can be negatively impacted by ADHD and impairments can be found across societal, familial, educational, occupational and health-care domains. Specifically, school-age children with ADHD are at risk from persistent educational difficulties including exclusion or drop out, stressful peer relationships, parental frustration and ineffective attempts at discipline (Ryan-Krause, 2010a, 2010b). According to the DSM-IV, typical behaviours observed at school are frequent conversation shift, not listening to others, severe social incompetence, frequently interrupting and intruding on other peoples conversation, blurting out answers to questions before the questions have been completed, clowning around, an inability to remain seated and initiating conversations at inappropriate times (First, et al., 2004).

School-age children with ADHD display elevated rates of noisy, disruptive and rule-violating behaviour (Landau & Moore, 1991) and are described by their peers as disruptive, unpredictable and aggressive (de Boo & Prins, 2007; Milich, Whitten, Landau, & Kilby, 1982; Pelham & Milich, 1984). Additional risks include a higher than average necessity for intervention from agencies such as child and family services (Kazdin, 1995; Loeber, Burke, Lahey, Winters, & Zera, 2000), entry into the criminal justice system (Loeber et al., 2001; Scott, Knapp, Henderson, & Maughan, 2001), and disruptive peer and family relationships (Carr, 2004; Mikami, Jack, Emeh, & Stephens, 2010). Adolescents with ADHD have a higher likelihood of developing anti-social and conduct disorder related behaviours and engaging in substance misuse (Biederman et al., 2006; Colledge & Blair, 2001; Lahey et al., 2000; Wilens, 2004). For these reasons, the investigation of ADHD is a highly relevant and significant area of research.

Comorbidities

The co-occurrence of additional types of psychopathology is very common because ADHD presents with great heterogeneity and therefore it is uncommon to find patients with *pure* ADHD (Taylor, Dopfner, et al., 2004). Consequently, ruling out other disorders is often difficult (Ryan-Krause, 2010a, 2010b). Symptoms of ADHD often overlap with symptoms of related disorders such as mood disorders (e.g., pediatric bi-polar disorder), dyslexia (reading and writing

difficulties), motor-coordination disorder (also known as dyspraxia), oppositional defiant disorder (ODD), conduct disorder (CD), emotional problems, anxiety disorders and bi-polar disorders (for a review see NICE, 2008) (Ryan-Krause, 2010a, 2010b). It is thought that approximately 50% to 60% of children with ADHD also fulfil criteria for an additional psychiatric disorder (Reiff & Tippins, 2004; Ryan-Krause, 2010a), with 57% of boys and 31% of girls meeting the criteria for ODD (Biederman, 2005; Ryan-Krause, 2010a). CD, which presents as a severe version of ODD, has been observed in 19% of boys and 8% of girls with the disorder (Biederman, 2005; Ryan-Krause, 2010a). The co-occurrence of ODD and CD is very common in children with hyperactivity and are sometimes best regarded as a complication rather than a differential diagnoses (Taylor, Dopfner, et al., 2004). Early longitudinal studies have suggested that the later development of CD is a risk factor even in primary school age children with pure hyperactivity and no initial conduct problems (Taylor, Chadwick, Heptinstall, & Danckaerts, 1996b).

Anxiety disorders are also found alongside ADHD with an estimated 25% of cases meeting the diagnostic criteria (Reiff & Stein, 2004; Ryan-Krause, 2010a). Symptoms of anxiety are however more subtle to detect as they are not as externalizing as ODD or CD (Reiff & Tippins, 2004; Ryan-Krause, 2010a). Some characteristics of anxiety include: school refusal, weight loss, sleep issues, irritability and greater social problems with friends although not in a disrupting sense (Ryan-Krause, 2010a). Pediatric bi-polar disorder is a more extreme mood disorder but presents with similar symptoms as ADHD. It is characterised by major mood swings, explosive behaviour and impairments in social interactions (Ryan-Krause, 2010a). It is further estimated that up to 90% of young people with bi-polar disorder have ADHD (Joshi & Wilens, 2009; Ryan-Krause, 2010a). Emotional problems also frequently co-exist in ADHD although much less is known about the reasons for this. It is however postulated that these stem as a consequence of academic breakdowns and difficulties in interpersonal relationships (Taylor, Dopfner, et al., 2004).

Treatments for ADHD

The first stage of intervention for school children are group-based education programmes and parent-training sessions (NICE, 2008). This may include psychological interventions such as social skills training and/or cognitive behavioural therapy (CBT) (NICE, 2008). Drug treatment is reserved for those young people with more severe symptoms and impairment, or those with

moderate severity who have declined other non-drug treatments or have not responded sufficiently to group psychological treatment or parent-training/education programmes (NICE, 2008). It is now recommended that before the commencement of drug treatment an electrocardiogram is carried out if there is a past history of serious cardiac disease in the family or of sudden death syndrome. A full pre-treatment assessment is also carried out which includes a mental health and social assessment alongside a medical history and physical examination (NICE, 2008).

Stimulant medication has been successfully employed to treat the symptoms of ADHD / "hyperkinetic disorder" since the 1930's (Biederman & Faraone, 2005). The two types of drugs commonly used in the management of ADHD include the psycho-stimulant drugs such as methylphenidate (MPH: e.g., Concerta or Ritalin) or amphetamine (e.g., Adderall or Vyvanse) and non-stimulants (e.g., Atomoxetine or Strattera). The drug Adderall is approved for use in children aged 3 years and older whereas other stimulant medications can be prescribed from the age of 6 years and over. Medications are adjusted to control symptoms or to manage adverse side effects according to the child's response. Younger children may only need a stimulant to assist their school performance during the day. On the other hand, adolescents may require medication after school, for example, during homework or study periods. Research studies investigating the effects of MPH have reported that the main symptoms of ADHD are reduced in the region of 65%-70% of children (Greenhill, Pliszka, et al., 2002; Swanson et al., 2004). According to NICE, treatment should be maintained for as long as it is effective and its efficacy should be monitored at least once per annum in children. Medication-free periods also known as "drug holidays", although not routinely recommended, are often employed by parents during school breaks and weekends.

Mechanism of Action

The central catecholaminergic system has a pivotal role in both motor activity and cognition (Wilens, 2008). ADHD is linked with disturbances in the function of the central catecholaminergic system (Wilens, 2008). Of the multiple etiologies associated with ADHD (e.g., environmental, genetic, neurobiological and neurochemical), catecholamine dysfunction, that is, noradrenergic and dopaminergic neurotransmission, is a significant under-lying feature (Wilens, 2008). MPH has a modulating affect of catecholaminergic tone, mainly impacting the striatum and prefrontal cortex regions of the brain (Wilens, 2008). Treatment with

methylphenidate results in elevated levels of dopamine (DA) signaling via several actions: (1) a obstruction of the DA reuptake transporter and intensification of DA response period, most prominently in striatal brain regions, (2) less inhibition of DA D2 auto-receptors and magnification of DA tone, and (3) turning on D1 receptors on the postsynaptic neuron (Wilens, 2008). The performance of MPH in the cortex may also be interceded by stimulation of the noradrenergic α_2 receptor and DA D1 receptor (Wilens, 2008). The function of other neurotransmitters such as serotonin, α-agonists, histamine and acetylcholine in regulating catecholamine pathophysiology in ADHD and medication for ADHD are less understood (Wilens, 2008). Ultimately, the alterations in catecholaminergic tone are most obvious clinically in patients with ADHD as improvements in motor hyperactivity, distractibility, and attention deficits (Wilens, 2008).

MPH treatment is estimated to reduce the core symptoms in 70% of children with ADHD (Greenhill, Pliszka, et al., 2002; Swanson et al., 2002). It is an indirect catecholamine agonist with most noticeable effects in frontal and striatal brain regions (Hannestad et al., 2010). A meta-analysis of placebo-controlled, clinical trials in children has verified the efficacy in improving (1) cognitive abilities, (2) behavioural and academic ratings and (3) inhibition (Arnold et al., 2004; Wigal et al., 2004). In ADHD, the most reliable effect of MPH on brain function is the upregulation and normalisation of decreased frontal and striatal activation (Epstein et al., 2007; Rubia et al., 2011; Rubia, Halari, Taylor, & Brammer, 2011; Shafritz, Marchione, Gore, Shaywitz, & Shaywitz, 2004; Vaidya et al., 1998).

Side effects of drug treatment

Although, stimulant medication is considered relatively safe, children and young people should be monitored for side effects. Adverse effects of stimulant medication include: slowing of linear growth, anorexia, sleep issues, irritability, headaches, abdominal pain, emergence of tics, a rebound effect (when the dose wears off) and subsequent heightened effect of ADHD symptoms, e.g., emotional lability and depressed affect (Faraone, Biederman, Morley, & Spencer, 2008; Tsai & Huang, 2010). The use of non-stimulants such as Atomoxetine, particularly during the initial months of treatment is linked to increased irritability and agitation, self-harming and suicidal behaviour, reduced appetite, resulting in weight loss and increased blood pressure and heart rate. There is also the rare potentiality for liver damage which usually presents as unexplained nausea, darkening of urine or jaundice along with abdominal pain (NICE, 2008).

Limitations of psychostimulants

However, despite the efficacy of psycho-stimulant medication it should be noted that circa 25% to 35% of young people with ADHD do not respond to treatment (Schweitzer, Cummins, & Kant, 2001; Wilens, 2008). Furthermore, some very efficient, short-term, longitudinal studies have reported long-term effects of MPH to be modest (Barbaresi, Katusic, Colligan, Weaver, & Jacobsen, 2007; Barbaresi et al., 2006), not helpful (Molina et al., 2009), or even damaging (Hazell, 2011). In addition, MPH does not alter the underlying disease process (Pliszka, 2009) . Long-term adverse effects of MPH have been documented including reduced growth, tremor, sleep and vegetative disturbances and irritability (Greenhill et al., 2001; Jensen et al., 2007). During the past decade prescriptions for stimulant medication for ADHD has escalated with a net drug cost to the NHS of circa £25, 000, 000[3] and approximately £30, 000, 000 for health care professionals including educational and social time. Consequently, concerns have been raised about the currently unknown effects of psychostimulant treatment to the developing brain. Animal studies have reported alterations in the dopaminergic system with long-term effects such as dysfunction of the reward system, to depression-like symptoms following the use of psychostimulants (Carlezon & Konradi, 2004; Grund, Lehmann, Bock, Rothenberger, & Teuchert-Noodt, 2006; Grund et al., 2007; Mague, Andersen, & Carlezon, 2005; McFadden, Yamamoto, & Matuszewich, 2011). In humans, there is evidence of long-term brain sensitisation to psychostimulants, particularly pronounced in subjects with novelty seeking traits typical for ADHD (Boileau et al., 2006). In light of the important side effects, parents are often reluctant to consent to their administration and seek instead *natural* alternatives.

Genetic inheritance and ADHD

ADHD is thought to be highly heritable within families with a 3-5 times greater risk in first-degree relations (Faraone et al., 2005; Taylor, Dopfner, et al., 2004). Considerable heritability is also found in twin studies with genetic factors accounting for 65% to 90% of the phenotypic variance in the population (Banaschewski et al., 2005; Thapar, Holmes, Poulton, & Harrington, 1999). Furthermore, several molecular investigations have identified genetic

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3 http://www.ic.nhs.uk/webfiles/publications/007_Primary_Care/Prescribing/Prescription_Cost_Analysis_England_2010/Prescription_Cost_Analysis_2010.pdf

variants within several genes implicated in the regulation of neurotransmitter systems which are in turn linked with an increased risk for ADHD (Brookes, Chen, Xu, Taylor, & Asherson, 2006a; Steinhausen, 2009). These genetic variations include the dopamine receptors 4 (DRD4 7-repeat allele) and 5 (DRD5 148bp-allele) and the dopamine transporter (DAT1 10-repeat allele) (Curran et al., 2001; Faraone, Doyle, Mick, & Biederman, 2001; Faraone, et al., 2005; Maher, Marazita, Ferrell, & Vanyukov, 2002). The 7-repeat allele of DRD4 appears to encode a receptor which is sub-sensitive to dopamine (Asghari et al., 1995), while the 10-repeat allele of DAT1 is linked to a greater manifestation of the transporter (Durston, 2008; Fuke et al., 2001; Mill, Asherson, Browes, D'Souza, & Craig, 2002; Taylor, Dopfner, et al., 2004). Other dopamine pathway genes which are also implicated include the dopamine D5 receptor and a synaptosomal-connected protein (SNAP-25), the latter is implicated in the vesicular release of neurotransmitters (Asherson, Kuntsi, & Taylor, 2005). Genes involved in serotonergic (5-HT) neurotransmission have additionally been implicated in ADHD (Baehne et al., 2009). These include the tryptophan hydroxylase gene (*TPH2*) and several single nucleotide polymorphisms (SNPs) (Millichap, 2008; Millichap, 2010). However, each of these alleles raises the relative risk only slightly with odds ratios of 1.2-1.9 (Millichap, 2008). The gene-environment reciprocal action is progressively being recognised as a significant mechanism in the cause, origin and development of ADHD, with some genes (e.g., *DAT1*) having an effect on the personal sensitivity to environmental etiologic factors (Millichap, 2008; Millichap, 2010; Thapar, Langley, Asherson, & Gill, 2007).

Submicroscopic structural chromosomal abnormalities, also known as copy number variants (CNVs), are a notable source of genetic variation (Wain, Armour, & Tobin, 2009; Williams, Zaharieva, et al., 2010). Large, rare CNVs are implicated in neurodevelopmental disorders including autism and schizophrenia (Abrahams & Geschwind, 2008; Consortium, 2008; Cook & Scherer, 2008). However, recent findings have now confirmed that a highly significant increase in the prevalence of CNVs exist in children with ADHD and those with intellectual delay compared to controls (Williams, Zaharieva, et al., 2010). However, although genetic influences are substantial, the chemical pathways involved are not yet fully worked out and further investigations in this field are clearly necessary.

Additional Biological Factors

Other biological factors are proposed as risk factors for the pathogenesis of ADHD which include (1) lead contamination (Braun, Kahn, Froehlich, Auinger, & Lanphear, 2006; Nicolescu et al., 2010; Tuthill, 1996), (2) cigarette and alcohol exposure in utero (Aronson, Hagberg, & Gillberg, 1997; Braun, et al., 2006; Knopik et al., 2006; Lehn et al., 2007; Linnet et al., 2003; Mick, Biederman, Faraone, Sayer, & Kleinman, 2002; Thapar et al., 2003), (3) pregnancy and delivery complications (e.g., toxemia, eclampsia), (4) low birth weight (Lou et al., 2004; Mick, Biederman, Prince, Fischer, & Faraone, 2002), (5) poor maternal nutrition including potential deficiencies in key micro-nutrients such as omega-3/6 fatty acids, zinc and iodine (Arnold et al., 2005; Arnold & DiSilvestro, 2005; Richardson & Montgomery, 2005; Vermiglio et al., 2004), (6) food additives (McCann et al., 2007) and increase in the "Western diet" (e.g., heavily processed foods rich in salt, sugars and saturated fats) (Howard, Robinson, Smith, Ambrosini, Piek & Oddy, 2010) and (7) psychosocial adversities (Taylor, 2010; Biederman, 2005; Mick , Biederman, Faraone, Sayer & Kleinman, 2002).

Nutrition and Deficiencies

The quality of the maternal diet is fundamental for the infant's brain development during pregnancy and post-birth via the transfer of human milk. Therefore, micronutrient intakes and status of trace elements such as iodine, iron, zinc, copper, iodine, vitamins A and B, choline, folate and LC-PUFAs including the omega-3, docosahexaenoic acid (DHA) and omega-6, arachidonic acid, are critical for brain development during the foetal and early postnatal stage when areas of the brain are undergoing rapid development (Carlson, 2001). Nutritional deficiencies (and for that matter excesses) may affect the infant's brain and subsequent development and behaviour permanently (Doyle & Rees, 2001; Georgieff, 2007; Rees et al., 2005). For example, iodine and iron deficiency (anemia) in infancy has been linked to (1) suboptimal human development demonstrated by poorer performance in global measurements of cognitive, motor and social-emotional behaviour (Grantham-McGregor & Ani, 2001; Lozoff, 2007; McCann & Ames, 2007; Sachdev, Gera, & Nestel, 2005; Venturi, Donati, Venturi, & Venturi, 2000) and (2) alterations in regulatory processes such as the sleep-wake cycle (Peirano et al., 2009). Iron deficiency in animal models have shown alterations in dopaminergic function with lower dopamine levels in the cerebrospinal fluid compared to controls (Coe, Lubach,

Bianco, & Beard, 2009). Iron deficiency is well documented to also affect other neurotransmitters and processes including neurometabolism in hippocampus and striatum, myelination, dendritogenesis and both gene and protein profiles (Beard & Connor, 2003; Georgieff, 2008; Lozoff et al., 2006).

There is persuasive evidence that deficiencies or metabolic abnormalities of polyunsaturated fatty acids (PUFAs) and the brain-selective nutrients, iodine, iron, cooper, selenium and zinc, necessary for their absorption, are associated with neurodevelopmental disorders including ADHD, which will be reviewed in closer detail in the next chapter of the thesis (Antalis et al., 2006; Colquhoun & Bunday, 1981; Richardson & Montgomery, 2005; Stevens, Zentall, Abate, Kuczek, & Burges, 2003; Ward, 2000).

Chapter Two: An introduction to LC-PUFA and literature review in relation to ADHD and comorbid disorders

The evidence presented in this literature review regarding the direct and indirect impact of dietary alteration(s) including omega-3 fatty acids to ADHD symptomology originates from three main sources: (1) animal and human studies which have shown the behavioural effects of omega-3 deficiency including reduced cognitive ability; (2) studies with children and young adults which have reported lower omega-3 fatty acids in the blood profiles of patients with ADHD compared to controls and (3) randomised controlled trials which have indicated some benefit of supplementation with omega-3 fatty acids in children with ADHD. This review will provide an overview of the evidence to date from randomised, placebo-controlled double blind, clinical trials and open-label studies in relation to the safety and efficacy of fatty acid supplementation thus far.

Long-Chain Polyunsaturated Fatty Acids (LC-PUFAs)

Essential fatty acids are strings of carbon atoms linked together. There are three classes of fatty acids and they are classified in accordance to the incidence of double bonds in the hydrocarbon chain. For example, saturated fatty acids contain no double bonds, monounsaturated fatty acids, have one carbon-carbon double bond and those fatty acids with 2 or more carbon-carbon double bonds are named polyunsaturated fatty acids (PUFAs) (Brenna & Diau, 2007b; Osher, Belmaker, & Nemets, 2006). The term *essential* derived from the pioneering research of George and Mildred Burr (1930) and their discovery that an absence of specific types of fatty acids resulted in clinical abnormalities in animal models (Burr & Burr, 1930). It is now well established that LC-PUFA's are vital for the optimal development of both the brain and retina (Eilander, Hundscheid, Osendarp, Transler, & Zock, 2007; Innis, 2008; Lauritzen, Hansen, Jorgensen, & Michaelsen, 2001; Ryan et al., 2010). They are abundant in the central nervous system and fulfil a pivotal function in neurotransmission, serotonergic and dopaminergic function (Chalon, 2009; McNamara & Carlson, 2006a; McNamara, Jandacek, et al., 2010; Yavin, Himovichi, & Eilam, 2009). There has been a small but growing body of research, spanning the past decade which has discussed the potential role of omega-3 and 6 essential fatty acids in relation to ADHD with specific reference to abnormalities in fatty acid blood profiles.

The association between LC-PUFA and brain development has steadily increased since the role of omega-3 PUFA in brain structure and function became apparent in the 1970's through the work of Crawford and his colleagues (Crawford, Hassam, & Williams, 1976). Crawford and Sinclair (1972) were the first to demonstrate that arachidonic (AA, c20:4n6) and docosahexaenoic (DHA, c22:6n-3) acids were essential for mammalian brain development (Sinclair & Crawford, 1972a, 1972b). Their pioneering work with primates provided experimental evidence for specific omega-3 fatty acid deficiency inducing behavioural pathology (Fiennes, Sinclair, & Crawford, 1973). Since then there have been indications that essential fatty acid supplementation may benefit several psychiatric, neurological and neurodevelopmental illnesses, such as depression, bi-polar disorder, schizophrenia, dementia, dyspraxia, dyslexia, autistic spectrum disorders and ADHD (Cyhlarova et al., 2007; Peet & Horrobin, 2002a; Richardson, 2004; Richardson, Cyhlarova, & Ross, 2003; Richardson & Montgomery, 2005; Richardson & Puri, 2002; Sinn & Howe, 2008; Stein, 2001; Stoll, Locke, Marangell, & Severus, 1999).

Long-chain polyunsaturated fatty acids (LC-PUFAs) are known as essential fatty acids because they unable to be made nor stored by the body for very long periods of time and therefore must be obtained via the diet (Elmadfa & Kornsteiner, 2009). The omega-3 PUFA α-linolenic acid (ALA, c18:3n-3) and the omega-6 PUFA linolenic acid (LA, c18:2n6) are the parent compounds or precursors to other omega-6 and omega-3 fatty acids (Brenna, Salem Jr, Sinclair, & Cunnane, 2009) and until the 1950's were collectively known as Vitamin F. LA is considered the main dietary PUFA in the Western diet commonly sourced from commercially manufactured foods including corn and sunflower oil. Typical intakes are estimated to range between 12 and 17 grams per day for both men and women. LA is a metabolic precursor to gamma-linolenic acid (GLA, c18:3n6) and then AA (Rett & Whelan, 2011b), connected biochemically via an elongase and 2 desaturases (see Figure 1). AA is a powerful bioactive molecule and when released from membrane phospholipids is converted to a variety of compounds called eicosanoids, known to be involved in the resolution of inflammation and tissue homeostasis but also associated with a number of chronic diseases (Rett & Whelan, 2011b). Dietary sources of AA include meat, eggs, fish and aquatic plants including algae (Wood et al., 2008). ALA can be found in green leafy vegetables, plants, nuts, vegetable oils and seeds (especially flax and canola). In contrast, the main dietary sources of DHA and EPA are marine

fish with mackerel, salmon, herring and sardine being particularly abundant in omega-3 fatty acids.

ALA is metabolised for incorporation into cell membranes, into the long chain omega-3 PUFAs eicosapentaenoic acid (EPA, c20:5n-3) and DHA (Brenna, et al., 2009). In order for the conversion process from the EFA precursor ALA to PUFAs, desaturase and elongation enzymes incorporate double bonds and elongate the carbon chains (Pawlosky, Hibbeln, Novotny, & Salem, 2001). There are 3 desaturases present in humans, delta 9, delta 6 and delta 5 (Nakamura & Nara, 2004). Delta 9 is responsible for the synthesis of monounsaturated fatty acids and oleic acid is a major product of delta 9 desaturase and the main fatty acid in human adipose triglycerides (Nakamura & Nara, 2004). In contrast, delta 5 and delta 6 desaturase are necessary for the synthesis of highly unsaturated fatty acids (HUFAs). Delta 5 desaturases, after desaturation and elongation by delta 6, add another double bond at the delta 5 position of 20-carbon fatty acids dihomo *y*-linolenic acid (c20:3n6) and eicosatetraenoic acid (c20:4n-3, see Figure 1) (Nakamura & Nara, 2004). Both AA and DHA are the two major HUFAs synthesised by the D6D/D5D pathway (Nakamura & Nara, 2004).

It is estimated that only .2% of ALA is converted to EPA, however the same enzyme (delta 5 desaturase) additionally changes docosapentaenoic acid (DPA) to the final product DHA but at a much higher conversion rate of 37% (Brookes, Chen, Xu, Taylor, & Asherson, 2006b; Pawlosky, et al., 2001). The metabolic pathway of fatty acids is complex (see Figure 1) and the conversion process is influenced by many factors including diet, oxidative stress, alcohol, smoking, age and genetic factors (Agostoni et al., 2008; Horrobin, 1987; Lands, 2008; Marangoni et al., 2004; Pawlosky & Salem, 2004). It is further suggested that an excessive ingestion of either ALA, LA or any other kind of PUFA could be disruptive, resulting in a containment of the metabolic pathway (Brookes, et al., 2006b; Nakamura & Nara, 2004). This has implications for the dietary balance of the ratio of omega-3/6 as an excessive intake of one fatty acid type may inhibit the conversion of the other kind (Nakamura & Nara, 2004).

The imbalance of omega-6/3, which is estimated to have risen from a ratio of 1:1 to approximately 16:1 in Western societies during the last century, is a focal point of much scientific debate and the current consensus is that the deficit in omega-3 is likely to negatively impact both physical and mental health (Simopoulos, 2002). The modern, Western diet has arguably evolved from a lower unsaturated to a high saturated fat ratio which is profoundly

different to that of our ancestors (Simopoulos, 2002). The change from our paleolithic heritage of whole and unrefined foods came about as a direct consequence of the Agricultural Revolution between the 17th century and the end of the 19th century and then continued post World War II in favour of mass food production resulting in the "modern refined diet", consumed today (Cordain et al., 2005; Eaton, Eaton, & Konner, 1997; Eaton, Eaton, Sinclair, Cordain, & Mann, 1998; Simopoulos, 2002). The modern Western diet is described as one with high sodium content, a low nutrient density in relation to the amount of minerals, vitamins and trace elements, anti-oxidants, fibre, phytochemicals, amino acids and unsaturated fatty acids per gram, and a high glycaemic load due to the presence of refined sugars and grain products (Cordain, et al., 2005). Furthermore, the 21st century has seen not only the introduction of a prolific number of chemicals in our environment but an abundance of new constituents in foods, including the global use of pesticides and fertilisers as opposed to crops developing and growing (or animals grazing) in a natural environment and a high concentration of long chain saturated fats from fatty domestic meats as opposed to wild mammals (Cordain, et al., 2005). Warnings about the physiological effects of a malnourished diet have been publicised during the last century by many leading authorities including (1) the works of Sir Robert McCarrison (1936; 1937) carried forward today by The McCarrison Society, (2) Hugh Sinclair (Magdalen College Oxford) was a doctor and expert of human nutrition who coined the expression "diseases of civilisation", and argued that deficiencies in essential fatty acids may lead to diseases such as atherosclerosis and myocardial infarction, (3) the Soil Association who promote healthy soil and organic food without the use of pesticides, (4) the Alliance for Natural Health who promote natural approaches to healthcare and (5) the work of Crawford and colleagues in the 1970's which demonstrated that omega-3 deficient diets lead to behavioural pathologies. More recently, the World Health Organisation (WHO) has made public the prediction that child mental ill-health is estimated to rise by 50% by 2020 (Global Forum for Health Research, online). The United Nations (UN) has also acknowledged a *new* type of malnutrition coined "Type B malnutrition", which derives as a result of multiple micronutrient depletion and is very likely the result of the globalisation of our Western food systems (Crawford, Bazinet, & Sinclair, 2009).

Several mechanisms of action have been proposed to account for the potential benefits of essential fatty acids, predominately related to their structural role in the brain and neurotransmission (Sinn & Howe, 2008). In relation to the brain, both DHA and AA are found in

elevated quantities in the grey matter of the cerebral cortex, in particular in the membranes of neuronal synapses (Gow, Matsudaira, Taylor, Rubia, Crawford, Ghebremeskel, Ibrahimovic, Vallee-Tourangeau, et al., 2009; McNamara, 2006; McNamara et al., 2009; McNamara, Jandacek, et al., 2010). Brain compositions of PUFA have been examined in neonate baboons and provided evidence for the distribution of DHA and AA in 26 different regions of the brain (Diau et al., 2005). DHA was found to be more abundant in the white matter of the brain and the greatest level found was in the corpus callosum at 7%. DHA ranged from 15.8% in the globus pallidus to 4.5% in the optic nerve while AA levels were highest in the hippocampus, cingulate, caudate, putamen, post-centralis, occipital, temporal, frontal and amygdala brain regions (Diau, et al., 2005) . Most of these brain areas have been found to be abnormal in structure and function in ADHD patients (Rubia, Halari, Taylor, et al., 2011; Valera, Faraone, Murray, & Seidman, 2007). The precursor of DHA, EPA, is also considered of importance in the brain, specifically in its role in eicosanoid production (Sinn & Howe, 2008). Both EPA and DHA are linked with many important functions related to neural activity such as neurotransmission, ion channel, myelination membrane fluidity, enzyme regulation and gene expression (Gow, Matsudaira, Taylor, Rubia, Crawford, Ghebremeskel, Ibrahimovic, Vallee-Tourangeau, et al., 2009; Lauritzen, et al., 2001; Sinn & Howe, 2008).

Inadequate supplies of DHA *in-utero* are associated with impaired learning and attention in addition to emotional irregularities such as increased depression, anxiety and aggression in animal models (Fedorova & Salem, 2006). Mathieu, Denis, Lavialle and Vancassel (2008) have shown that these irregularities are in part related to alterations in neurotransmission function (Mathieu, Denis, Lavialle, & Vancassel, 2008). They demonstrated that dietary induced deficiencies in DHA result in a deregulation of the meso-cortico-limbic dopaminergic pathway which is implicated in emotion and reward processes (Zimmer et al., 2002). Mathieu et al. (2008) also reported that in rodents receiving a chronically deficient omega-3 diet a reduction in the release of acetylcholine and serotonin in the hippocampus is observed. Furthermore, the amount of omega-3 PUFA in the diet influenced the serotonergic receptor and the muscarinic receptor binding (Mathieu, et al., 2008). These results were thought to negatively contribute to poor performance across a series of cognitive tasks in the group that were given an omega-3 deficient diet compared to controls (Mathieu, et al., 2008). In addition, Clements and colleagues (2003) reported diminished amounts of neuronal omega-3 fatty acids in the spontaneously hypertensive

rat (SHR) which is deemed an ideal animal model for ADHD (Clements, Girard, Xing, & Wainwright, 2003; Sagvolden, 2000).

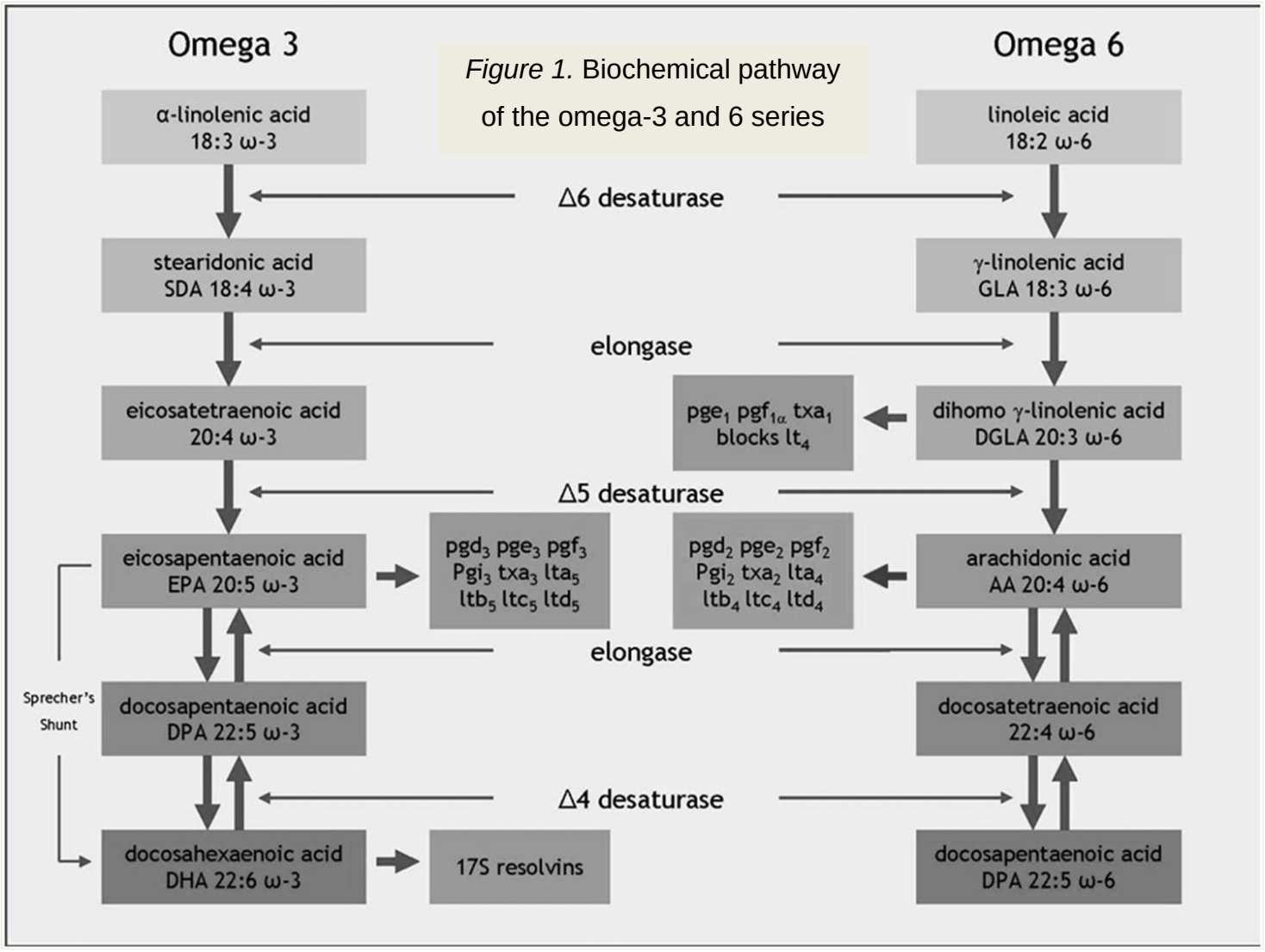


Figure 1. Biochemical pathway of the omega-3 and 6 series

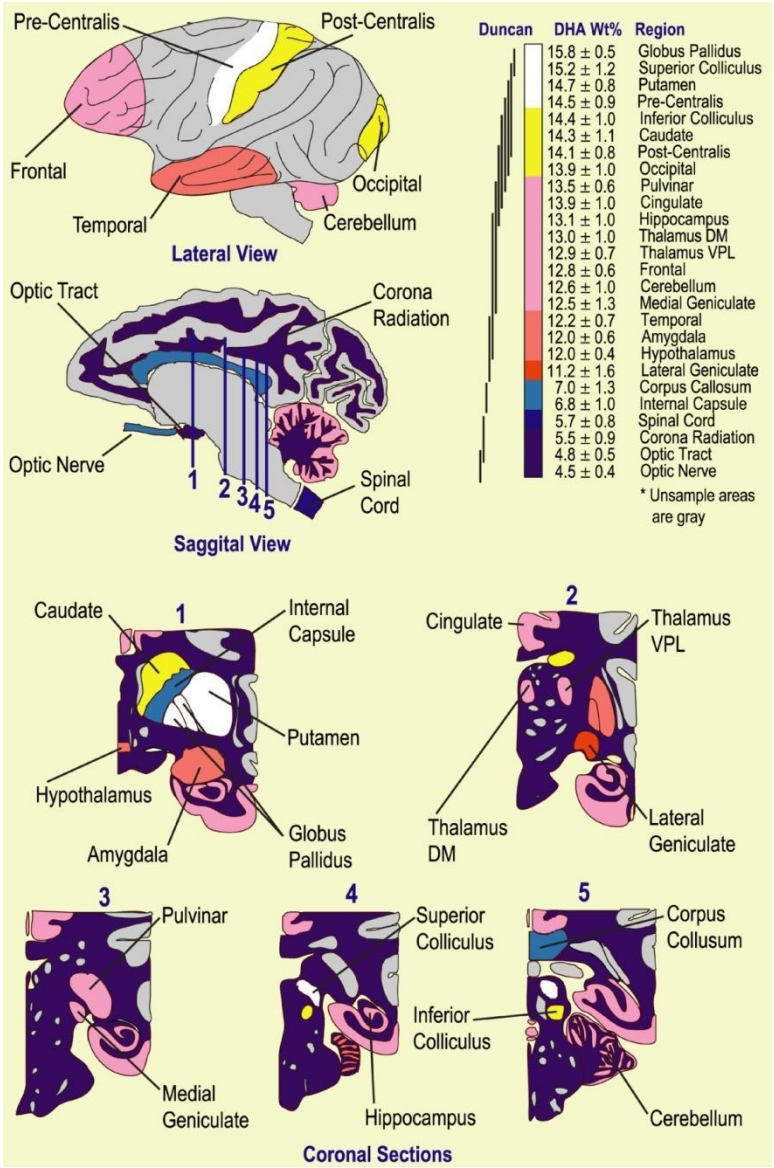


Figure 2. The Central Nervous System (CNS) DHA Map of a Baboon Neonate. DHA concentrations are colour coded and rank ordered from highest (white) to lowest (dark blue). Numbered lines in the parasagittal section refer to coronal sections; the right side is illustrated with the majority of the right hemisphere removed for clarity. "Duncan" refers to Duncan's multiple range test; means sharing a line are not statistically different ($p < 0.05$). The colours each span 10% of the DHA concentration range (with permission for reprint from Brenna et al., 2007).

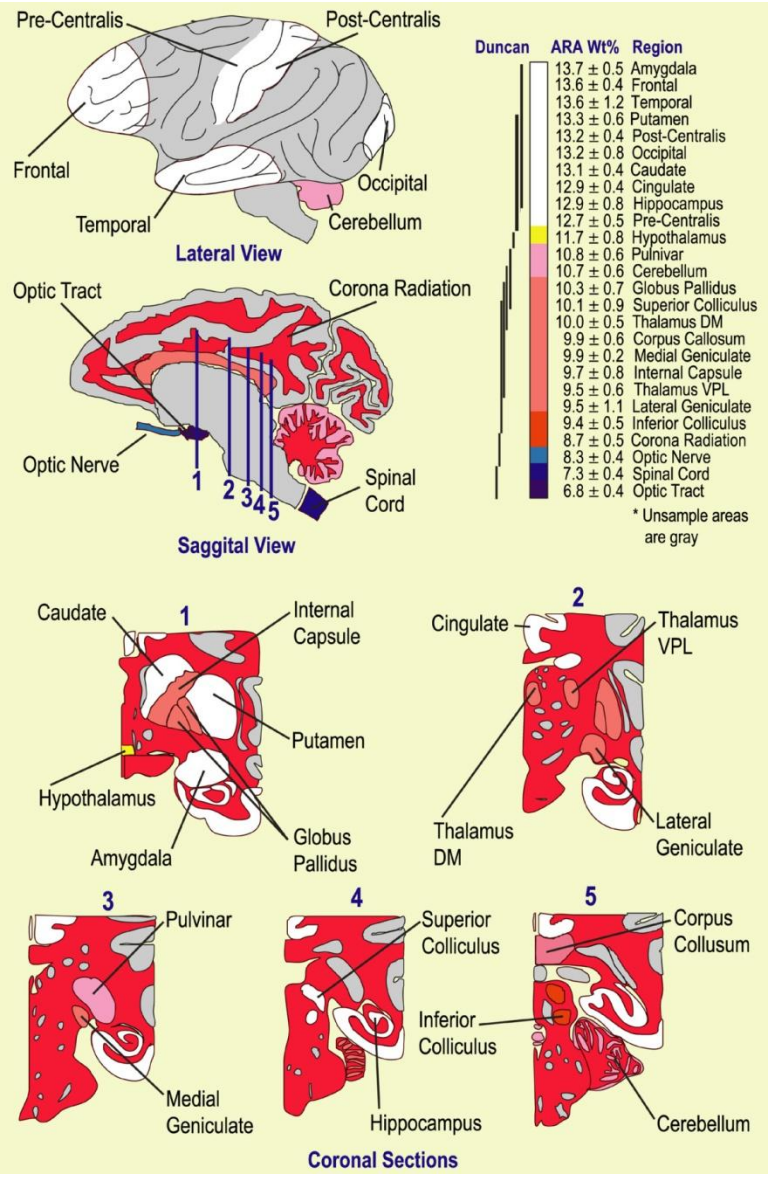


Figure 3. The CNS Arachidonic Acid (AA) Map of a 4 week old baboon central nervous system. AA concentrations are colour coded and ranked highest (white) to lowest (dark blue). Printed with permission from Brenna et al., 2007.

PUFA and cognitive functions in healthy children and adults

Cumulative research suggests that LC-PUFAs have an important role in the neurodevelopment of both cognitive and emotional function (Marszalek, & Lodish, 2005) and that, in respect of the latter, supplementation of fatty acids, particularly EPA, can elevate mood and alleviate depression (Fontani, Corradeschi, Felici, Alfatti, Bugarini, et al., 2005; Freeman et al., 2006; Gow, Matsudaira, Taylor, Rubia, Crawford, Ghebremeskel, Ibrahimovic, Vallee-Tourangeau, et al., 2009; Peet & Horrobin, 2002a). However, there are very few publications exploring the relationship between LC-PUFA and neurocognitive development (e.g., neuropsychological measures of brain function including executive function processes such as self-regulation measuring inhibitory control, attention control, forethought, planning, goal-directed behaviour, delay of gratification, working memory, problem solving, cognitive flexibility, the inhibition of inappropriate behaviours or acts, sustaining and selective attention, and affect processing) in children/adolescents groups and thus the literature to date is extremely limited.

There are a small number of epidemiological studies of LC-PUFA intake in childhood (e.g., prospective follow-up studies from birth to 4 years of age) (Ryan, et al., 2010) and measures of cognitive performance (e.g., motor skills, digit span/working memory, problem solving and IQ) showing some significant effects (Aberg et al., 2009; Bakker, Hornstra, Blanco, & Vles, 2007; Hibbeln et al., 2007; Oken, Osterdal, et al., 2008; Oken, Radesky, et al., 2008; Willatts, Forsyth, DiModugno, Varma, & Colvin, 1998; Zhang, Hebert, & Muldoon, 2005) while others finding no results (Bakker et al., 2003; Ghys, Bakker, Hornstra, & van den Hout, 2002). For example, Bakker and colleagues (2003) assessed the relationship between LC-PUFA measures of DHA and AA (taken from phospholipid blood samples at 2 time points: at birth via umbilical measures and then again at 7 years of age) and cognitive ability (using the Kaufman Assessment Battery for Children). There were no significant associations between any of the measures (Bakker, et al., 2003), for a review see Ryan et al., (2010).

Aberg and colleagues (2000) examined the fish intake, socio-economic and disease status in Swedish adolescents (n = 18, 158) aged 15 years of age. A proportion of data from male responders was later merged with measures of intelligence quotient taken at the age of 18 recorded by the Swedish Military Conscription Service. This later analysis found that fish consumption of $>$ 1 time per week was positively correlated with higher scores in composite IQ

and both verbal and visuospatial performance at 18 years of age in contrast to those consuming fish less than once a week (Aberg, et al., 2009).

Epidemiological studies have also examined LC-PUFA intake during pregnancy and later neurodevelopment. For example, the Avon Longitudinal Study of Parents and Children (ALSPAC) also referred to in the U.K. as *Children of the 90's*, is a longitudinal study of health care outcomes with Bristol University. In 1991, over 14,000 mothers registered during pregnancy and the developmental and health trajectory of their children has been charted ever since. One particular publication by Hibbeln et al., (2007) that has arisen from the project and subsequently published in the Lancet found that maternal seafood consumption in pregnancy was significantly negatively associated with developmental outcomes. For example, the lower the seafood consumption the lower the developmental scores of fine motor skills, verbal IQ, social development and pro-social behaviour in children aged 8 years and under (Hibbeln, et al., 2007).

Whalley and colleagues (2004) carried out an observational investigation of 120 participants born in 1936 whose cognitive abilities were assessed in 1947 (aged 11) and then for a second time in 2000-2007 (at 64 years). They reported that a measure of the total amount of omega-3 fatty acids, alongside an elevated ratio of DHA/AA as measured in the red blood cells of participants were significantly positively correlated with IQ at 64 years (but not at age 11). Total omega-3 fatty acids, the ratio of omega-6/3 and DHA/AA ratio were also significantly positively correlated with cognitive scores on the Raven's Progressive Matrices, digit symbol scores and block design tests (Whalley, Fox, Wahle, Starr, & Deary, 2004). The sample was further divided into 2 groups: those who consumed fish oil versus nonusers and observed separately. In this sub-group there were positive associations between childhood IQ and erythrocyte omega-3 PUFAs, specifically EPA (Whalley, et al., 2004).

The only published study to date, to investigate the outcomes of omega-3 supplementation on physiological and cognitive factors was carried out by Fontani, Corradeschi, Felici, Alfatti, Migliorini and Lodi (2005) in healthy subjects. During both Go/NoGo and sustained attention tasks a reduction in RT was observed in the active supplementation group. This was coupled with a shift towards theta and alpha waves in electrophysiological studies (EEG). Simultaneously, omega-3 supplementation had a positive effect on mood as recorded by self-rated measures of depression, anxiety and anger alongside an increase in vitality. However, although omega-3 was implicated in enhancements in both attentional and physiological

functions, a direct comparison of placebo and active groups was not undertaken. Rather, the researchers examined the two timepoints, baseline and endpoint in each group (i.e., placebo and then active separately and did not contrast between placebo and active groups) (Fontani, Corradeschi, Felici, Alfatti, Migliorini, et al., 2005).

More recently, McNamara and colleagues (2010) explored the mediating effects of DHA supplementation in healthy school children (aged 8-10 years) on brain activity during a sustained attention task using fMRI. The boys were randomly allocated to receive 1 or 2 measures of DHA (i.e., 400 mg versus 1200 mg) or placebo. Cortical brain activity was recorded at two time points: (1) at the start of the study prior to supplementation and (2) at endpoint, 8 weeks later. DHA erythrocyte membrane composition had increased at 8 weeks in those receiving the 400 mg dose by 47% and by 70% in those receiving the 1200 mg dose compared to the placebo group (McNamara, Able, et al., 2010). The two active groups both displayed greater alterations in activation of the dorsolateral prefrontal cortex (DLPFC) relative to placebo (McNamara, Able, et al., 2010). Furthermore, there were greater decreases in activation from baseline to end point in the occipital and cerebral cortex in both low and high dose groups compared to placebo (McNamara, Able, et al., 2010). The 1200 mg dose group in comparison to the 400 mg dose group resulted in greater decreases in activation in the bi-lateral cerebellum (McNamara, Able, et al., 2010). At both baseline and endpoint, the blood measures of DHA were positively associated with activation in the DLPFC and negatively associated with RT (McNamara, Able, et al., 2010). This is the first study to demonstrate that dietary intake of DHA and respective increases of RBC measures are in turn associated with changes in cortical attention networks in healthy boys (McNamara, Able, et al., 2010).

LC-PUFA in ADHD

Several lines of enquiry have suggested a link between ADHD and omega 3/6 fatty acids (Antalis, et al., 2006; Richardson & Montgomery, 2005; Richardson & Puri, 2002; Stevens, Zentall, et al., 2003). One of the first studies to suggest an association was published by Colquhoun and Bundy (1981). They surveyed the diets of 214 children (161 boys) that attended The Hyperactive Children's Support Group (HACSG) and suggested that boys with ADHD-type symptoms displayed signs of fatty acid deficiency. For example, they reported that two thirds of the children suffered from polydipsia (excessive thirst), had zinc values below the normal range and suffered from allergies including eczema, asthma with intolerances to milk and wheat; all of

which are symptoms directly or indirectly related to fatty acid deficiency. Although this study was not a clinical trial, it was published as a medical hypothesis and facilitated the closer investigation by other researchers into the potential link between PUFA deficiency and hyperactive behaviour.

Abnormal percentage values of fatty acids have also been found in the erythrocytes of children and adults with ADHD (Antalis, et al., 2006; Chen, Hsu, Hsu, Hwang, & Yang, 2004; Colter, Cutler, & Meckling, 2008; Germano et al., 2007; Mitchell, Aman, Turbott, & Manku, 1987; Mitchell, Lewis, & Cutler, 1983). However, the precise nature of this association is not yet apparent and further research is necessary to establish whether this is due to a deficiency in PUFAs, or an abnormality in metabolism (Antalis, et al., 2006). The publications in this area are limited but the trends persistently demonstrate abnormal fatty acid profiles. Stevens, Zentall, Abate, Kuczek and Burgess (1995) reported that approximately 40% of male children between 6 and 12 years of age with a clinical diagnosis of ADHD (n = 53) showed thirst and skin problems and furthermore that their arachidonic acid (AA) and DHA quantities were significantly lower than matched controls (n = 43) (Stevens et al., 2003; Stevens, Zentall, Abate, Kuczek, & Burges, 1996). However, it was not evident that blood levels of EFAs observed in this sample were either indicative of a deficiency or confirmation that they consumed less dietary PUFAs than controls (Stevens et al., 1995). Following on from this initial research, Stevens and colleagues (1996) used the same cohort but combined the ADHD and controls together and classified them into "low" or "high" groups according to their omega-3 and omega-6 status. The researchers then used parental reports of the children's behaviour, learning and health to compare the groups (Stevens, et al., 1996). They found that lower concentrations of omega-6 were associated with classic signs of fatty acid deficiency such as skin problems, frequent urination, excessive thirst, rough and dry skin/hair, frequent colds and antibiotic use thus supporting the earlier work of Colquhoun and Bundy (1981). The low omega-3 group was also correlated with fatty acid deficiency symptoms alongside learning difficulties and behavioural difficulties such as hyperactive-impulsive behaviour, anxiety, temper tantrums and conduct disorder symptoms (Stevens, et al., 1996).

On the basis of their previous findings, Stevens and colleagues (2003) carried out a pilot study evaluating the efficacy of the PUFA supplementation on blood levels of essential fatty acids and behaviour in 50 children with ADHD. The participants were of both gender and aged

between 6 and 13 years with a previously given clinical diagnosis of ADHD (Stevens, Zhang, et al., 2003). Parents were asked to complete a fatty acid deficiency questionnaire and those who scored 4 or greater for their thirst/skin problems were recruited for the study. A double-bind, placebo-controlled design was employed and children were randomised into 2 treatment groups: active (DHA: 60 mg, EPA: 10 mg, AA: 5 mg, GLA: 12 mg and Vitamin E: 3 mg) or placebo (0.8 mg of olive oil per capsule). Participants were instructed to take 8 capsules per day for a 4 month period. The findings confirmed that supplementation with PUFA resulted in a sizeable increase of both plasma and red blood cell (RBC) levels of EPA, DHA and α-tocopheryl. In the placebo (olive oil) group, plasma levels of ALA (c18:3n-3) were also elevated. There were significant improvements in a range of parent-rated outcomes in both groups; however the PUFA treatment group did not show a clear benefit in improvements in ADHD symptomology. The treatment difference was significant on only 2 out of 16 measures: parental ratings of conduct problems and teacher ratings of attention (Stevens, Zhang, et al., 2003). In the active PUFA group, significant improvements were observed in children with oppositional defiant disorder type symptoms which changed from a clinical to non-clinical range in comparison to the placebo group (Stevens, Zhang, et al., 2003).

To investigate the fatty acid deficiency research hypothesis in ADHD further, Antalis and colleagues (2006) focused on the potentiality of a deficiency in metabolism as an explanation for the changed fatty acid phenotype. They did this by selecting a population of male students (*M* = 24.3 years in age) diagnosed with ADHD and compared them to control adults (*M* = 22.3 years in age) without a behaviour disorder. In addition, comparisons were drawn between the fatty acid levels and blood markers, for a range of conditions and nutrients which may influence long-chain polyunsaturated fatty acid status and abnormal behaviour (Antalis, et al., 2006). In plasma the ADHD group had a higher proportion of total saturated fatty acids and total monounsaturates compared to controls. In terms of PUFAs, there were no significant differences for the majority of individual family members of the omega-6 series in plasma, however the total omega-6 ratio was approximately 6% higher in controls (Antalis, et al., 2006). In contrast, in the erythrocytes only levels of AA varied between the 2 groups which was found to be approximately 10% greater in the ADHD group compared to the control group (Antalis, et al., 2006). In relation to the omega-3 series acids, DHA levels were lower in the ADHD group compared to controls by 53% in plasma and 36% less in erythrocytes (Antalis, et al., 2006). An identical pattern was also

observed for total omega-3 ratio (Antalis, et al., 2006). In plasma, levels of ALA were greater in ADHD however all ALA metabolites were lower (Antalis, et al., 2006). The total omega-6/omega-3 ratio (i.e., the sum of total omega-6 over the total of omega-3) was higher in both plasma and erythrocytes in ADHD compared to controls (Antalis, et al., 2006). Additionally in the ADHD group, the AA/EPA ratio was 36% higher in erythrocytes compared to the controls (Antalis, et al., 2006). This association was also similar in plasma although the difference was not significant. Correlational analysis was conducted to assess the strength of the relationships between behaviour and omega-3 fatty acids in both plasma and erythrocytes. Conners Rating subscales for inattention and impulsive-hyperactive symptoms of ADHD were found to be significantly negatively associated with plasma levels of DHA (Antalis, et al., 2006). Similar patterns were found for the total omega-3 ratio in both plasma and erythrocytes blood samples, implying that lower omega-3 is linked with increased severity of ADHD symptoms (Antalis et al., 2006).

Other studies including Colter et al., (2008) have assessed fatty acid status and behavioural symptoms of ADHD in young people (Colter, Cutler & Meckling, 2008). Colter et al., sought to examine whether potential differences existed in dietary ingestion of fatty acids and if any relationships existed between fatty acid indices and Conner Parent Rating Scales (CPRS-L). Their sample size was limited with a total of 23 participants of which 11 had a clinical diagnosis of ADHD and 12 were healthy controls. The fatty acid deficiency symptom check reported an average of 0-9 symptoms for the ADHD group versus 0-4 symptoms in the control group (Colter, et al., 2008). Analysis of the Conners' Rating Scales in the ADHD group revealed specific components of diet such as total energy (i.e., % of calories from saturated fat levels) were positively correlated with oppositional and hyperactive behaviours (Colter, et al., 2008). Both levels of total fat and saturated fat were significantly positively associated with scores of oppositional, problematic and hyperactive behaviours (Colter, et al., 2008). Phospholipid analysis of red blood cells found that compared to controls, DHA (M = 3.12 $\pm$ 0.75 vs. M = 4.39 $\pm$ 1.34) and total omega-3 fatty acids (M = 5.79 $\pm$ 1.39 vs. M = 7.42 $\pm$ 1.64) were significantly lower in the ADHD group (Colter, et al., 2008). DHA fractions in RBC were negatively correlated with various parent rated subscales of the Conners Index including oppositional behaviour, problematic behaviour, cognitive problems, hyperactivity, restlessness, DSM-IV inattention and DSM-IV total (Colter, et al., 2008). Total omega-6 levels were significantly

positively correlated with problematic and oppositional behaviour, restlessness, DSM-IV inattentive, DSM-IV total and ADHD index scales (Colter, et al., 2008). Overall, this group of ADHD adolescents had lower concentrations of total omega-3 and DHA. They also had higher levels of LA compared to age matched controls. It was theorised that the lower levels of DHA may be due to a higher frequency of oxidation (Colter, et al., 2008). The diet records did not reveal differences in total omega-3 fatty acid consumption. It could be theorised then that the differences found in the blood samples cannot be attributed to diet. However, it is noted that 50% of the ADHD group were taking vitamin and mineral supplementation (non-specified) compared to 25% of the controls. This study was also limited by the small sample size. Furthermore there was a gender imbalance within groups which is relevant as males can differ from females in the metabolism of fatty acids (Colter, Cutler, & Meckling, 2008).

Researchers in Taiwan have also examined the dietary patterns of children (aged 4-12) with a clinical diagnosis of ADHD (n = 58) and found lower LA, AA and DHA fatty acid compositions in red blood cells compared to controls (n = 52). The children with ADHD also had significantly higher iron levels in their blood compared to controls. (Chen, et al., 2004).

Ross and colleagues (2003) have also reported abnormal fatty acid metabolism in ADHD. For example, they reported a significantly higher degree of ethane exhalent which are a marker of fatty acid oxidation levels (i.e., the degradation of fatty acids prior to their incorporation into cell membranes) in ADHD children relative to controls (Ross, McKenzie, Glen, & Bennett, 2003).

Associations have also been found between ADHD and fatty acid desaturase genes in a genetic study (Brookes, et al., 2006a; Brookes, et al., 2006b). The authors investigated 3 genes that encode desaturase enzymes for the metabolism of fatty acids by searching for genetic links between 45 SNPs and ADHD (Brookes, et al., 2006b). The results demonstrated a significant relationship between ADHD and *SNP rs498793* in the fatty acid desaturase 2 (*FADS2*) (Brookes, et al., 2006b). On the basis of their finding and assuming the detection of a true association, the authors further discussed that the *rs498793* may be connected with decreased activity of this desaturase enzyme resulting in lower levels of omega-3 being incorporated into the cell membrane (Brookes, et al., 2006b). Following this conjecture, the build up of omega-3 fatty acid precursors will be subsequently rerouted for breakdown to the oxidation pathway thus also providing a conceivable explanation for the rise in ethane levels exhale observed by Ross et al

(2003) (Brookes, et al., 2006b). However, the authors note that further examination of the genes that control fatty acid metabolism relative to ADHD would be necessary to refute or confirm their preliminary observations (Brookes, et al., 2006a; Brookes, et al., 2006b).

Randomised Controlled Trials of EFA supplementation in ADHD patients

Several randomised, placebo controlled, double blind trials have explored the plausibility of omega-3 supplementation to ADHD symptoms but with varying design methods and consequently results (see Table 1 below). The Oxford-Durham study (2005) provided important evidence linking omega-3/6 supplementation and improvements in behaviour and concentration in underachieving mainstream schoolchildren (who had symptoms of ADHD and Developmental Coordination Disorder (DCD is also known as dyspraxia) (Richardson & Montgomery, 2005). This randomised clinical trial (RCT) was placebo controlled and double blind. It recruited 117 school children aged 5-12 years old and supplemented them either an omega-3/6 fatty acid treatment or placebo during a three month period. At 15 weeks the placebo group was crossed over onto the active supplement while the active group continued with the active supplement for a further 15 weeks (Richardson & Montgomery, 2005). The main objective of the study was to assess whether those schoolchildren receiving fatty acid supplementation, compared to placebo, would show significant improvements in key areas of functions relative to learning (using standardised and age-adjusted methods to assess literacy including word reading and spelling, motor-skills, teacher ratings of behaviour and learning difficulties linked to ADHD) (Richardson & Montgomery, 2005). Children were assessing for ADHD symptoms using the Conner's Teacher Rating Scale (CTRS-L) and at baseline, mean scores were just over 1 *SD* above the mean, 31% of the sample scored 2 SD above the mean on the DSM-IV total CTRS scale (Richardson & Montgomery, 2005). None of the children recruited had received a previously given clinical diagnosis of ADHD (Richardson & Montgomery, 2005). All children met the diagnostic criteria for DCD according to the DSM-IV but none were receiving intervention for the condition (Richardson & Montgomery, 2005). Exclusion criteria included both mental and physical conditions as verified by the child's G.P. (Richardson & Montgomery, 2005).

These results showed no effects for motor skills as determined by the Movement Assessment Battery for children, however significant improvements were observed for spelling, reading (as measured by the Wechsler Objective Reading Dimensions battery) and behaviour (as

rated by the CTRS-L) over three months of treatment in the treatment (fish oil) group relative to the placebo group (Richardson & Montgomery, 2005). The treatment group in the first three-month period of omega-3/6 supplementation made a 6-month reading gain and a 3-month gain in spelling (Richardson & Montgomery, 2005). The crossover from placebo to active groups showed similar changes while the active group continued to make improvements up to the end of the trial at six months (Richardson & Montgomery, 2005). The findings of this study support the theory that omega-3/6 supplementation may be an efficient treatment consideration for children with behavioural and educational difficulties associated with DCD (Richardson & Montgomery, 2005).

A further two trials have attempted to replicate the Oxford-Durham since in ADHD populations. The first by Sinn and Bryan (2007) recruited 132 South Australian children aged 7-12 who scored 2 *SD* higher than the mean (i.e., over the 90th percentile) on Conners abbreviated ADHD Index (which assesses the severity of attentional/cognitive, hyperactive and impulsive problems) (Sinn & Bryan, 2007). Children who were already taking stimulant medication were not included in the study (Richardson & Montgomery, 2005). At 15 weeks of supplementation, the study revealed significant, medium to strong effects in the active group compared to placebo as measured by the following subscales of the CPRS: Connors ADHD Index, Conners Global Index: Restless-Impulsive, Cognitive Problems/Inattention, both DSM-IV symptoms subscales Oppositional Behaviour and Inattentive and Hyperactive-Impulsive (Sinn & Bryan, 2007). The placebo group were crossed over to treatment at week 16 and continued with the active supplement until week 30. Their scores were compared at weeks 15 and 30 and significant improvements were found in the following subscales of the CPRS: Conners ADHD Index, Hyperactivity, Cognitive Problems/Inattention, Global Index: Restless-Impulsive, both DSM-IV symptoms subscales Inattentive and Hyperactive-Impulsive as well as the Social Problems and Perfectionism (Sinn & Bryan, 2007). At 30 weeks, both PUFA group continued to show significant improvements on Cognitive Problems/Inattention, Connors ADHD Index, Conners Global Index: Restless-Impulsive, both DSM-IV symptoms subscales Inattentive and Hyperactive-Impulsive and on the Hyperactivity (Sinn & Bryan, 2007). However, no significant effects were found for any of the behaviour measures on the CTRS at either 15 or 30 weeks, nor were there any significant differences with or without micronutrients. The dose of micronutrients (20 mg) was quite low and may account for the lack of findings.

Table 1. Clinical Trials with LC-PUFA in children with ADHD

Author & Journal	Target Group	Type of Formula	ALA	LA	Total fish oil	EPA	DHA	GLA	Vitamins	Daily dose	Duration	*N* =	M / F
Richardson & Montgomery *Pediatrics* (2005)	Met DSM-IV criteria for Developmental Coordination Disorder	natural triglyceride	n/a	n/a	400 mg	186 mg /2 capsules	58 mg	20 mg	3.6 mg	x 6	6 mo.	117	Both
Sinn & Bryan *J Dev Behav Pediatr* (2007)	Scores 2SD above general population average (> 90th percentile on Conners abbreviated ADHD index	natural triglyceride	n/a	n/a	400 mg	186 mg /2 capsules	58 mg	20 mg	3.6 mg	x 6	6 mo.	132	Both
Johnson, Östlund, Fransson, Kadesjö & Gillberg *J Attention Disorders* (2008)	Met DSM-IV criteria for diagnosis of ADHD of any suybtype, scoring at least 1.5 SD above age norm for their their agnostic subtype using norms for the ADHD Rating Scale IV Parent version	natural triglyceride	n/a	n/a	400 mg	186 mg / 2 capsules	58 mg	20 mg	3.6 mg	x 6	6 mo.	75	Both
Voigt et al. *J Pediatr* (2001)	Diagnosis of ADHD by a physician included those with commorbidities of ODD and CD	algae-derived triglyceride capsule	n/a	n/a	n/a	n/a	345 mg	n/a	n/a	x 3	4 mo.	63	Both
Hirayama et al. *Eur J Clin Nutr* (2004)	Diagnosed or suspected as ADHD according to DSM-IV and dioagnostic interviews including behavior observation by psychiatrists	fortified foods (soybean milk/ bread rolls)	n/a	n/a	n/a	700 mg /week	3.6 g /week	n/a	n/a	n/a	2 mo.	40	Both
Raz et al J Child Adolesc Psychopharmacol (2009)	Clinical diagnosis	soft gel capsule	60 mg	240 mg	n/a	n/a	n/a	n/a	n/a	x 1	7 weeks	78	M
Arnold et al. Biol Psychiatry (1989)	Diagnosis according to DSM-III	Evening Primrose Oil	n/a	350 mg	n/a	n/a	n/a	40 mg	13 IU	x 8	1 mo.	18	M

Sinn and colleagues (2008) also employed neuropsychological measures of cognition and reported significant advancements in the capability to switch and control attention (as measured by the Creature Counting Task) in the same group of children (aged 7 to 12 years) with ADHD receiving omega-3/6 polyunsaturated fatty acids at 15 weeks compared to placebo (Sinn, Bryan, & Wilson, 2008). Following a cross-over from the placebo into the active group at 16 weeks (from week 16 -30) a significant improvement in this measure was also observed. The authors noted that enhancement in cognitive performance mediated the parent rated improvements in ADHD symptoms (namely, hyperactivity, impulsivity and inattention) reported previously (Sinn, Bryan & Wilson, 2008).

The second study by Johnson, Östlund, Fransson, Kadesjö & Gillberg, (2009) recruited 75 children and adolescents (comprising 64 boys and 11 girls) with ADHD (35 with ADHD combined type and 40 with ADD inattentive subtype) aged between 8 and 18 years of age. The participants were recruited across 3 clinical centres in Sweden and had already received a diagnosis of ADHD. Eligibility into the study included those who had met criteria for a diagnosis of any subtype of ADHD according to the DSM-IV, scoring at least 1.5 *SD* above their age norm as per the ADHD Rating Scale IV Parent Version (Johnson, Ostlund, Fransson, Kadesjo, & Gillberg, 2009) . Participants were screened for any comorbidity such as learning difficulties (LD) and their reading and writing ability (RWD) using standardised tests. Exclusion criteria were diagnosis of autism (although Asperger syndrome and/or autistic traits were not excluded), mental retardation, alcohol or substance misuse, stimulant medication or fish oil supplementation in the previous 3 months, psychosis, bi-polar disorder, seizures or other significant medical conditions (Johnson, et al., 2009). The two primary outcome measures were the investigator-rated ADHD-RS-IV-Parent version and the Clinical Global Impression (CGI) severity scales (Johnson, et al., 2009). Overall, this study was negative due to a lack of statistically significant differences in either of the outcome measures between active and placebo groups at 3 or 6 months (Johnson, et al., 2009). There was however a non-significant trend for a greater reduction in ADHD-RS scores in the active group compared to placebo for 2 subscales measuring total symptoms of ADHD and Inattentive and Hyperactive-Impulsive scores in the omega-3/6 treatment compared to the placebo group in the full sample of ADHD children and adolescents (Johnson, et al., 2009). However, at the end of the first study phase, there was a subgroup (26%)

within the full sample who responded to treatment resulting in a clinically significant improvement in key ADHD symptoms (i.e., a decrease of 25% on the ADHD-RS and a reduction of CGI scores to within close to the normal range). Within this group, there was a small proportion of responders (N = 7) who demonstrated a 50% reduction in symptoms (Johnson, et al., 2009). At phase 2 of the study, 47% (28/59) were responders compared with the first phase and of these 12% (7 participants) displayed a 50% reduction in ADHD-RS scores measuring symptom severity (Johnson, et al., 2009). Following analysis of the diagnostic subtypes, a higher number of responders were in the ADD group (p = .03) compared to the ADHD combined type group, and more likely to have a comorbid developmental disorder (e.g., RWD, LD, autistic traits) (Johnson, et al., 2009). None of the responders were found in patients with comorbid CD, ODD, depression or anxiety (Johnson, et al., 2009).

An RCT by Richardson and Puri (2002) also explored the effects of highly unsaturated fatty acids (HUFAs) to both learning and behavioural difficulties connected with ADHD. They recruited 41 children with both above average ratings for symptoms of ADHD and specific learning difficulties. The children were already known to teachers as having literacy problems (Richardson & Puri, 2002). None of the participants had a formal diagnosis for either ADHD or another psychiatric disorder (Richardson & Puri, 2002). All participants were assessed by the investigator to ensure they met they fulfilled diagnostic criteria for dyslexia and then randomly assigned to either an active (EPA: 186 mg, DHA: 480 mg, GLA: 96 mg, vitamin E: 60 IU, cis-linoleic acid: 864 mg, AA: 42 mg and thyme oil: 8 mg) group (N = 22) or placebo (N = 19) comprised of olive oil (Richardson & Puri, 2002). The dose was 8 capsules a day for 12 weeks. The CPRS-L was employed at two time points (baseline and follow up at 12 weeks) to evaluate the severity of behavioural problems linked with ADHD (Richardson & Puri, 2002). The results of the study demonstrated that the treatment group had significantly lower scores on 2 global scales: DSM Inattention and Conners Global Total as measured by the CPRS-L than the placebo group (Richardson & Puri, 2002). Three of the sub-scales, namely: Psychosomatic, Cognitive Problems and Anxious/Shy were also significant at trend level (Richardson & Puri, 2002). There were no reported improvements on any scale in the placebo group at end point compared to baseline (Richardson & Puri, 2002). There were several limitations to this study discussed by the authors (1) an active placebo was employed, olive oil contains a psychoactive lipid called oleamide which is derived via oleic acid, (2) the small sample size clearly lacked statistical

power, (3) diagnostic issues, none of these children were diagnosed with ADHD, furthermore, only parent rating scales were used and generally should be used in partnership with the CTRS-L to be meaningful, and (4) the choice of HUFA as previous research in other psychiatric populations such as schizophrenia has shown greater efficacy with EPA alone (Richardson & Puri, 2002).

More recently, Milte and colleagues (2011) recruited 75 adolescents aged 7 -12 years of age with ADHD and with or without learning difficulties to investigate the effects of omega-3 PUFAs (DHA and EPA) versus an omega-6 (LA) control on ADHD symptoms, cognition and literacy. The participants gave blood samples and undertook various cognitive assessments. Conner Rating Scales were also collected from parents of the children. The baseline data from a placebo-controlled three-way crossover trial (after controlling for covariates) revealed that the higher PUFA group predicted lower anxiety/shyness; higher DHA predicted better word reading and elevated omega-6 poorer levels of reading, vocabulary, spelling and attention. Furthermore, 36% of the sample with learning difficulties had lower erythrocyte DHA status than those with just ADHD alone (Milte et al., 2011). Of note, the authors employed correlation analysis and corrections for multiple testing do not seem to have been employed, as the alpha criterion was reported at $p < .05$.

Other clinical trials investigated PUFA in ADHD have reported little or no effect of omega-3 supplementation (Arnold et al., 1989; Belanger et al., 2009; Hirayama, Hamazaki, & Terasawa, 2004; Raz, Carasso, & Yehuda, 2009; Voigt et al., 2001). These trials will not be individually discussed here due to their non-significant findings but it should be noted that there are many variables that could be accountable for the wide variation in findings and hence replicability of these trials. Primarily, there are few trials which employ the same design, supplement, dose and duration of supplementation. The three clinical trials mentioned earlier which show arguably the most promise for PUFA efficacy have employed the double-blind, one-way crossover design for a six month period and all have used the same formula with is a combination of omega-3 and 6 (Johnson, et al., 2009; Richardson & Montgomery, 2005; Sinn & Bryan, 2007). The least successful trials collectively have a number of notable misguidings in their design. For example, they have (1) employed a fortified (fish oil) food product (e.g., fermented soybean milk, steamed bread and bread rolls, see Hirayama et al., 2004) as opposed to a direct source of PUFA and arguably lowered the bioavailability of the product, (2) lacked

statistical power due to small sample size (Arnold, et al., 1989; Belanger, et al., 2009), (3) have used DHA only supplementation (Voigt, et al., 2001) without first checking the micronutrient and fatty acids status of the individual at baseline to assess for high/low status or variability within the omega-3 index (von Schacky & Harris, 2007) or (4) have used omega-6 only (e.g., GLA) for a very short duration (e.g., 1 month) (Arnold, et al., 1989) which does not tally with the duration necessary (i.e., approx 6 weeks) to physiologically alter the RBC fatty acid status.

One example of the null findings is the RCT carried out by Voigt and colleagues (2001). Their group recruited 63 children with a clinical diagnosis of ADHD aged 6-12 years old, who were already successfully receiving stimulant medication, and randomly assigned them to two groups (1) an algae-derived triglyceride treatment group (the formula containing 345 mg of DHA only) or (2) a placebo group for 4 months (Voigt, et al., 2001). There were no significant improvements in the outcome variables of attention and impulsivity (using the Test of Variables of Attention and the Children's Color Trails test) or parent rating's of behaviour (as measured by the Child Behaviour Checklist and Conners' Rating Scales: CRS). Although, physiological changes were noted (e.g., the active group was 2.6 fold higher in DHA levels (in plasma phospholipid measures) at completion of the study compared to the placebo group) the findings concluded that a 4-month period of treatment with DHA did not decrease ADHD symptoms (Voigt, et al., 2001).

Raz and colleagues (2009) tested the efficacy of short-chain EFA supplementation (short-chain are fatty acids with carbon atoms from 3 to 7) on measures of attention, hyperactivity-impulsivity using Parent-Teacher Abbreviated Conners rating scales and a computerised continuous performance test in 78 male children (M = 10.48 years) in the context of a randomised, placebo-controlled double-blind trial. All children had received a clinical diagnosis of ADHD prior to the trial. Comorbidities such as LD, ODD, CD, DCD, OCD, Tourette Syndrome, Sleep disorder, or Tic disorder were not excluded (Raz, et al., 2009). The active supplementation was 1 daily capsule comprising: LA: 240 mg, ALA: 60 mg, mineral oil: 95 mg and α-tocopherol: 5 mg (anti-oxidant). The dose provided a total of 600 mg of EFA. The placebo was a 1000 mg capsule of vitamin C. Supplementation was given for 7 weeks only. Blood samples were taken at the beginning of the study to test for total cholesterol, high and low-density lipoprotein, triglycerides, iron, ferritin, B_{12} and haemoglobin (Raz, et al., 2009). There were no measures of baseline fatty acid status and reasons for this were not given. The results

showed no significant effects of treatment between groups. The group by treatment interaction was significant at trend level only for parent ratings of DSM-IV inattention subscales with increased improvements in the placebo group (Raz, et al., 2009). The authors concluded that short-chain EFA are not effective as a primary intervention for ADHD (Raz, et al., 2009). However, there are some limitations of this study that should be noted; fundamentally the formula/choice of supplement is unusual. There is adequate evidence to demonstrate that Western diets are already overloaded with dietary LA as it is present in almost all commonly consumed commercial foods and typical intakes are 12-17 grams per day for women and men (Rett & Whelan, 2011b). Furthermore, dietary sources of ALA do not provide substantial quantities of the key omega-3, EPA and DHA and the metabolic pathway is both complex and low in efficiency.

A small number of open-label studies have reported some improvements in behavioural ratings of ADHD however the sample sizes in all the studies were small, i.e., < 30 (9, 16, 20 and 30 respectively) and therefore need replicating to ensure validity (Germano, et al., 2007; Harding, Judah, & Gant, 2003; Joshi et al., 2006; Sorgi, Hallowell, Hutchins, & Sears, 2007). For example, Harding and colleagues (2003) recruited 20 children, aged 7 to 12 years of age, with a clinical diagnosis of ADHD and allocated them into 2 groups of parental choice: a pharmacological treatment (MPH) group (N = 10) or a dietary supplementation group (N = 10). The alternative treatment comprised of a combination of amino acids, phospholipids, essential fatty acids, vitamins, minerals, probiotics and phytonutrients (see Harding et al., 2003) for a period of 4 weeks. Children with ODD or CD were not included in the study. The Ritalin was prescribed to be taken 2 to 3 times a day at a dose of 5-15 mg as determined by a physician. Assessments included the CPRS, revised, long version and the Intermediate Visual and Auditory/Continuous Performance Test (IVA/CPT). The IVA/CPT is a standardised computerised task which assesses attention and response inhibition, it additionally tests for visual and auditory distractibility. The results of the study showed improvements in 4 dimensions of the IVA/CPT task (visual response control, auditory response control, visual attention and auditory attention control) following supplementation. Improvements in attention and self control were equivalent in both treatment groups. To date, this study has not been replicated but offers preliminary evidence that a combination of vitamins, minerals, probiotics, fatty acids, amino acids and phospholipid may be as effective as psycho-stimulant medication in some children

with ADHD (Harding, et al., 2003). Although, it should be noted that due to the small sample size and open-label status thus lacking randomisation and blindness, the results should be interpreted with caution.

PUFA supplementation in non-clinical populations

Osendarp and colleagues (2007) assessed the effects of a micronutrient intervention (zinc, iron, vitamins A, B6, B12, and C, folate, DHA: 88 mg per day and EPA: 22 mg per day) by way of a fortified drink in 2 groups of school children, defined as well nourished and marginally nourished, aged 6-10 years of age (Osendarp et al., 2007). They found no significant improvements in general intelligence, attention or cognitive assessments but a significant difference for measurements of verbal learning and memory in the treatment group compared to placebo (Osendarp, et al., 2007). Arguably the dose of EPA and DHA was relatively low, especially for marginally nourished Indonesian children allowing just 770 mg per week.

The first published randomised placebo controlled trial to investigate the efficacy of omega-3 intervention on the cognitive ability and behaviour in U.K. mainstream school children aged 8 to 10 years of age (Kirby, Woodward, Jackson, Wang, & Crawford, 2010b) was recently published. There were 117 compliant children in the active group (supplement was a chewable orange flavour gelatin capsule containing 400 mg fish oil of which 260 mg were omega-3 fatty acids, DHA: 200 mg and EPA: 28 mg, vitamin A: 400mg RE, vitamin C: 30 mg, vitamin D: 2.5mg, and vitamin E:1.5 mg a-TE) and 118 in the placebo group. The placebo supplements were made up of Italian olive oil and were identical in appearance and flavour to the active supplements (Kirby, et al., 2010b). The study employed cheek cell analysis to assess the fatty acid status due to its non-invasive method. Phase 1 entailed all participants to ingest supplementation (active or placebo) in a randomised, double-blind method for a period of 16 weeks. Following the first phase of the study, was a one-way crossover from placebo supplements to the treatment supplements within the context of an "open-label" study (weeks 17-24) (Kirby, et al., 2010b). The objective of the study was to evaluate the effects of omega-3 on cognitive performance (as measured by IQ using the Kaufman Brief Intelligent test, reading and spelling as measured by the WIAT-II, Wechsler, 2005, hand writing using the Computerised Penmanship Evaluation Tool: ComPET, working memory using the Working Memory Test Battery for Children: WMTB-C, attention as measured by the Creature Counting a subset of the

TEA-Ch, impulsivity and visual attention using the Matching Familiar Figures Task: MFFT) and behaviour ratings (using the Strengths and Difficulties Questionnaire: SDQ and SNAP-I: a rating scale for ADHD) at baseline and follow up at 16 weeks (Kirby, et al., 2010b). In spite of the wide range of assessments employed, only 3 significant findings were observed between groups at 16 weeks and one of these, teacher-rated measures of pro-social behaviour, was in support of the olive oil (placebo) group. In the active group, there was a significant improvement in relation to the number of correct responses made to trials in the MFFT compared to the placebo in the per-protocol analysis (Kirby, et al., 2010b). Specifically cheek cell PUFA DHA and EPA levels were correlated with faster responses and greater accuracy on both the MFFT and creature counting subtest and had a seemingly protective outcome against the decline of pro-social behaviour (Kirby, et al., 2010b). As a group, the children also obtained elevated WIAT-II scores which may be possibly due to learning effects (Kirby, et al., 2010b). However, the authors advised that the results should be approached with caution because they were found in the per-protocol analysis and not in the intention-to-treat analysis of the data. Furthermore, they were practically isolated among a wide range of outcome measures (Kirby, et al., 2010b). It should be noted that buccal cell analysis is fairly limited in its ability to accurately assess a stable fatty acids status over a period of time. This is due to the rapid turnover in cheek cells and consequently the results are likely to be indicative of a dietary daily PUFA status. Furthermore, it could be argued that the placebo was not a true placebo as Italian olive oil is bioactive, is known for its anti-inflammatory effects and is rich in anti-oxidants (Beauchamp et al., 2005) .

The same group at the University of Wales also investigated the association between fatty acid deficiency symptoms as measured by the FADS and PUFA status in cheek cell analysis in 450 school children aged 8-10 years (Kirby, Woodward, Jackson, Wang, & Crawford, 2010a). The FADS measure the existence and severity of seven known fatty acid deficiency symptoms including brittle nails, dry skin, small skin bumps, dandruff, excessive thirst and frequent urination (Kirby, et al., 2010a). Essentially, they assessed for relationships between the FADS scores and attention and behaviour ratings, somatic complaints and cognitive test performance (Kirby, et al., 2010a). The results revealed that symptom severity of fatty acid deficiency scores were not connected to any of the omega-3/6 fatty acid levels in buccal cell samples (Kirby, et al., 2010a). There were significant associations however between parent ratings of child behaviour and FADS, in other words, the more severe the behaviour - the higher the FADS (Kirby, et al.,

2010a). The authors concluded that employing FADS as an indicator of fatty acid deficiency may not be suitable in a cohort of typically developing children (Kirby, et al., 2010a).

Two further randomised, placebo controlled trials have been conducted in healthy school children both with DHA supplementation. The first by Kennedy et al (2009) examined the cognitive effects and mood of 8 weeks supplementation with 400 mg or 1000 mg in healthy school children (n = 90) aged 10 – 12 years of age. The results from 10 individual tests of cognition and mood found no effect of either high or low doses of DHA (Kennedy et al., 2009). Of note, is the dose formulation choice (DHA only) as the existing literature demonstrates that it is EPA which seems to be most beneficial in alleviating symptoms of ADHD alongside low mood and depression (Bloch & Qawasmi, 2011; Freeman, et al., 2006; Jazayeri et al., 2008; Peet & Horrobin, 2002b). In relation to the duration of dose, 8 weeks is a relatively short period especially if baseline DHA status was not assessed prior to randomisation as there is a 7 fold variation in the population alone (Von Schacky, 2010). The second study by Ryan and colleagues (2008) assessed the efficacy of DHA versus placebo (high oleic sunflower oil) on cognitive functions including measures of sustained attention, memory, vocabulary acquisition and impulsivity in healthy, pre-school children (n = 175) for 4 months. The results of this study demonstrated no statistically significant benefit of DHA supplementation for each of the cognitive tests between active and placebo groups in this cohort of healthy school-children. Regression analysis however showed a positive association between DHA and elevated scores on the PPVT which is a test of listening comprehension and receptive vocabulary (Ryan & Nelson, 2008). The authors retrospectively reported some issues with 2 of the tests employed measuring sustained attention and impulsivity, e.g., they found a ceiling effect with 100% scores both at baseline and study end implying these tests were too easy for the children and more challenging tests should have been considered (Ryan & Nelson, 2008).

Depression, Aggression, Nutrition and Essential Fatty Acids

There is cumulative evidence that lower PUFA and/or multi-vitamin levels may also be linked with behavioural problems that are commonly associated with ADHD such as anti-social behaviours (including aggression), low mood and depression (Edwards, Peet, Shay, & Horrobin, 1998; Gesch, Hammond, Hampson, Eves, & Crowder, 2002; Nemets, Nemets, Apter, Bracha, & Belmaker, 2006; Osher, et al., 2006; Peet, Murphy, Shay, & Horrobin, 1998). Several lines of

research have established an association between major depressive disorders and omega-3 fatty acids in adults (Logan, 2003). Fish and seafood are the main nutritional source of LC-PUFAs, and irregular fish intake across cultures is linked with depression in health related studies (Sontrop & Campbell, 2006). Research in this area has focused on links between specific nutrients and depression. The most studied nutrients, and for which the evidence is strongest, is omega-3 fatty acids and folic acid (Papakostas et al., 2004; Peet, et al., 1998). High levels of fatty acids lead to an increase in the fluidity of membranes which in turn enhances the transfer of serotonin into the endothelial cells (Block & Edwards, 1987; Freeman, et al., 2006; Hibbeln et al., 1998). People suffering from depression are reported to have reduced serotonin uptake and therefore the role of fatty acids in depression has important implications.

The only placebo-controlled double-blind, pilot study thus far to investigate PUFAs and childhood depression was carried out by Nemets, Nemets, Apter, Bracha and Belmaker (2006). They recruited 28 children and randomised them into groups to receive one 1000 g of fish oil containing 190 mg EPA and 90 mg of DHA (active supplement) or placebo containing olive oil. Participants were recruited via the depression clinic or child psychiatry clinic in Israel. Supplementation lasted 16 weeks and evaluations of depression using the, Childhood Depression Inventory (CDI), Clinical Global Impression (CGI) and Childhood Depression Rating Scale (CDRS) were taken at baseline, 2, 4, 8, 12 and 16 weeks (Nemets, et al., 2006). From the 28 children recruited, 20 completed at least 1 month's ratings and were included in the data analysis (n=10 active and n=10 placebo). The results found significant effects of omega-3 fatty acids on self rated measures of depression employing the CGI, CDRS and CDI concluding that they may have therapeutic benefits in childhood depression. Further research is needed with larger sample sizes to replicate these findings (Nemets, et al., 2006).

Other studies have been conducted investigating the efficacy of omega-3 to aggressive (which is linked to both depression and anxiety) and psychopathic behaviours (Corrigan et al., 1994; Gesch, et al., 2002; Zaalberg, Nijman, Bulten, Stroosma, & van der Staak, 2010). Gesch and colleagues (2002) examined the effect of supplementary essential fatty acids (in conjunction with a vitamin supplement) in a young adult UK prison (aged 18 – 21 years) population using a randomised, double blind design. The aim of the study was specifically to investigate whether alterations in diet by way of supplementation could reduce anti-social behaviours among inmates. Two hundred and thirty one young offenders took active (LA: 1260 mg, GLA: 160 mg,

EPA: 80 mg and DHA: 44 mg) or placebo supplementation for an average period of 142 days (Gesch, et al., 2002). The results revealed a marked reduction (37%) in anti social behaviour and violent offences for active versus placebo. This trial concluded that the supplementation of minerals, vitamins and EFAs reduces anti-social behaviour in a prison population which may have wider implications for the community (Gesch, et al., 2002).

The link between diet and aggression is also documented cross-culturally. For example, in Japan, Itomura and colleagues (2005) carried out a randomised, placebo-controlled, double blind trial in school children aged 9 -12 years of age with the aim of examining the efficacy of fish oil in reducing aggression. The findings showed a statistically significant decrease in physical aggression in the active group (3600 mg of DHA, 840 mg of EPA in fortified foods) compared to placebo (Itomura et al., 2005). In the USA, Schoenthaler (1991) carried out an experimental study substantially reducing the sugar content in the diets of 3000 imprisoned juveniles. Instead, the young offenders were given healthier snack options containing reduced sugar and refined foods. The results of the study over a 12 month period showed a 21% reduction in antisocial behaviour, 25% reduction in assaults, 75% reduction in physical restraints by staff and a 100% reduction in suicides (Schoenthaler, 1991a). In a similar study by the same author with 402 Californian prisoners those given 100% of the U.S. recommended daily intake of vitamins committed fewer offences than those given 300% implying that the *correct* dose is crucial for optimum brain function (Schoenthaler, 1991b).

Schoenthaler and Bier (2000) have also investigated the efficacy of vitamin and mineral supplementation on juvenile delinquency in the context of a randomised, double-blind placebo-controlled trial in 468 U.S. schoolchildren, aged 6 to 12 years of age. Supplementation of vitamins and minerals at 50% of the U.S. recommended daily allowance or placebo was given daily (Schoenthaler & Bier, 2000). The outcome measure was the number of violent and non-violent delinquent acts as measures by the schools official disciplinary records. The active group in the research sample compared to placebo produced lower rates of anti-social behaviours including threats/fighting, conduct problems, defiance, obscene behaviour, refusal to engage, being a risk to other and vandalism (Schoenthaler & Bier, 2000).

Conclusion

In conclusion, the research thus far collectively suggests that deficiencies of PUFA and vitamins may pose a preventable neurodevelopmental risk factor for the later emergence of psychopathology (McNamara & Carlson, 2006a). Future studies should investigate further the potential benefits of combined LC-PUFA and multi-vitamin supplementation to establish which formulas are most beneficial in relation to symptom improvement. Epidemiology studies have previously reported an association between reduced DHA levels and disorders such as ADHD and depression (Maes et al., 1996; Tanskanen, Hibbeln, Tuomilehto, et al., 2001; Young, Conquer, & Thomas, 2005; Young, Maharaj, & Conquer, 2004). Comparably, prophylactic effects of omega-3 ingestion for mood disorders have been reported in a meta-analysis (Freeman, et al., 2006). Affective impairment as seen in ADHD may underlie co-morbid depression, anxiety and/or conduct disorder. Low levels of LC-PUFA, particularly omega-3 fatty acids in blood measures have been associated with a variety of mood and behavioural disorders including ADHD. The research in omega-3/6 fatty acids and non-clinical (i.e., typically developing) child/adolescent populations is widely under-researched and consequently less understood. Future research should focus on well designed, ideally longitudinal studies employing sensitive parameters to assess treatment benefits in both groups of children/adolescents especially with neuroimaging techniques such as fMRI.

Overall, the findings in fatty acid supplementation studies in ADHD reviewed in this chapter are wide ranging, due to multi-factorial reasons as mentioned previously (e.g., variation in dose, length of supplementation, dose, participant pool) and therefore problematic for any firm conclusions to be drawn. Due to the wide variation in methodology and design of the research thus far, replicability is also challenging. The recent meta-analysis by Block and Qawasmi (2011) however demonstrated that supplement efficacy in the treatment of ADHD was significantly associated with the EPA dose within supplements. Other epidemiology studies have revealed an association between reduced omega-3 levels and cognitive abilities in both typically developing children/adolescents and those with a clinical diagnosis of ADHD; a similar pattern is also found in depression (Aberg, et al., 2009; Hibbeln, et al., 2007; Maes, et al., 1996; Tanskanen, Hibbeln, Tuomilehto, et al., 2001; Young, et al., 2005; Young, et al., 2004). Arguably, one of the major confounds is not taking into consideration the nutritional status of the

sample at baseline, prior to randomisation, because of individual variability. For example, there is a 7 fold range of DHA variation in the blood within the normal population and for these reasons the omega-3 index should always be assessed prior to randomisation (von Schacky & Harris, 2007).

The American Psychiatric Association reviewed the evidence in this area in 2006 and formulated several recommendations for the use of omega-3 fatty acids. Namely, 1) that all adults should eat 2 portions of fish per week 2) patients with mood, psychotic disorders or impulse control should consume 1 to 9 grams of EPA and DHA per day and 3) supplementation should be considered in patients with mood disorders (between 1 to 9 grams) with doses over 3 grams per day monitored by a physician (Freeman, et al., 2006). Recommendations by the Food Standards Agency regarding fish consumption for children less than 16 years of age are currently set at 2 portions of fish per week, one of which should be oily. However, children under 16 are advised not to eat shark, marlin and swordfish due to potential risk of mercury contamination.

Ultimately, the evidence presented in this literature review from (1) animal and human studies which document the behavioural effects of omega-3 deficiency including reduced cognitive ability; (2) studies with children and young adults with ADHD which have presented with lower levels of omega-3 fatty acids in their blood profiles compared to controls and (3) randomised controlled trials (which have indicated some benefit of supplementation with omega-3 fatty acids at least in children with ADHD) warrant further investigation.

This PhD study will add to the existing research by providing novel evidence about the relationship between LC-PUFA and brain activity as recorded by EEG/ERP's in children and adolescents with and without ADHD. It will further establish whether omega-3 and 6 levels in venous blood samples differ between both these groups for the first time in a U.K. population. Finally, it will explore relationships between behavioural measures characteristic of ADHD and LC-PUFA.

Chapter Three: The Neuropsychology of ADHD

Introduction

The neuropsychological profile of ADHD has been widely researched in the main employing tests of executive function (EF). EF are a valuable construct which refer to a broad range of 'top down' cognitive processes important for mature adult goal-directed behaviour, such as functions of self-regulation, i.e., motor and interference inhibition control, attention control i.e., selective and sustained attention, forethought, planning, delay of gratification, working memory, problem solving and cognitive flexibility (Baddeley, 1996; Stuss & Alexander, 2000). Indeed, children and adolescents with ADHD have been found to be impaired in a range of EF compared to typically developing controls (Lansbergen, Kenemans, & van Engeland, 2007; Mullane, Corkum, Klein, & McLaughlin, 2009; Overtoom et al., 2002; Packwood, Hodgetts, & Tremblay, 2011; Willcutt, Doyle, Nigg, Faraone, & Pennington, 2005).

Barkley (1997) suggested that a fundamental deficit in the control of inhibitory processes (e.g., the ability to suppress or interrupt a response) accounts for the many deficits observed in ADHD patients, including many EF and in turn this could explain the dysfunctional behaviours related to ADHD (Barkley, 1997). However, several studies report disruption to cognitive processes aside from inhibition or EF, for example, in timing processes as well as motivation and reward related cognitive functions (Losier, McGrath, & Klein, 1996b; Martinussen, Hayden, Hogg-Johnson, & Tannock, 2005; Nigg & Casey, 2005; Nigg, Willcutt, Doyle, & Sonuga-Barke, 2005; Oosterlaan, Logan, & Sergeant, 1998; Rubia, Smith, Brammer, & Taylor, 2007; Sonuga-Barke, Sergeant, Nigg, & Willcutt, 2008; Willcutt, et al., 2005). Thus EF deficits are neither sufficient nor necessary for the presence of ADHD. This chapter will discuss the difference between hot and cool EF and review the literature to date in ADHD. It will further discuss the limitations of the EF literature alongside a summary of non-EF deficits in ADHD. The final section will first present a summary of the literature in two EF tasks relevant to this PhD which have been found to be impaired in ADHD; namely sustained attention and motor response as well as conflict (interference) inhibition. The chapter concludes with a brief summary of the literature in emotion processing, which is also measured in this PhD.

Hot and Cool Executive Functions in ADHD

Recent development theorists have distinguished between "cool" and "hot" executive functions (Kerr & Zelazo, 2004; Zelazo & Müller, 2002). Cool (and more abstract) cognitive executive functions include those mentioned above that measure working memory and fluency, planning, attention, cognitive flexibility, vigilance, self-regulation, and inhibition and are associated with networks engaging the prefrontal cortex (in particular implicating the lateral inferior and dorsolateral frontostriatal and frontoparietal structures), basal ganglia and parietal lobes in children and adults (Booth et al., 2003; Christakou et al., 2009; Rubia, Hyde, Giampietro, & Smith, 2010; Rubia, Smith, Taylor, & Brammer, 2007; Rubia et al., 2006b). In contrast, hot executive functions involve emotionally relevant EF processes such as reward related decision making processes that involve incentive, reward or punishment (Kerr & Zelazo, 2004). These are documented as interceded by orbitomedial, paralimbic and ventromedial frontolimbic regions, which form part of the prefrontal cortex, in children and adults (Christakou, Brammer, Giampietro, & Rubia, 2009; Fellows & Farah, 2005; Hampton, Adolphs, Tyszka, & O'Doherty, 2007; Northoff et al., 2006; Remijnse, Nielen, Uylings, & Veltman, 2005; Schoenbaum, Roesch, & Stalnaker, 2006).

Other connected areas involved in the regulation of emotion and motivation include the ventral striatum, hippocampus and hypothalamus, anterior cingulate, amygdala and insula (Davidson, Jackson, & Kalin, 2000; Davidson, Putnam, & Larson, 2000b). The amygdala has an important role in the processing of threat and negative affect and alongside the ventral striatum mediates the link between stimulus-reward and motivational processes (Dolan, 2007; Goto & Grace, 2008; Haber, 2008; Haber, Kim, Mailly, & Calzavara, 2006). Furthermore, lesion, animal studies and imaging studies have linked orbitofrontal and temporal lobes with aggression and impulsivity (Bechara, Tranel, & Damasio, 2000; Bechara & Van der Linden, 2005; Durston et al., 2003; Rubia, 2010).

The neuropsychological literature has consistently shown that children and adults with ADHD show abnormalities in cool executive functions, most prominently in motor response inhibition, interference inhibition, cognitive flexibility, sustained attention and working memory (Marchetta, Hurks, De Sonneville, Krabbendam, & Jolles, 2008; Martinussen, et al., 2005; Rubia, Smith, Brammer, et al., 2007; Sergeant, Geurts, & Oosterlaan, 2002; Valko et al., 2010;

Willcutt, et al., 2005). Deficits in this population, however, have also more recently been found in "hot" EF such as tasks of delay discounting, gambling and reward-related decision making (Antrop et al., 2006; Bitsakou, Psychogiou, Thompson, & Sonuga-Barke, 2008; Dalen, Sonuga-Barke, Hall, & Remington, 2004; Luman, Oosterlaan, & Sergeant, 2005; Luman, Tripp, & Scheres, 2010; Marco et al., 2009; Rubia, 2010).

Deficits in ADHD children in sustained attention, working memory, motor response inhibition, interference inhibition and emotion processing

For this PhD project, I have chosen two EF functions that have consistently been found to be dysfunctional in ADHD patients, namely (1) sustained attention (SA) as measured by the CPT task, that also had a small load on WM and (2) response conflict inhibition as measured by the Go/NoGo task, which measured motor response as well as conflict (interference) inhibition. In addition, this study investigates emotion processing in ADHD for 2 main reasons (1) social/emotional dysfunction is arguably under-researched in the ADHD child and adolescent literature and (2) omega-3 fatty acids, particularly EPA, are in current therapeutic use in emotional lability-mood disorders and the later are in turn implicated in ADHD. Therefore, the following section will provide a brief summary of the literature to date in these cognitive functions specifically, SAT, WM, motor and interference inhibition, and emotion processing.

Sustained Attention and WM. Children with ADHD have consistently been found to be impaired in performance during selective and sustained attention tasks, most commonly measured in the continuous performance task (CPT) (Losier, McGrath, & Klein, 1996a; Rubia, Smith, Brammer, et al., 2007; Sonuga-Barke, Bitsakou, & Thompson, 2010; Willcutt, et al., 2005). The CPT task, originally a long, complex and prolonged clinical assessment, was designed by Rosvold, Mirsky, Sarason, Bransome and Beck (1956) to specifically measure *vigilance* or *sustained attention*; however shorter and simpler versions have since been developed (Robertson, Manly, Andrade, Baddeley, & Yiend, 1997). Attention describes a set of procedures which enhance motor, sensory and cognitive processing (Pasini, Paloscia, Alessandrelli, Porfirio, & Curatolo, 2007). Sustained attention can be broadly summarised as the ability to concentrate on a specific stimulus over a period of time while excluding the distraction of other irrelevant stimuli. It requires the ability to maintain performance (as measured by the behavioural response) during incessant activities which rely on the capacity to detect a stimulus,

vigilance and the ability to resist distraction and among the wide-range of tasks employed to study attention, the CPT is among the most prevalent (Pasini, et al., 2007; Shalev, Ben-Simon, Mevorach, Cohen, & Tsal, 2011). Typically, CPTs necessitate the recognition of low probability items such as the letter X or successions of targets for example A-X randomly presented among high frequent alphabetical strings (Corkum & Siegel, 1993). The more difficult A-X version of the CPT is called a cued CPT and presents a cue such as the letter A (a warning stimulus) followed by the target stimulus (e.g., X) or a non-target stimulus (non-X). These stimuli can be controlled by increasing the probability of either the target or the non-target item after the cue (Dias, Foxe, & Javitt, 2003). Essentially, the CPT task measures rates of omission (i.e., the failure to respond to target trials) which are considered to be an estimate of poor sustained attention and errors of commission (incorrect hits to non-target trials) which are thought to reflect impulsiveness (Halperin et al., 1998, Rubia et al., 2007). Children with ADHD during CPT tasks have typically more errors of commission, omission and display more variable reaction times (Holcomb et al., 1986; Castellanos & Tannock, 2002; Rubia et al., 2001, 2007, Willcutt et al., 2005; Levy & Hobbes, 1997; Strandburg, Marsh, Brown, Asarnow, Higa et al., 1996). Moderate to large effect sizes have been found in sustained attention deficits in ADHD as evidenced by meta-analytic reviews of neuropsychological function (Willcutt, et al., 2005). Furthermore, it is thought that the heterogeneity within cross-sections of child/adolescent patients with ADHD is likely to explain and contribute to the contradictory findings in sustained attention deficits to date (Johnson et al., 2007).

Working Memory

Working memory (WM) is vital to conscious thought as it allows internal representations of information (e.g., rules) to steer overt behaviour (e.g., responses) and decision making during pursuits so that behaviour is not governed by instant sensory cues in ones surroundings (Martinussen, et al., 2005). The most influential model of WM is arguably Baddeley's (1986) multi-component model consisting of both verbal and visuospatial storage systems and a central executive (CE) that regulates and directs the 2 storage systems (Baddeley, 1996; Baddeley & Della Sala, 1996; Martinussen, et al., 2005). Deficits in verbal storage are associated with the acquisition of language weaknesses including word decoding and vocabulary whereas weaknesses in visuospatial storage are associated with poor academic achievement in literacy,

comprehension and numeracy (Baddeley, Gathercole, & Papagno, 1998; Gathercole & Pickering, 2000). The CE element of WM is thought to regulate and manipulate the stored information alongside acting on information retrieved via long-term memory to support complex cognitive tasks including language and reading comprehension, mental calculation and generation of text (Baddeley, et al., 1998; Gathercole & Pickering, 2000; Martinussen, et al., 2005). Baddeley and Hitch (1974) demonstrated the role of sequencing in WM. They argued that the phonological or acoustic presentation of sequences was significant for correct recall. For example, similar sounding letters such as B, V, C were less likely to be recalled correctly than a sequence of letters less alike such as T,W, R (Ross, 2006). Children with ADHD have also demonstrated considerable difficulty correctly encoding social cues which may in turn be related to poor WM processes, alongside inhibition (Best & Miller, 2010; Castellanos & Tannock, 2002a; Keage et al., 2006; Martinussen, et al., 2005; Miyake et al., 2000; Shallice et al., 2002; Stevens, Quittner, Zuckerman, & Moore, 2002; Willcutt, et al., 2005; Willcutt et al., 2001). However a meta-analysis of WM processes demonstrated that deficits are present in multiple components of WM in children with ADHD independent of comorbidity with language disorders and limitations in general intellectual ability. Both of the latter have been identified as among the potential confounders likely to be accountable for the discrepancies in the WM findings published to date (Martinussen, et al., 2005).

WM processes are considered to be dependent on frontostriatal (thought to underlie the "central executive" as defined by Baddeley's WM model) and cerebellum brain regions and converging data from neuropsychological and neuroimaging studies implicate frontostriatocerebellar dysfunctions in ADHD (Castellanos et al., 2002; Durston, van Belle, & de Zeeuw, 2011; Rubia, 2011). It is also known that dopaminergic and noradrenergic systems modulate WM processes (Goldman-Rakic, Castner, Svensson, Siever, & Williams, 2004) and in children with ADHD WM deficits may be linked to both frontostriatocerebellar dysfunction and/or dopaminergic dysregulation (Levy & Swanson, 2001; Martinussen, et al., 2005).

Motor Response Inhibition

An important measure of EF is motor response inhibition (Logan, Schachar, & Tannock, 2000; Packwood, et al., 2011) . This is typically quantified in tasks of Go/NoGo or STOP tasks (Nigg, 2003). In the Go/NoGo task, participants have to respond to a Go stimulus and inhibit

responses to a NoGo stimulus. The task measures selective attention and selective motor response inhibition. The Stop signal (SST) is a visual choice reaction time task used to study motor response inhibition. Participants are requested to respond with a button press to the high frequency Go trials, but to suppress a response upon presentation of an unexpected Stop signal occurring at random intervals after the Go stimulus (Schachar & Logan, 1990). Children and adolescents with ADHD have consistently been shown to be impaired in this task where they have a flatter slope of the inhibition function (percentage of unsuccessful inhibitions as a result of Go-Stop delay interval) and increased Stop signal reaction times (SSRT) (Oosterlaan et al., 1998, Lijffijt et al., 2005). Stop tasks like Go/NoGo tasks measure the assessment of two processes: (1) the simple RT and accuracy during Go trials, i.e., the response execution process and (2) the inhibitory processes during Stop trials. The latter is typically expressed in a measure of inhibitory speed, the stop-signal RT (SSRT) which is deduced from the distribution of RTs and the number of stops correctly achieved (Lijffijt, Kenemans, Verbaten, & van Engeland, 2005).

Impairments in the inhibitory measures of the Go/NoGo task (Gomez, 2003; Rubia, Taylor, et al., 2001; Schachar et al., 2007; Schachar et al., 2005) and the Stop task is one of the most reliable findings of the ADHD neuropsychological literature (Durston, 2003; Lijffijt, et al., 2005; Oosterlaan, et al., 1998; Pennington & Ozonoff, 1996; Rubia, Smith, Brammer, et al., 2007; Rubia, Taylor, et al., 2001; Rubia, Oosterlaan, Sergeant, Brandeis, & von Leeuwen, 1998; Schachar, Mota, Logan, Tannock, & Klim, 2000; Willcutt, et al., 2005).

In ADHD groups, longer SSRT's have been found in Stop tasks relative to healthy controls (Bidwell, Willcutt, Defries, & Pennington, 2007; Bitsakou, et al., 2008; King, Colla, Brass, Heuser, & von Cramon, 2007; Lijffijt, et al., 2005; Nigg, Blaskey, Stawicki, & Sachek, 2004; Oosterlaan, et al., 1998; Seidman et al., 2006; Waldman et al., 2006; Willcutt, et al., 2005). In Go/NoGo tasks, higher commission errors have been found in ADHD relative to healthy controls (Gomez, 2003; Rubia, Taylor, et al., 2001; Schachar, et al., 2007; Schachar, et al., 2005; Slaats-Willemse, Swaab-Barneveld, de Sonneville, van der Meulen, & Buitelaar, 2003) .

A meta-analysis carried out in 1998 of 8 studies found good effect size's (ESs) for the differences between ADHD and controls between 0.49 and 0.64 for mean simple Go RT (MRT) and SSRT correspondingly, reflecting both a longer MRT and SSRT for the ADHD group relative to matched controls (Oosterlaan, et al., 1998). Oosterlaan and colleagues concluded that

because the ES was greater for SSRT than MTR, the premise of impaired inhibitory motor control as a key feature of ADHD was substantiated. Their supposition was further supported by additional investigations of the Stop task literature (Nigg, 2001; Sergeant, et al., 2002). More recently, however this hypothesis was challenged by a meta-analytic review by Lijffijt and colleagues (2005). They undertook a review of a further 33 studies published using this task following the meta-analytic review by Oosterlaan et al (1998). The main question posed by Lijffijt and colleagues was whether ADHD principally involves a deficient inhibitory motor control or is typified instead by a broader cognitive deficit (Lijffijt, et al., 2005). They proposed that a deficit in attention would be demonstrated by longer RTs, more lapses of attention and longer SSRT whereas inhibitory deficits would be demonstrated by a disproportionately elongated SSRT relative with MRT. Twenty nine studies were included in the meta-analytic review comprising data from 977 children and adults with ADHD and 1,078 controls (see Lijffijt et al., 2005 for a review). Their findings reported that both children and adult groups with and without ADHD significantly differed in SSRT. Children with ADHD also had a significantly longer basic Go RT relative to controls, but this finding was not observed between the adult groups. In addition, a significant interaction was observed in adults but not in children with ADHD between the elongation of the latency to stop and act in response. The authors concluded that inadequate inhibitory motor control may be less vital in younger age groups compared to adults with ADHD (Lijffijt, et al., 2005).

Collectively, the research suggests that ADHD participants show impaired performance on measures of inhibitory control as demonstrated by both the higher commission errors displayed in Go/NoGo tasks and larger SSRT in Stop tasks.

Conflict response inhibition

There is some evidence that ADHD patients are impaired in tasks of conflict inhibition although the evidence is more controversial than for motor inhibition deficits (Boonstra, Oosterlaan, Sergeant, & Buitelaar, 2005; King, et al., 2007; Rubia, Taylor, et al., 2001; Sergeant, 2002). There are various tasks which measure both conflict response inhibition (also referred to as interference inhibition) and selective attention and these include the Stroop task (Stroop, 1935), the Simon task (Simon, 1969) and the Erikson Flanker task (Eriksen & Schultz, 1979).

The Stroop task examines the aptitude to inhibit interfering information in a stimulus response incompatibility setting. The congruent condition requires the participant to name the colour in which words were written e.g., "red", "yellow", "green" and "blue" written in a colour congruent with the word they have to read. In the interference condition the ink of the colour in which words are written conflicts with the colour word (e.g., the word "red" but printed in green ink). This condition is more difficult as the subject has to inhibit the interference from the predominant response propensity to read the word as opposed to name the ink in which it is written. The incongruent condition elicits larger MRT than the congruent condition which is called the Stroop effect. This task is considered to measure interference (conflict) inhibition and selective attention (MacLeod, 1991). Although, there are multiple variations of the Stroop but the standard version was designed by Stroop (1935). The standard version for evaluating Stroop interference is to establish the difference in scores between the colour and colour-word items, the total number of scored items and time per item. The theory is that the lower the difference in scores, the less the amount of interference from incongruent words during the naming of colours process in the colour-word condition. An adapted version of this quantification exists by Golden (1978) but will not be discussed here.

Three meta-analysis of Stroop performance have been conducted in child and young adult ADHD populations and discovered moderate to large group effect sizes for the incongruent colour-word measure of standardised clinical Stroop tasks (Homack & Riccio, 2004; Pocklington & Maybery, 2006; van Mourik, Oosterlaan, & Sergeant, 2005). Two more recent meta-analyses of Stroop interference in ADHD have been carried out since then (Lansbergen, et al., 2007; Schwartz & Verhaeghen, 2008). The first of these meta-analyses by Lansbergen and colleagues (2007) involved 19 studies across all age ranges, included computerised versions of the task and studies that had previously recruited those with a clinical diagnosis of ADHD and a control group. The findings revealed a mean effect size for interference deficits in ADHD relative to controls of 0.24 across all studies. In the time per item studies, that is, when the dependent variables were quantified as RT per item or the amount of time taken to read each card, the effect size was 1.11. Furthermore, when differences were assessed on accomplishment on the word task in base-word reading, the ADHD groups were found to be systematically slower in this aspect of the task. Overall, this meta-analysis demonstrated increased interference in the Stroop task in

patients with ADHD compared to controls concluding that this feature of behavioural inhibition is reliably disturbed in ADHD (Lansbergen, et al., 2007).

The second recent meta-analysis by Schwartz and Verhaeghen (2008) aimed to investigate whether interference inhibition is a sensitive parameter in ADHD and if so whether maturation has any influence on the attentional feature of ADHD. These authors evaluated data from 25 studies which had employed the Stroop word colour test in children and adults, aged 9 to 41, with and without ADHD. The strength of the Stroop effect was assessed using a hierarchical approach. They further evaluated whether the Stroop effect varied according to age. The results revealed that the relationship between RT of colour and colour-word conditions was indistinguishable across age groups and ADHD status. Specifically, the Stroop interference effect did not covary with age once baseline differences in colour RT were factored into the equation and was the same for both children and adults with ADHD and control participants. Furthermore, the data did not lend support for differences in maturation rates in either of the Stroop conditions between the ADHD and control groups. In both groups, RT declined as a function of age and therefore the Stroop effect seems to be resistant to age, irrespective of ADHD diagnosis (Schwartz & Verhaeghen, 2008).

Simon and Eriksen Flanker Tasks

The Simon test (Simon, 1990) is a nonverbal computerised task which measures interference control using RT. In this task, a single item (e.g., an arrow) is presented to the left or right side of the screen. Participants have to respond to the item by pressing either a left or right button which corresponds with the direction of the arrow (i.e., right button response for right arrow direction). In the congruent condition the arrow is presented on the same side as it points. In the difficult incongruent condition, the arrow points to the opposite side of the screen in which it appears. The participant has to inhibit the predominant response tendency to respond to the spatial information (i.e., press on the same side of the screen) in order to follow the iconic information (i.e., press on the side where the arrow points to). RT is typically faster when the arrow direction presented is congruent with the side of the screen on which it is presented as opposed to in incongruent trials, where they conflict. The longer RT to incongruent as opposed to congruent trials is called the Simon effect.

The Eriksen Flanker test (Eriksen & Eriksen, 1974) is based on the same principle as the Simon task. The task requests a participant to press a button according to an arrow direction,

which in congruent trials is flankered by same pointing arrows, but in incongruent trials is flankered by arrows that point in the opposite direction. The aim is for the participant to indicate the direction of the target (central) arrow, via a speedy button press response, pointing to the left or right side of a computer screen. On incongruent trials, the participant must filter out the distracting and irrelevant information from the flanking arrows, a process considered to involve interference or conflict inhibition (Mullane, et al., 2009). Longer RT on incongruent trials reflects the necessity for extra attentional processing during the filtering out process. In a similar way, accuracy has a tendency to be poorer on incongruent trials supporting the theory that the cognitive processing necessary during these trials requires greater effort (Mullane, et al., 2009; Ridderinkhof & van der Stelt, 2000a).

The Stop, Simon and Flanker tasks are advantageous for the investigation of interference control in children and adolescents with ADHD for a number of reasons. Firstly, the computerised versions of these tasks are non-verbal and do not require participants to read or provide verbal answers which eradicates possible reading related confounds (Golden & Golden, 2002; Mullane, et al., 2009). Secondly, the tasks provide a measure of anterior cingulate (although not restricted to that region) activity, which in turn is the brain region associated with conflict monitoring and interference control (Fan, Flombaum, McCandliss, Thomas, & Posner, 2003; Ridderinkhof, Ullsperger, Crone, & Nieuwenhuiss, 2004).

Mullane and colleagues (2011) recently reviewed and summarised the existing literature on the interference control abilities of 272 children (comprising 12 studies) with ADHD (*M* age = 9.28 yrs) and typically developing children (*M* age = 9.38 yrs) using both the Flanker and Simon tasks (Mullane, et al., 2009). In ADHD, specific performance disadvantages were found in relation to RT, number of errors made and inverse efficiency (IE) on incongruent trials compared to congruent trials, implying ineffective interference control in this group compared to typically developing populations (Mullane, et al., 2009). Overall, their findings suggest that in ADHD, the executive control deficit surpasses response suppression however may in addition encompass interference control (Mullane, et al., 2009).

Limitations of the EF literature in ADHD

It is important to note that while impairments have been found in some children with ADHD in these functions, these findings are not consistent throughout the literature (Nigg, et al., 2005; Sonuga-Barke, et al., 2010). It is further reported that there are subgroups of children with

ADHD that demonstrate impairments in either "hot" or "cool" EF or in temporal and perceptual processes with only a proportion of these displaying overlapping deficits (Cubillo, Halari, Smith, Taylor, & Rubia, 2011a; Nigg, et al., 2005; Sonuga-Barke, et al., 2010). Various hypothetical models have been put forward to explain this heterogeneity including the presence of multiple developmental pathways in ADHD which have functional and structural irregularities but inseparable functional networks underlying the detected deficits (Cubillo, et al., 2011a; Makris, Biederman, Monuteaux, & Seidman, 2009; Nigg & Casey, 2005; Sonuga-Barke, et al., 2010; Willcutt, et al., 2005). Furthermore, although deficits in these cognitive functions are associated with ADHD, the effect size (approximately .5 standard deviations) is not large enough to be a defining feature of the disorder as effects of this size are also observed in other childhood disorders (Taylor, 2008).

Deficits in non-EF in ADHD

ADHD patients are not only impaired in EF, but have also shown to be impaired in several other functions. For example, other behaviour impairments reported in ADHD include an inconsistent style of response (Leth-Steenson, Elbaz, & Douglas, 2000; Rubia, Taylor, et al., 2001), a speed-accuracy trade off favouring speed (Douglas & Parry, 1994), abnormal reaction times and irregular timing processes such as abnormal delay aversion (Bitsakou, Psychogiou, Thompson, & Sonuga-Barke, 2009; Sonuga-Barke, et al., 2010; Sonuga-Barke, 2002, 2003), abnormal motor timing (Rubia, Taylor, Taylor, & Sergeant, 1999; Rubia, Noorloos, Smith, Gunning, & Sergeant, 2003) and deficits in estimation of timing (Mullins, Bellgrove, Gill, & Robertson, 2005; Rubia, Halari, Christakou, & Taylor, 2009; Smith, Taylor, Lidzba, & Rubia, 2003; Smith, Taylor, Rogers, Newman, & Rubia, 2002), (for a review on timing deficits see Rubia, Halari, Christakou & Taylor, 2009). In addition, patients with ADHD display consistently premature responses across all tasks measured in a large battery which showed high sensitivity and specificity to discriminate ADHD from controls (Rubia, Smith, Brammer, et al., 2007; Rubia, Taylor, et al., 2001).

Differences in RT during executive processes are typically reported between participants with ADHD and healthy controls in a number of tasks (Fallgatter et al., 2004; Leth-Steensen, King Elbaz, & Douglas, 2000). Some studies report an overall slowing of RT (Carte, Nigg, & Hinshaw, 1996; Schachar, et al., 2005; Tamm, Menon, Ringel, & Reiss, 2004; van Mourik, et al., 2005) signifying wide-ranging generic and task-unspecific difficulties in ADHD participants

(Fassbender & Schweitzer, 2006) while others, however, reporting no differences depending on diagnostic subtype (Derefinko et al., 2008). RT variability is thought to reflect frontal cortex function and potentially associated with sustained attention deficits (Johnson, et al., 2007). One of the most consistent and discriminating ADHD associated neuropsychological deficit is increased intra-individual response variability (Castellanos et al., 2005; de Zeeuw et al., 2008; Epstein et al., 2011; Klein, Wendling, Huettner, Ruder, & Peper, 2006; Leth-Steenson, et al., 2000; Rubia, Smith, Brammer, et al., 2007). For example, Castellanos et al (2005) found in their study that children with ADHD demonstrate significantly more variability in reaction time as demonstrated by the fast Fourier transform technique (FFT) compared to controls (Castellanos, et al., 2005). Johnson et al., (2007) also employed FFT to distinguish performance between ADHD and typical developing children during 2 adaptations of a sustained attention to response task (SART). Both tasks had a Go/NoGo design employing 9 digits, children were instructed to respond to all the digits with the exception of 1 via a button press. In the first adaptation of the task, the order of the stimuli was utterly conventional and repetitive right the way through the task; in the second version, the stimuli were randomised and more consistent with a standard Go/NoGo paradigm. In both tasks the children with ADHD displayed both increased fast and slower (e.g., throughout the course of the task) variability (Johnson, et al., 2007). Epstein and colleagues (2011) recently provided evidence for elevated RT variability in children with ADHD across a number of different tasks including the addition of reward and event rate manipulations. They found that children with ADHD displayed great RT variability across all 5 tasks (Choice Discrimination task, Child Attentional Network task, Go/NoGo task, Stop Signal task and N-Back task) but there were minimal differences in RT variability across the ADHD subtypes. Compared to control children, those with ADHD had poorer task accuracy across all tasks with the exception of the Choice Discrimination task. Finally, it was observed that although the manipulation of error rates and rewards showed some effect of children's RT and task accuracy, it failed to differentiate ADHD children from controls (Epstein, et al., 2011). Inconsistent reaction times have also been found to be one of the best discriminators between ADHD and controls in a large battery of EF tasks (Rubia et al., 2007).

Emotion processing

Children with ADHD do not only endure hyperactive-impulsive and/or attention deficit symptoms but frequently have added difficulty with affect such as emotional dysregulation,

extreme emotional reactivity and emotional instability (Schlochtermeier et al., 2011). There is some evidence that emotion dysfunction exists in children with ADHD on a behavioural level as evidenced by an inability to correctly identify the facial expressions of others especially emotions of fear, anger and sadness (Pelc, Kornreich, Foisy, & Dan, 2006; Singh et al., 1998; Yuill & Lyon, 2007). Deficits have also been observed in facial affect recognition (Braaten & Rosen, 2000) and in the capacity to disconnect emotion from cognitive processes (Friedman et al., 2003). Facial expressions provide significant non-verbal social cues to affective states and are instant indicators of emotional dispositions in other people (Eimer & Holmes, 2002). In ADHD, impaired interpersonal relationships have been observed and in children this is manifested within peer, sibling, teacher and parental relationships (de Boo & Prins, 2007; Greene et al., 2001; Pelc, et al., 2006). Both lesion and neuroimaging research has illustrated that the amygdala and orbitofrontal cortex play an important role in the processing of affective facial expressions (Berlin, Rolls, & Kischka, 2004; Rolls, 2000). Other prefrontal regions such as the right anterior cingulate, right inferior parietal cortex, inferotemporal cortex, hippocampus and ventromedial occipitotemporal cortex are also implicated in the evaluation and examination of faces and facial expressions (Adolphs, Damasio, Tranel, & Damasio, 1996; Blair, Morris, Frith, Perrett, & Dolan, 1999; Eimer & Holmes, 2002).

Conclusions

In conclusion, as this review has demonstrated, ADHD patients have consistently been found to be impaired in EF, in particular in motor and interference inhibition and sustained attention but are also impaired in non-EF such as emotion processing. In consideration of the literature to date, three tasks were chosen for this PhD program of study, namely (1) a hybrid Stroop interference inhibition and motor response inhibition task (Go/NoGo), (2) a sustained attention CPT and finally (3) an emotion processing task, which theoretically should capture potential deficits in these areas in ADHD relative to healthy control children/adolescents. The Go/NoGo task[4] employed in this PhD study was a hybrid between a Stroop-type interference inhibition and a Go/NoGo motor response inhibition task (Rowe et al., 2007). In the task, the word "PRESS" was flashed alternately in green and red colours on a computer screen. The participants were instructed to respond to the word "PRESS" when presented in the colour green

[4] BRAINnet@brainresource.com; Discovery@brainresource.com

(Go stimuli) by pressing a button box with left and right index fingers as fast and accurately as possible and not to press when they see the word "PRESS" presented in red (NoGo stimuli). There were disproportionally more Go stimuli (presented 6 times in a row for 21 out of 28 sequences/blocks) than NoGo stimuli (of which there are 7 blocks presented pseudo-randomly) presented. Essentially the aim of the task was to inhibit the interference between the meaning of the word (and thus the prepotent tendency to follow the word meaning and press a button) and the instructions (i.e., not to press the button when written in red ink), attending only to the colour of the stimuli. Therefore, the task was adept for measuring both motor response and interference inhibition. The task provides behavioural measures of performance including RT, intra-subject response variability and omission to Go trials as measures of the execution process and commission errors to measure the inhibitory process.

The sustained attention (CPT) is a target detection task chosen in this PhD study (Keage et al., 2008b) that measures mainly sustained/selective attention, as well as processes involved in the orienting reflex, categorisation, contextual updating, and has a small load on WM. A series of letters (B, C, D or G) are presented to the participant who is requested to press two buttons with the index finger of each hand to the target stimuli. The majority of letters are non-target letters (i.e., background letters), while a small proportion of stimuli consist of 20 pseudo-randomly presented, “1-back”, target letters (i.e., repetitions of the previous letter), and 20 distracter stimuli consisting of checkerboard patterns (black and white 1 x 1 cm checkerboards). Participants have to ignore all distractor letters and respond to target letters only. The checkerboards were interleaved randomly with the letter stimuli and participants were asked to ignore the “checkerboards”. Behavioural measures of performance include RT to targets, errors of omission (thought to reflect inattention) and errors of commission (thought to reflect impulsiveness).

The emotion perception task involved the recording of EEG data while 48 grey scale stimuli of facial expressions were presented covertly on a computer screen. The stimuli represented 3-D facial expressions of happiness, sadness, anger, fear and disgust relative to neutral faces and were chosen from a standandised set of stimuli (Gur et al., 2002). The 192 stimuli were made up of eight different individuals representing each expression, shown pseudo-randomly, recurring 4 times. This task measures non-conscious and automatic processing of

emotional stimuli. Ultimately, this specific task has been employed in a number of published research papers (see Williams et al., 2006; Williams et al., 2008).

Chapter Four: Structural and Functional Imaging in ADHD

Structural imaging in childhood ADHD

Although there are a variety of imaging methods, only magnetic resonance (MRI) imaging can measure brain structure and is furthermore the imaging technique with the best spatial resolution. MRI has been used over the past 2 decades to investigate differences in the structure of brains of both healthy and clinical populations. Neuroimaging in ADHD has advanced substantially to unravel the neural systems engaged in its pathophysiology (Reed & Warner-Rogers, 2008). Particular consideration has been paid to neural circuits linking the basal ganglia and prefrontal cortex due to their implication in modulating response inhibition and also to the cerebellum due to its involvement in both motor coordination and executive functions such as planning (Krain & Castellanos, 2006). Such research has confirmed structural deficits in children with ADHD in comparison to typically developing control children in the basal ganglia, frontal lobes, cerebellum, and parietotemporal lobes (Krain & Castellanos, 2006). A recent meta-analysis of structural imaging studies using regions of interest (ROI) analysis in children with ADHD has shown that the largest deviations were observed in cerebellar regions, splenium of the corpus callosum, total and right cerebral volume and right caudate (Valera, et al., 2007). Structural abnormalities of the caudate nucleus and putamen, which serve as entry points to the basal ganglia, have consistently been associated with this disorder. In fact, volumetric and asymmetry differences have been found in the caudate nucleus, globus pallidus and putamen between ADHD and controls (Castellanos, et al., 2002; Ellison-Wright, Ellison-Wright, & Bullmore, 2008; Hill et al., 2003; Krain & Castellanos, 2006; Valera, et al., 2007).

A meta-analysis using whole brain analysis identified reductions in gray matter in the right putamen and globus pallidus region. No brain regions were detected with increases in gray matter in children and adolescents with ADHD compared to controls (Ellison-Wright, et al., 2008). The most recent meta-analysis by Nakao and colleagues (2011) was conducted in 378 ADHD patients and 344 healthy controls. The findings revealed that the ADHD group had global reductions in gray matter volumes, as well as reduced gray matter in the right lentiform nucleus that extended to the caudate nucleus. The proportion of patients taking stimulant medication as well as increasing age was found to be independently associated with more usual values in this

region. In addition, slightly greater gray matter volumes were also found in the left posterior cingulate cortex in ADHD compared to control patients (Nakao, Radua, Rubia, & Mataix-Cols, 2011).

Brain Maturation Delay in ADHD

Longitudinal imaging studies have observed structural abnormalities in the fronto-parietal and fronto-striato-cerebellar systems, which are hypothesised to be due to a late structural maturation of these brain areas. Most cortical regions in ADHD compared to healthy controls were showing a delay in their cortical thickness maturation with some regions being particularly delayed such as temporal and frontal areas which were delayed by up to 4-5 years respectively. On average, the peak of cortical thickness maturation was deferred in children with ADHD by about 3 years (Shaw et al., 2007).

There is therefore now neuro-anatomical substantiation for the *maturational delay hypothesis* of ADHD as opposed to a deviance of typical brain development (Rubia, 2007; Shaw, et al., 2007). The maturation delay theory was initially discussed during the early classification stages of ADHD, partly from observations that the brain activity in response to cognitive tasks and during the *at rest* state in children with ADHD was similar to their slightly younger but typically developing peers (El-Sayed, Larsson, Persson, Santosh, & Rydelius, 2003; Rubia et al., 2000; Rubia et al., 1999). Other researchers, however, have reported a quantitatively different neurophysiology in both EEG (Barry, Johnstone, & Clarke, 2003a; Clarke, Barry, McCarthy, & Selikowitz, 2001a) and functional imaging studies (Castellanos & Tannock, 2002b; Dickstein, Bannon, Castellanos, & Milham, 2006) supporting the notion that ADHD may be a deviation from typical development.

Further evidence which supports the brain maturational delay hypothesis include (1) that symptoms of ADHD are inclined to progress with age in up to 80% of children, (2) cross-sectional imaging findings show volumetric reductions in cortico-striatal brain regions which are documented to mature in late adolescence and (3) functional brain imaging studies demonstrate reductions in brain activity in regions whose functions are known to progressively develop with age (Rubia, et al., 2000; Rubia, Smith, Brammer, Toone, & Taylor, 2005; Smith, Taylor, Brammer, Toone, & Rubia, 2006b). The study of Shaw which was the first to provide direct evidence for the hypothesis of a delay in brain maturation in childhood ADHD, showed that the most prominent delays were in prefrontal brain regions which are in turn associated with EF and

emotion control (Shaw, et al., 2007). In further support of the maturation delay hypothesis it was also demonstrated by the same research group that children in late adolescence who had shown normalization of the decrease in cortical thickness of left cerebellar hemisphere and parietal lobes were more liable to *grow out of* the disorder and showed a better clinical outcome (Mackie et al., 2007; Shaw et al., 2006).

Another magnetic resonance imaging technique is diffusion tensor imaging (DTI) which can be used to investigate the architecture of myelinated tracts. White matter fibers comprise approximately 50% the brain's total volume and keep on developing throughout childhood, adolescence and early adulthood, providing a measure of anatomical connectivity amid cortical regions (Murias, Swanson, & Srinivasan, 2007; Paus et al., 2001). In ADHD, reductions in volumes of within-hemisphere (cortico-cortical) and callosal white matter have also been reported (Castellanos, et al., 2002; Durston et al., 2004). Other studies such as that carried out by Nagel and colleagues (2011) have also found alterations in a range of tested white matter microstructure in young children, between 7 and 9 years, with ADHD (Nagel et al., 2011). Nagel and colleagues (2011) concluded that their results demonstrate that even prior to adolescence ADHD is a disorder of altered structural connectivity of the brain. Furthermore, maturing frontolimbic pathways were also atypical, potentially due to a decrease or delay in myelination (Nagel, et al., 2011).

Another study by Silk and colleagues (2009) found abnormal white matter (WM) tracts in children with ADHD, with specific differences in fronto-striatal and fronto-parietal circuits. Additional alterations were found between groups (ADHD and controls) in WM tracts attributed to developmental changes occurring in the caudate nucleus with age (Silk et al., 2009). Other studies in children with ADHD have reported decreased WM tracts in areas associated in the pathophysiology of ADHD namely left cerebellum, left middle cerebellar peduncle, and left parieto-occipital, right premotor, right cerebral peduncle and right striatal and prefrontal tracts (Ashtari et al., 2005; Pavuluri et al., 2009). In addition, reductions in WM tracts have been observed in the corticopinal tract and the superior longitudinal fasciculus (Hamilton et al., 2008). Furthermore, deficits observed in functional activity have also been associated with variability in the myelination and regularity of right prefrontal structures in parent-child dyads with ADHD (Casey et al., 2007). In adults reductions in size of right-hemispheric fiber tracts in the cingulum bundle connecting the anterior cingulate with the dorsolateral prefrontal cortex as well as in

fronto-striatal fiber tracts and the superior longitudinal fasciculus that links prefrontal and parietal regions have been observed (Konrad et al., 2010; Makris et al., 2008)

Collectively the structural connectivity research using DTI in patients with ADHD suggest merging support for WM pathology and disturbed anatomical connectivity which has led some researchers to suggest that the differences observed may represent a developmental delay (Hamilton, et al., 2008; Nagel, et al., 2011) which starts to normalise during the path of adolescence (Silk, Vance, Rinehart, Bradshaw, & Cunnington, 2009). There are however fairly substantial methodological limitations that should be considered in relation to the analysis of connectivity measures which will not be discussed here. In the future, longitudinal studies may be able to ascertain at what developmental phase alterations in neural networks surface. For an extensive review of this field see Konrad and Eickhoff (2010).

fMRI in childhood ADHD

fMRI is well-suited for exploring neurofunctional processes that underlie the performance and behaviour deficits that are detected in ADHD. Meticulous focus has been paid to EF, in particular to tasks of motor and interference inhibition and attention (Durston, et al., 2011; Paloyelis, Mehta, Kuntsi, & Asherson, 2007; Rubia, 2011). The functional neuroimaging literature in childhood ADHD compared to controls has reported consistent deficits within fronto-striatal and frontoparietal circuits during these cognitive paradigms measuring inhibitory control, working memory, selective and sustained attention (Bush, 2011; Cubillo, et al., 2011a; Cubillo & Rubia, 2010; Dickstein, et al., 2006; Durston, et al., 2011; Paloyelis, et al., 2007; Rubia, 2011). These reductions in activation have been observed especially in the IFC, caudate, anterior cingulate and additionally in temporo-parietal areas during tests measuring interference inhibition (Cubillo, et al., 2011a; Konrad, Neufang, Hanisch, Fink, & Herpertz-Dahlmann, 2006; Rubia et al., 2010; Rubia, Halari, et al., 2009; Vaidya et al., 2005), motor response inhibition (Booth et al., 2005; Durston, Mulder, Casey, Ziermans, & van Engeland, 2006; Durston, et al., 2003; Epstein, et al., 2007; Pliszka et al., 2006; Rubia et al., 2008; Rubia, Overmeyer, et al., 1999; Rubia, et al., 2005), attention allocation (Rubia, Smith, Brammer, et al., 2007; Smith, Taylor, Brammer, Toone, & Rubia, 2006a; Stevens, Pearlson, & Kiehl, 2007; Tamm, Menon, & Reiss, 2006; Tamm, et al., 2004) and of selective, flexible and sustained attention (Dickstein, et al., 2006; Rubia, 2010) (for a review see Dickstein et al., 2006; Rubia, 2010). Reductions in activation have also been found in dorsal and ventrolateral prefrontal, cerebellar and cingulate

regions of the brain during temporal processes together with tasks of temporal foresight and unpredictability, time discrimination and motor timing (Durston et al., 2007; Rubia, Overmeyer, et al., 1999; Rubia, Taylor, et al., 2001; Rubia, Halari, Christakou, et al., 2009; Smith, Taylor, Brammer, Halari, & Rubia, 2008). During tasks measuring attention the cerebellum has also been observed to be affected by impairment in ADHD (Cubillo, et al., 2011a; Rubia, Cubillo, et al., 2010; Rubia, Halari, Cubillo, Mohammad, & Taylor, 2009).

Neurofunctional deficits have recently been investigated during tasks of motivation, and abnormalities have been observed during reward related processes in ventral striatum, cingulate and orbitofrontal cortices (Cubillo, Halari, Smith, Taylor, & Rubia, 2011b; Rubia, Halari, Christakou, et al., 2009; Rubia, Smith, et al., 2009; Scheres, Milham, Knutson, & Castellanos, 2007). However, it should be noted that there are a number of potential confounding variables in the imaging studies to date in ADHD that warrant consideration. These confounds include (1) that the screening of participants for comorbid disorders such as ODD or CD is not always undertaken and therefore the sample do not contain *pure* cases of ADHD, (2) the issue of previous medication history because of its modifying long-standing effects on both brain structure (Shaw et al., 2009) and function (Konrad, Neufang, Fink, & Herpertz-Dahlmann, 2007) and finally the inclusion of both males and females with ADHD as they are known to substantially fluctuate in their brain activation (Cubillo, et al., 2011a; Cubillo & Rubia, 2010; Valera et al., 2009).

More recently, other associative features of the disorder such as emotional salience, reward/anticipation and resting state are increasingly been studied showing deficits also in orbitofrontal and limbic areas (Krauel et al., 2007; Paloyelis, et al., 2007; Scheres, et al., 2007; Tian et al., 2006).

Recent interest has been on functional connectivity deficits in ADHD. Functional connectivity essentially relates to the temporal association of spatially isolated neurophysiological occurrences in the same subject(s) (Friston, Frith, Liddle, & Frackowiak, 1993; Konrad & Eickhoff, 2010). Essentially, regions are deemed to be functionally connected if their activity is correlated in some way, regardless of the mechanism underlying the correlation (Friston, et al., 1993). Functional connectivity has become ever more predominant since a paradigm shift in research focus from an assumed pathology due to regional brain alterations to impairments within dispersed network organisation (Konrad & Eickhoff, 2010) . The pathology

of neuropsychiatric disorders is now progressively more understood in terms of a systems viewpoint in which function surfaces from an interface of regionally specialised parts (Konrad & Eickhoff, 2010) . Resting state refers to a task-free paradigm with the participant lying still in the scanner with their eyes closed and mind wandering (Konrad & Eickhoff, 2010). This state provides a measure of brain activity independent of task related cognitive processes and can provide insights into the *default mode network* (DMN) which is a wide-ranging vigorously replicable system of brain areas during rest (Konrad & Eickhoff, 2010). In healthy participants, the DMN is progressively attenuated down during the shift from rest-to-task states although it is not extinguished and fervent deactivation is connected with enhanced task difficulty (Eichele et al., 2008; Greicius & Menon, 2004; Singh & Fawcett, 2008). Persistent DMN activity, i.e., problems with the deactivation of the DMN, in ADHD patients during EF tasks is associated with inaccuracies in the STOP signal (Li, Yan, Bergquist, & Sinha, 2007) and flanker tasks (Eichele, et al., 2008). Furthermore, an inability to attenuate the DMN in ADHD patients is also associated with attention lapses as measured by longer RTs and imprecise performance during tasks of attentional control (Konrad & Eickhoff, 2010; Weissman, Roberts, Visscher, & Woldorff, 2006). In ADHD patients, problems with deactivation of the DMN during cognitive tasks has been associated with attention lapses (Broyd et al., 2009).

Interregional functional connectivity has been examined in child patients with ADHD with fMRI during the resting state and reductions have been observed in functional connectivity compared to controls in frontoparietal, frontostriatal, temporoparietal and frontocerebellar networks (Liston, Cohen, Teslovich, Levenson, & Casey, 2011; Rubia, 2011; Tian, et al., 2006; Wang et al., 2009; Zang et al., 2007) (for a review see Konrad et al., 2010). Two studies looking at functional connectivity in young patients with ADHD relative to typically developing controls during cognitive tasks found (1) reductions in connectivity during tasks of interference inhibition and time estimation between frontoparietal and frontocerebellar regions respectively (Vloet et al., 2010), (2) a decreased level of functional connectivity during a test of sustained attention in ADHD compared to controls between parietal lobes and cerebellum, IFC (inferior frontal cortex) and the basal ganglia in addition to cerebellum, parietal and striatal brain regions in ADHD compared to controls (Rubia, Halari, Cubillo, et al., 2009).

Chapter Five: Electroencephalography (EEG) & Event Related Potentials (ERPs) in ADHD

Electroencephalography (EEG): A Historical Introduction

In 1829, the German scientist Hans Berger was the first to carry out a large number of controlled experiments and provide detailed observations of the recording of the brain's electrical activity in humans, consequently coining the term *Electrocephalogram*. Berger proceeded to name the large-amplitude rhythm (circa 10 waves per second or 10 Hertz) generated by eye closure and during the *at rest* state, *alpha* and the faster, smaller amplitude waves, present when the eyes were open, *beta* (Buzsáki, 2006). The application of Berger's non-invasive recording technique in humans progressed to become one of the most widespread methods in clinical and psychological laboratories worldwide (Buzsáki, 2006). In 1947, the American EEG Society was established and around the same time, the First International Congress was organised in London (Sanei & Chambers, 2007). EEG signals were analysed, throughout the early stages of EEG measurement, by applying Fourier analysis to EEG sequences (Sanei & Chambers, 2007). However, EEGs are now recorded using fully computerized systems furnished with numerous signal processing tools with sensitive and precise electrodes for measurement and sufficient capability to retain several hours of recordings (Sanei & Chambers, 2007).

Neural Activities and the Generation of EEG

The central nervous system (CNS) comprises neurons and glial cells which are positioned among neurons (Sanei & Chambers, 2007). Nerve cells generate responses to impulses and in turn communicate information over lengthy areas. Each nerve cell is made up of axons, dendrites and cell bodies. Dendrites are linked to either the dendrites of other cells or the axons. Their role is to pick up impulses and/or transmit signals from one nerve cell to another. Each nerve is linked to in the region of 10, 000 other nerves primarily via dendritic connections in the human brain (Sanei & Chambers, 2007). The activities of the CNS are predominantly linked to the synaptic currents conveyed between the synapses of dendrites and dendrites of cells or axons and dendrites (Sanei & Chambers, 2007). It is possible to record, an action potential with negative polarity of 60-70 mV beneath the membrane of a cell body. This intracellular potential alters

depending on alterations in synaptic activity, and is accompanied by an opposing change in the extracellular potential . For example, when an action potential journeys down the fiber that terminates in an excitatory synapse, an excitatory post-synaptic potential (EPSP) takes place in the next neuron (Sanei & Chambers, 2007). Whereas if a fiber ends in an inhibitory synapse then hyperpolarisation occurs, signifying an inhibitory postsynaptic potential (IPSP) (Sanei & Chambers, 2007). The number of neurons at birth amount to approximately 10^{11} and the amount of synapses per neuron enhances with age, whereas the amount of neurons diminishes with age. The average adult brain has about 5×10^{14} synapses (Sanei & Chambers, 2007).

Essentially, EEG provides verification of the oscillations of brain extracellular electric potential via electrode recordings on the human scalp (Nunez & Srinivasan, 2006). One electrode can provide an approximation of synaptic action averaged over tissue masses made up of approximately 100 million and 1 billion neurons (Nunez & Srinivasan, 2006). The physiological basis of the signal originates from extra cellular postsynaptic currents of apical dendrites extending from pyramidal cells, as opposed to axonal currents associated with the action potential (Ward, 2000). The pyramidal cell is the most prevalent in the cerebral cortex which has 5,000 – 50, 000 postsynaptic receiving sites. A number of basic requirements must be met for an electric signal to be detectable at the scalp. First, an entire population of neurons must be simultaneously active in order to generate a large enough electrical field and secondly they must be aligned in parallel so that they can summate rather than cancel out (Ward, 2000). This yields a dipolar magnetic field with positive and negative charges which enables the current to flow between; such arrangements are referred to as *open fields* due to their parallel alignment (Rugg & Cole, 2002). Neurons are arranged in this way fortunately in the cerebral cortex; however this cannot be extended to all regions of the brain (Ward, 2000). For example, due to the orientation of neurons in the thalamus, activity here is relatively invisible to this recording method (Ward, 2010).

EEG is measured as the difference in voltage between two electrode sites (Rugg & Coles, 1996). It is general practice to employ a recording procedure referred to as a *common reference*. This entails creating circuits that connect each component of an assortment of scalp electrodes to a solo reference made up of either a pair of electrodes or one other electrode. A common reference for researchers is the *linked mastoid* which is made up of a related pair of electrodes placed on the mastoid bone situated behind each ear (Rugg & Coles, 1996). This reference site is

chosen so it does not interfere with the electrical activity of investigational concern. Recordings are sourced on the variation in voltage between the reference electrode(s) and each exploring one (Rugg & Coles, 1996). Electrode locations are normally described in relation to the 10-20 system (Jasper, 1958; and see Figure 1 below).

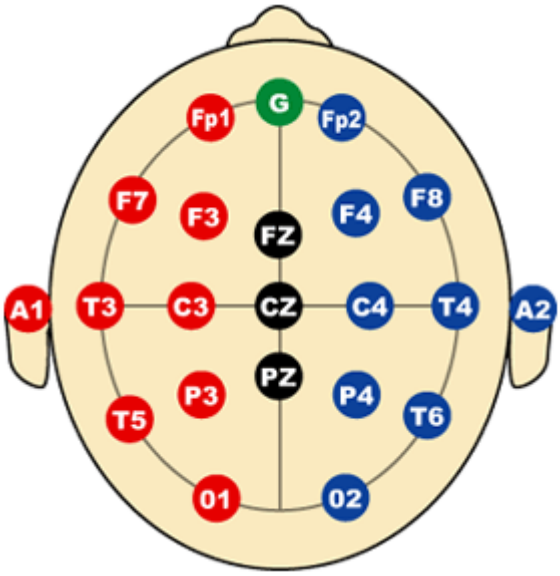


Figure 1. The International 10-20 system of electrodes used in a typical ERP experiment[5]

In the 10-20 system, the position of the electrode is stipulated in relation to its closeness to specific brain regions (e.g., occipital, frontal, central, temporal and parietal) and according to its position in the lateral plane (e.g., odd numbers are assigned for the left hand side, the subscript z is given for midline and even numbers are allocated for the right hand side) (Rugg & Coles, 1996). Therefore, according to this system, Fz represents a midline electrode site over the frontal scalp regions while T3 defines a left temporal site. It is important to note that although, these electrode descriptors relate to specific scalp regions, electrical activity recorded at particular scalp areas are not automatically ascribed to activity in brain regions in close nearness to that site, due to electric conductivity through biological material (e.g., meninges, skull, scalp) (Rugg & Coles, 1996).

[5] Source: http://www.immrama.org/eeg/electrode.html

EEG Rhythms

Typically, EEG measures are computed via fast Fourier transform and referred to as are *the relative* or *absolute power* in spectral analyses (Taylor & Baldeweg, 2002). There are 5 primary oscillatory frequencies named alpha (α), theta (θ), beta (β), delta (δ) and gamma (γ), differentiated by their low to high frequency ranges respectively. The frequencies and amplitudes of these signals alter depending on human states, for example, during wakefulness and sleep and also vary depending on age (Sanei & Chambers, 2007).

Alpha waves have a frequency range of 8-13 Hz and are usually observed over occipital scalp regions. They can also be indentified over all posterior lobe areas. Alpha waves are thought to be indicative of a relaxed awareness without concentration or attention (Sanei & Chambers, 2007). Theta waves have a frequency range between 4-7.5 Hz and normally during shallow sleep stages (e.g., stage II) and can be observed in children (Sanei & Chambers, 2007). Theta waves are also associated with meditation, creativity and unconscious material (Aftanas & Golosheikin, 2003; Gruzelier, 2009). Beta waves are most prominent during alert wakefulness and range from 14-26 Hz. Increased beta is linked to attention and aroused states including panic in humans and is predominately located over the frontal and central regions (Arruda, Amoss, Coburn, & McGee, 2007; Loo, Hopfer, Teale, & Reite, 2004; Sanei & Chambers, 2007). The gamma range is sometimes referred to as the fast beta wave and refers to activity between 30-70 Hz (although with a focal point of 40 Hz). Finally, delta waves have a frequency of between 0.5-4 Hz and occur in children and during deep sleep. The EEG power spectrum is generally computed from numerous EEG origins due to the topographic localisation of the frequency bands (e.g., the alpha band is characteristically observed above occipital scalp regions). The relative power constitutes the percentage of the summed EEG signal encompassed in every frequency band (Taylor & Baldeweg, 2002).

EEG rhythms and clinical application

EEG signals can be used to investigate pathophysiological brain disorders in addition to functional and particular brain abnormalities such as those found in ADHD. In the diseased, damaged or poorly developed brain, EEG may (1) become slower in frequency (referred to as EEG slowing), (2) may present with altered topography (for example, alpha waves at temporal regions), (3) may display higher amplitudes (often referred to as hypersynchronisation) and (4)

display a distinct slow rhythm in delta frequency (1-3 Hz) (Kropotov, 2009). Increase of slow activities (theta, delta) and decrease of faster frequencies (e.g. beta, gamma) in local EEG can indicate low metabolic activity in underlying mechanisms (Kropotov, 2009). It should be noted, however, that all these EEG rhythms change dramatically with age reflecting different stages of cortical development, see section titled "Developmental Differences in Neurophysiology", for a short review (Taylor & Baldeweg, 2002).

EEG investigations in children with ADHD have been characteristically associated with higher theta activity occurring predominantly in the frontal regions, alongside increases in delta rhythms in posterior regions and lower alpha and beta activity, also most prominent posteriorly (Barry, Clarke, & Johnstone, 2003; Clarke, et al., 2001a; Clarke, Barry, McCarthy, & Selikowitz, 2001b; Janzen, Graap, Stephanson, Marshall, & Fitzsimmons, 1995; Lazzaro et al., 1998; Mann, Lubar, Zimmerman, Miller, & Muenchen, 1992). Of all the EEG frequency bands, the gamma range is relatively under-researched in children and adolescents with ADHD. A recent study by Barry and colleagues (2010) explored EEG profiles of children with ADHD in the resting state, eyes closed condition, and reported increased levels of absolute delta and theta power along with reduced levels of absolute beta and gamma relative to healthy control children (Barry et al., 2010). In relation to relative power measures, children with ADHD in comparison to typically developing controls, displayed enhanced theta and delta activity alongside reductions in beta and gamma bands. Furthermore, measures of inattention rated by Parents using the Conner's scales were negatively associated with absolute gamma. This study confirmed that the widely replicated fast-wave EEG deficits in children with ADHD extends to the gamma rhythm and in turn associates that with higher scores of inattention (Barry, et al., 2010).

The occurrence of EEG clusters has also been investigated in 184 boys with ADHD and 40 age matched controls (Clarke, et al., 2001a). In this study, 3 distinct EEG patterns were found in children with ADHD which were characterised by (1) elevated slow wave activity and lacking of fast wave (2) elevated high amplitude theta with insufficient beta activity and (3) a surplus beta group. These findings confirm that children with ADHD do not present as a uniformed group in terms of their EEG profile. This also has significant repercussions for efforts directed at utilizing EEG as a discriminate tool for ADHD and typically developing children in clinical practice (Clarke, et al., 2001a).

The Event Related Potential (ERP*)*; Methods & Measurement

Event Related Potentials (ERPs) refer to those EEGs signals which directly quantify the electrical response of the cortex to cognitive, sensory or affective events, time-locked to the stimulus onset or behavioural response (e.g., a button press) (Gow, Matsudaira, Taylor, Rubia, Crawford, Ghebremeskel, Ibrahimovic, Vallee-Tourangeau, et al., 2009). ERPs are typically generated in response to external or peripheral stimulations and appear as visual, somatosensory and auditory brain potentials or as gradually evolving neural activity observed prior to voluntary movements or during anticipation of conditional stimulation (Sanei & Chambers, 2007). Each psychological process concerns the temporal inhibition and activation of neuronal patterns in a specific brain area (Kropotov, 2009). The result is a number of simultaneously produced and event-locked post-synaptic potentials recorded at the scalp in the shape of an ERP deflection: a potential wave which is both temporally confined and spatially localised (Kropotov, 2009). ERPs studies are able to expose both psychophysiological precursors and associated poor performance consequently revealing disparities in concealed information processing, even when no overt differences in performance are apparent (Brandeis, van Leeuwen, & Rubia, 1998; van Leeuwen, Steinhausen, Overtoom, 1998). Indeed, ERPs are useful tools for defining both psychiatric and neurological conditions including ADHD (van der Stelt, van der Molen, Boudewijn Gunning & Kok, 2001). Although the evaluation of ERPs peaks do not result in a dependable diagnosis, they have been employed to address a wide range of research questions in cognitive and emotion functions using experimental manipulations (Sanei & Chambers, 2007).

Relative to background EEG activity, ERPs are quite small (1-30 μV) hence the requirement for a signal-averaging procedure to improve signal to noise ratio (Sanei & Chambers, 2007). Traditionally, the ERP waveforms are measurable across 3 aspects (1) amplitude, (2) latency and (3) scalp distribution (Sanei & Chambers, 2007). Amplitudes provide an indicator of the degree of neural activity in response to functionally relevant stimuli (Friedman, Cycowicz, & Gaeta, 2001a). The latency is essentially a measure of the point in time at which the peak amplitude arises, e.g., in relation to the activation time or speed of processing. The scalp distribution reflects the pattern of the voltage slope of a deflection above the scalp at any instantaneous time (Friedman, et al., 2001a; Sanei & Chambers, 2007). The scalp recorded ERP voltage activity is a reflection of the computation of cortical and subcortical neural activity

within each time window. There are several ways to measure the amplitude of an ERP component. Three of these are called a *peak amplitude measure*, *mean amplitude measure* and *area-under-the-curve (AUC) amplitude measure*. The first, a peak amplitude measure is calculated by measuring amplitude at the time point the deflection reaches its maximum (or minimum) amplitude, i.e., quantifying the amplitude at its peak latency (Handy, 2005). The second measurement is to quantify the *mean amplitude measure*. This is essentially involves averaging an amplitude over a time window which comprises the component of interest resulting in a value which is the mathematical mean of all time points within the defined window (Handy, 2005). The number of timepoints is adequately controlled in order to avoid this using time points in adjacent components in the waveform and thus when making a mean amplitude measure the time window normally centres on the peak latency (Handy, 2005). The third measure, the AUC amplitude measure is the sum of the voltages at each time point within the measurement window (Luck, 2005a). Using the AUC measure (the integral of the curve over a specified time range), the space between the ERP waveform and baseline is divided into multiple time-windows that can be mapped onto ERP component time-windows. Area and mean are identical, except that area is multiplied by the duration of the time range. The use of larger windows of activity and AUC as opposed to indentifying a single peak is advantageous as it avoids the distortions created by the signal-averaging process by reducing biases in identifying the ERP signal amongst noise and also takes into account trial-by-trial activity variation in peak amplitude (Jongen & Jonkman, 2008). Given that ADHD has been associated with greater variation in the speed of response to stimuli during attention-related tasks (i.e., greater standard deviation of reaction time) (Rubia, Smith, Brammer & Taylor, 2007b), this method may well provide a better indication of underlying activity. For these reasons, in most cases, measurements of mean amplitude are superior to measurements of peak amplitude (Luck, 2005). Furthermore, few ERP studies in ADHD adolescents have used the area-under-the-curve (AUC) data analyses methods (Jonkman, 2006; Jonkman, Sniedt, & Kemner, 2007). This PhD for the reasons given has employed AUC amplitude measures for all ERP analyses.

ERP components are traditionally named based on their polarity and their average time of occurrence in millisecond post-stimulus. For example, the ERP wave form consists of either positive (P) or negative (N) voltage deflections called components or peaks (Gow, Matsudaira, Taylor, Rubia, Crawford, Ghebremeskel, Ibrahimovic, Vallee-Tourangeau, et al., 2009). The

letter P, indicates positive going, and is followed by the time represented in terms of milliseconds after the onset of audio, visual or somatosensory stimuli. For example, P200 and P300 are both positive peaks occurring at 200 and 300 ms respectively. Another type of classification other than their positive or negative class is their functional significance. There are quite a few ERP waves which are elicited during specific types of behavioural concepts and some have particular names related to their associative function (Kropotov, 2009). These include, for example, (1) the mismatch negativity (MMN) which is an indication of a detection in change in auditory (i.e., in sound) or visual (i.e., a change in letter following a repetitive string of letters); (2) the processing positivity (PN), a marker of purposeful attention to a specific sensory channel; (3) error related negativity (ERN) which is an marker of errors made during a sustained attention (CPT) task; (4) N2 NoGo component as a marker of motor suppression during a Go/NoGo task (Kropotov, 2009). The P3b component is widely considered as an indicator of the process in updating working memory; and P3a component as an index of automatic switching of attention (Kropotov, 2009; Luck, 2005a). Those components specifically relevant to the tasks employed in this PhD study e.g., the N2 and P3 family will be reviewed in more detail in later sections.

The next section of this chapter will firstly provide a general non-specific account of the main ERP components encountered in cognitive neuroscience research. This is followed by a brief account of the ERP findings to date in childhood ADHD focusing on the literature relevant to the experiments carried out in this PhD. In other words, it will summarise the main findings from tasks measuring motor response and interference inhibition such as the Go/NoGo (which is used to measure a participant's ERP response during inhibitory processes), sustained attention and affect processing in relation to the most relevant ERP components employed in this PhD study. The review will conclude with a section on developmental differences in neurophysiology.

A Brief Review of Key ERP Components

As previously emphasised, ERP components have allocated descriptions such as P1 or N1 that relate to their place and polarity within the waveform (Luck, 2005a). However, it is important to note that one cannot presuppose that these descriptions are related in someway to the nature of the brain activity lying underneath (Luck, 2005a). The auditory P1 positive-going and N1 negative-going deflections, for example, bear no specific relationship to the visual P1 and N1 deflections other than they are generated by respective sensory cortices and are predominantly exogenous, i.e., indicate their dependence on external as opposed to internal factors (Luck, 2005a). Later waves, for instance the P3 family are predominantly modality-independent; however despite this the P3 component may have modality-specific subcomponents (Crottaz-Herbette & Menon, 2006; Luck, 2005a). The following review focuses on visual stimuli as opposed to auditory.

P1 Modulations

The P100 or P1 wave usually onsets 60-90 ms post-stimulus peaking between 100-130 milliseconds and is largest at lateral occipital electrode sites (Luck, 2005b). P1 onset is considered problematic to measure due to its overlap with the early C1 component (Luck, 2005a). In addition, the latency of P1 will also differ considerably on stimulus contrast (Luck, 2005a). Several research investigations have made efforts to localise the P1 component using mathematical modeling methods alone or together with co-localisation with fMRI effects (Luck, 2005a). The results of these studies have suggested that the early part of the component occurs from dorsal extrastriate cortex, specifically in the middle occipital gyrus, and the subsequent part originates more ventrally from the fusiform gyrus (Di Russo, Martinez, Sereno, Pitzalis, & Hillyard, 2002; Luck, 2005a). However, within the first 100 ms post-stimulus at least 30 different visual regions are activated and a lot of these areas are likely to add to the voltages recorded in the C1 and P1 latency series (Luck, 2005a). The P1 is thought to originate in the visual cortex, is sensitive to changes in stimulus parameters such as the direction of spatial attention, and arousal states (Luck, 2005a; Vogel & Luck, 2000).

N1 Modulations

The N1 or N100 follows the P1. There are a number of visual N1 subcomponents with the earliest peaking at anterior electrode sites, 100-150 milliseconds post-stimulus (Luck, 2005a). There are no less than 2 posterior N1 waves the first occurring from the parietal cortex; the other arising from the lateral occipital cortex (Luck, 2005a). Both typically peak around 150-200 ms post-stimulus (Luck, 2002). Various research investigations have demonstrated that spatial attention manipulates these waveforms (Luck, 2005a).

Additionally, the lateral occipital N1 subcomponent seems to be larger during discrimination tasks as opposed to detection tasks leading researchers to infer that this subcomponent reflects some sort of discriminative processing (Hopf, Vogel, Woodman, Heinze, & Luck, 2002). The N1 is larger in response across the right hemisphere to meaningful pictures such as those of faces relative to cars (Rossion & Caharel, 2011).

P2 Modulations

The P200 or P2 waves go after N1 deflections occurring at anterior and central scalp regions, with a maximum at fronto-central sites (Luck, 2005a). This early positive wave has been associated with the suppression of sensory input from additional processing by way of automatic recognition of stimuli and discrimination (Hegerl & Juckel, 1993) or the inhibition of alternative outlets of attention competing for further processing and attention (Oades, 1998; Wiersema, van der Meere, Roeyers, Van Coster, & Baeyens, 2006). It is thought to be larger for target stimuli and increased when targets are fairly irregular (Luck, 2005a; Luck & Hillyard, 1994). From this perspective the P2 component is comparable to the P3a wave; yet, the anterior P2 effects seem to only take place when the target is characterised by rather easy stimulus features (Luck, 2005a). The posterior P2 is frequently problematic to identify due to other overlapping waves namely the N1, N2 and P3 components and as a result is not well understood (Luck, 2005a).

N2 Modulations

Naatanen and Picton (1986) have described that a recurring, non-target stimulus will generate a *basic* N2 (N200) component, although it will contain many subcomponents (Luck, 2005a; Naatanen & Picton, 1986). The N2 is a frontal negative component arising at a latency of 200–450 milliseconds post-stimulus (Dimoska, Johnstone, & Barry, 2006; Johnstone et al., 2007). It is thought to be related to inhibitory processes (Johnstone, et al., 2007), process monitoring (Kratz et al., 2011), categorization and discrimination difficulty (Senkowski &

Herrmann, 2002), working memory (Missonnier et al., 2007) and conflict management (van Veen & Carter, 2002b). A later N2 component referred to as N2b can also be observed and is produced by both visual and auditory deviants, if task relevant. For visual stimuli, this component is largest over posterior regions and largest for auditory stimuli over central regions (Luck, 2005a). It is considered to be an indication of the categorisation process and is larger for less frequent processes. (Luck, 2005a).

In tasks measuring motor response inhibition for example the Go/NoGo or Stop-Signal tasks the N2 deflection is enhanced in frontal areas and greater on trials where inhibition is failed (Smith, Johnstone, & Barry, 2007). The source of the N2 component has been persistently localised in the ACC (Bekker, Kenemans, & Verbaten, 2005; van Veen & Carter, 2002a) and it is considered to reflect activity of prefrontal areas which modulate processes implicated in the orienting of attention and the preparation of a motor response (Banaschewski & Brandeis, 2007; Eimer, 1993; Falkenstein, Hoormann, & Hohnsbein, 1999; Gow, Matsudaira, Taylor, Rubia, Crawford, Ghebremeskel, Ibrahimovic, Vallee-Tourangeau, et al., 2009; Kok, 1986), as well as conflict monitoring (Donkers & van Boxtel, 2004; Nieuwenhuis, Yeung, van den Wildenberg, & Ridderinkhof, 2003). This theory proposes that conflict monitoring processes are activated each time conflict arises between the prepotent response and the current, obligatory response (Jonkman, 2006).

The P3 (P3a/P3b) Family and the Oddball Paradigm

At approximately 250 – 400 ms post-stimulus presentation a group of late positive over-lapping deflections occur that comprise a component referred to as P3 or P300. Despite countless published P3 experiments and an appreciable progress of the literature over the past 25 years, the precise neural origins and neuropsychological meaning of the role of this component remains unclear (Polich & Criado, 2006). While, a significant amount is known about the effects of a range of experimental manipulations on P3 amplitude and latency there is still no clear, general agreement regarding which cognitive / neural processes the P3 deflection imitates (Luck, 2005a; Polich & Criado, 2006). The standard paradigm for eliciting the P3, named the *oddball task,* consists of a series of events comprising two classes presented to the participant (Rugg & Coles, 2002). The first class is a frequently occurring or standard stimulus. The second class is an infrequently occurring oddball or deviant (Rugg & Coles, 1996). The task is manipulated so that

one class is commonly rarer than the other and the participant is instructed to respond to the more infrequent stimuli (Rugg & Coles, 1996). In this task, the P3 latency is considered to be a measure of stimulus appraisal whereas the amplitude of the wave varies accordingly to the likelihood of occurrence, that is, the more infrequent the event the larger the P3 (Rugg & Coles, 1996). These findings led to the theory that P3 reflects and responds to alterations in several aspects of attention , relying on widespread neural mechanisms including frontal, temporal, parietal and subcortical regions (Donchin & Coles, 1988; Friedman, et al., 2001a; Friedman, Goldman, Stern, & Brown, 2008; Polich & Criado, 2006; Polich & Margala, 1997).

Seminal work in this area was carried out by Donchin and Coles (1988). They suggested that P3 replicates a process of memory or context updating in which the existing environmental model is altered as a function of inward bound information (Donchin & Coles, 1988; Kopp & Wolff, 2000). Evidence of this orienting response can be found in a different kind of oddball task named the *Novelty oddball* (Friedman, et al., 2001a). This involves the introduction of a third *novel* auditory stimulus into the oddball paradigm, such as a dog barking or a door slamming in high and low tones (Friedman, et al., 2001a). The result of this addition elicits a large positivity with an earlier latency than that of an oddball target-evoked P3. This wave tends to have a more frontal distribution and consequently is regarded as the frontal P3 or the P3a as compared to the typical P3 which is parietally distributed (Rugg & Coles, 1996). The P3a has been associated with procedures concerned in the spontaneous capture of attention by prominent novel events (Rugg & Coles, 1996) and is also influenced by lesions in the frontal cortex (Knight, 1991).

Deviant, rare events, occurring within a succession of same type stimuli can draw out a orienting response (Friedman, et al., 2001a). The resulting ERPs are sequentially made up of the MMN and the novelty P3 (Friedman, et al., 2001a). The latter term is used in relation to the P3 wave drawn out by events in which the participant has no fore warning of (e.g., environmental sounds as previously mentioned, a dog bark, etc) (Friedman, et al., 2001a). The term *target P3* refers to the event elicited P3 during which the participant has already been directed and to which some type of response is required (e.g., a speeded RT) (Friedman, et al., 2001a).

Distinguishing between P3a and P3b

The labels P3a and P3b refer to ERP deflections which are elicited as a result of variations in stimuli, including those classified as targets in addition to novel stimuli, and can co-occur within the same waveform (Friedman, et al., 2001a). Squires, Squires and Hillyard (1975) reported the first main difference between the frontally maximal P3a and parietally maximal P3b deflections. Both components were obtained following irregular and rare alterations in the pitch or intensity of the tone, however the P3b wave was only current when these alterations were related to the task (Luck, 2005a; Squires, Squires, & Hillyard, 1975).

P3a. P3a is elicited by novel or distracting stimuli, including sudden changes in sensory stimulation, and has been associated with the orienting response (Friedman, et al., 2001a; Soltani & Knight, 2000). Unexpected tones elicit a large positive P3a, occurring as early as 280 milliseconds after stimulus on-set indicating that the novelty of the sound has involuntary seized attention and the focus of attention is likely at that time point (Friedman, Cycowicz, & Gaeta, 2001b). The P3a is thought to rely heavily on anterior cingulate function, giving it a frontocentral distribution. However, P3a also contains posterior aspects (Friedman, et al., 2001a).

P3b. P3b is enhanced in response to target detection, often requiring a motor or cognitive response (Friedman, et al., 2001a). It is also elicited by infrequently occurring events which typically involve a decision or are task relevant. By contrast with the P3a, it almost always provides a scalp distribution over posterior parietal scalp (Friedman, et al., 2001a). Luck (2005) argues that it is the likelihood of the task-defined stimulus type which is important and not the likelihood of the physical stimulus (Luck, 2005a). For instance, if the letter C is the target and occurs on 10% of the trials, and the non-targets are presented randomly from other alphabetical strings, the target (i.e., the letter C) will draw out an extremely large P3b component despite the fact that the target letter is about 4 times more likely than any other non-target letter (Luck, 2005a; Vogel, Luck, & Shapiro, 1998). Additionally, P3b amplitude is larger when more effort is dedicated to a task implying that P3b amplitude can be considered a quantification of resource allocation (Isreal, Wickens, Chesney, & Donchin, 1980; Luck, 2005a). When a participant is unsure whether a given stimulus is a target or non-target, P3b amplitude is smaller. However, if the task difficulty is increased this may result in an increased P3b amplitude, on the other hand, it may also decrease by creating uncertainty in the participant regarding the category of a particular stimulus (Luck, 2005a). It has been proposed by Johnson (1986) that the variables of probability

(P), uncertainty (U) and resource allocation (R) unite to have an impact on P3b amplitude and can be presented in the following equation: P3b amplitude = U x (P + R) (Johnson, 1986; Luck, 2005a).

In Go/NoGo tasks, distinctions have been observed between Go and NoGo ERPs related to the anteriorisation of the P3 amplitude which usually has a maximum at centroparietal sites in NoGo compared to Go trials (Bokura, Yamaguchi, & Kobayashi, 2001). This effect is thought to be associated with inhibitory response processes and the activation of specific prefrontal regions, especially the anterior cingulate cortex (ACC) (Fallgatter et al., 2004; Strik, Fallgatter, Brandeis, & Pascual-Marqui, 1998).

N170 and Vertex Positive Potential (VPP)

ERPs are also used to study face processing including emotional responses to different facial expressions. The process of face recognition begins very early, approximately 180 ms after the presentation of the stimulus (Bentin, Allison, Puce, Perez, & McCarthy, 1996; Botzel & Grusser, 1989; Maurer, Grand, & Mondloch, 2002). The first perceptive stage, during which the individual constructs the 'structural code' of the face, is believed to occur independently from complex facial information such as emotional significance (Balconi & Pozzoli, 2003; Bruce & Young, 1986). Jeffreys (1989) was one of the first researchers to find a disparity, at central and midline electrode sites, between 150 and 200 milliseconds when comparing ERP responses to illustrations of faces and non-faces and named this the vertex positive potential (VPP) (Jeffreys, 1989; Mouchetant-Rostaing & Giard, 2003). Rossion et al., (1999) proposed that the VPP reflects the same dipole as the N170, although opposite sides and this has been confirmed in more recent studies (Joyce & Rossion, 2005). An evaluation of the early face processing literature during childhood demonstrates that the N170 component is significantly elevated in amplitude for both inverted and upright faces but that there are developmental changes during childhood sensitive to task demand (Kropotov, 2009; Taylor, Batty, & Itier, 2004; Williams, Palmer, Liddell, Song, & Gordon, 2006).

Holmes, Vuilleumier and Eimer (2003) argue that the N170 wave is enhanced by attention (Holmes, Vuilleumier, & Eimer, 2003) while others report it is enhanced by the valence of emotional expressive stimuli such as faces over occipital-temporal sites (Balconi & Pozzoli, 2003; Williams, et al., 2006). N170 is thought to be associated with an initial monitoring process

which decodes the relevance of incoming information and occurs before the detection of emotional stimuli which may be mirrored in the N250 and P3 responses (Gow, Matsudaira, Taylor, Rubia, Crawford, Ghebremeskel, Ibrahimovic, Vallee-Tourangeau, et al., 2009; Williams, et al., 2006). Studies by Williams and colleagues (2006) have demonstrated a clear right-hemisphere effect for N170 independent of valence (Gow, Matsudaira, Taylor, Rubia, Crawford, Ghebremeskel, Ibrahimovic, Vallee-Tourangeau, et al., 2009; Williams, et al., 2006). However, other studies propose that negative stimuli (such as fearful faces) are lateralised to the right while positive stimuli (e.g., happy faces) to the left (Balconi & Lucchiari, 2005). It is pertinent to consider that the VPP (and seemingly the N170) is also elicited for other highly well-known stimuli such as in the recognition of common words (Luck, 2005a; Schendan, Ganis, & Kutas, 1998). To date, the face specificity of the N170 remains a subject of great discussion (Bentin & Carmel, 2002; Carmel & Bentin, 2002; Luck, 2005a; Rossion, Curran, & Gauthier, 2002).

Summary

To summarise, the evidence to date suggests that the N1 component is linked to spatial attention and/or discriminative processing whereas the P2 is involved in the gating of unimportant sensory information supposedly safe-guarding higher-order cognitive processes from overload (Lijffijt et al., 2009). The N2 is considered to be linked to inhibitory processes, conflict monitoring, categorisation and discrimination and working memory. The P3 family are linked to both novel and distracting stimuli and the later P3b to target detection, decision making and effortful processes. In relation to face processing, the early N170 is implicated in the first perceptive phase of face recognition and enhanced to emotional expressive stimuli. This PhD has selected specific area-under-the-curve (AUC) time windows for each ERP task, the details of which will be provided in the relevant methods sections.

Developmental Differences in Neurophysiology

It is pertinent to note that the development of EFs including inhibition, attention and flexibility progress gradually from early childhood to adulthood (Davidson, Amso, Anderson, & Diamond, 2006; Jonkman, 2006; Ridderinkhof & van der Stelt, 2000b; Williams, Ponesse, Schachar, Logan, & Tannock, 1999). This is paralleled by fMRI studies which demonstrate that fronto-striatal and fronto-parietal brain activation during response inhibition and other executive control processes undergoes intense development between late infancy and adolescence which

has been attributed to the late structural development of these regions (Jonkman, 2006; Luna, Garver, Urban, Lazar, & Sweeney, 2004; Luna et al., 2001; Rubia, Smith, Taylor, et al., 2007; Rubia, et al., 2006b) (for a review see Geier et al., 2010). Knowledge concerning the typical development of executive control procedures and their neurobiological origin is central in the comprehension of critical stages in development which in turn, is vital for the identification of abnormal behaviour such as those reported in ADHD (Jonkman, 2006).

Patterns of cortical development have been directly associated with cognitive maturation (Krain & Castellanos, 2006; Reiss, Abrams, Singer, Ross, & Denckla, 1996; Sowell et al., 2004). For example, verbal functioning is negatively correlated with left dorso-frontal and parietal cortical gray matter thickness. Furthermore, IQ has been found to be positively associated with volumetric prefrontal cortical gray matter (Reiss, et al., 1996; Sowell, Thompson, Leonard, et al., 2004). Electrical brain activity has demonstrated sensitivity to alterations in cortical structure (Whitford et al., 2007). Collective evidence further demonstrates that absolute EEG power decreases in each and every frequency bands and this relative decrease persists into adulthood, although at a smaller speed than in teenage years (Dustman, Shearer, & Emmerson, 1999; Gasser, Verleger, Bacher, & Sroka, 1988; Matsuura et al., 1985; Whitford, et al., 2007). Whitford and colleagues (2007) investigated brain maturation in adolescence using EEG recordings and MRI and found that gray matter volume decreased across the age bracket of 10 to 20 years (n = 138) in the frontal and parietal regions with the largest change taking place in adolescence. EEG activity, especially in the slow-wave band revealed a comparable curvilinear decline to gray matter volume in related cortical areas. In the parietal lobes, an opposite pattern of curvilinearly was observed with increasing white matter volume (Whitford, et al., 2007). The gray matter reduction is thought to mirror a decrease of neuropil (i.e., the region between the blood brain barrier and neuronal cell bodies in the gray matter of the brain) with the parallel abolition of active synapses thought to reflect the subsequent reduction in EEG power (Whitford, et al., 2007).

Age-related changes have also been long reported in the P3 component (Bauer & Hesselbrock, 1999; Bauer & Hesselbrock, 2003; Hill et al., 1999; Johnstone, et al., 2007; Segalowitz & Davies, 2004; van der Stelt, Kok, Smulders, Snel, & Boudewijn Gunning, 1998). In healthy adults the P3b amplitude, normally maximal at Pz electrode sites, is large in response to the *to be attended* to stimuli whereas its amplitude is smaller when the memory burden is

greater (Gomarus, Althaus, Wijers, & Minderaa, 2006; Kok, 2001; Mulder, Gloerich, Brookhuis, van Dellen, & Mulder, 1984). In relation to its topography, it is reported to change with age from amplitudes which are maximal at occipital scalp regions in the younger age groups to a parietal maximum in the older age groups. In addition, its latency onset has been shown to decrease with age (Gomarus, et al., 2006; van der Stelt, et al., 1998). In children, larger P3 amplitudes are noticeable with a posterior midline topography, this is followed by the appearance of the midline frontal P3a occurring in late adolescence (Davies, Segalowitz, & Gavin, 2004; Polich & Criado, 2006; Segalowitz & Davies, 2004). Other studies have demonstrated that component latency, amplitudes and topography varied as a function of age (Bauer & Hesselbrock, 2003; Bishop, Hardiman, Uwer, & von Suchodoletz, 2007; van der Stelt, et al., 1998). For example, in children aged 7 and 9 and 10 to 12 years relative to adults the early frontal selection positivity (FSP) (circa 150 and 200 ms) onset has been shown to be delayed and the early, occipitally maximum, selection negativity (normally found in adults in selective attention tasks) hardly or not visible (Gomarus, et al., 2006; van der Stelt, et al., 1998). The N2b component (circa 250 – 300 ms) which in adults has a central distribution has also demonstrated a late latency for young children localised to more fronto-temporal scalp regions (Gomarus, Wijers, Minderaa, & Althaus, 2009; van der Stelt, et al., 1998). Younger children compared to adults have also shown reductions in specific ERP components, such as the novelty P3, linked with the pre-frontal cortex (PFC) and this immaturity continues into late adolescence (Segalowitz & Davies, 2004). Other components such as the N2 has also been associated with age-related changes and higher amplitudes have been observed in passive oddball tasks in children aged between 10 and 11 years of age relative to adults, and although a comparable negativity is observed in adults it tends to be smaller and more focally distributed (Bishop, Hardiman, et al., 2007). The N1 and P1 are also known to develop with age. For example, the P1 component has also been found to have marked developmental changes appearing around 70 ms with a fronto-central positive distribution in children aged less than 11 years of age. The N1, appearing at approximately 100 ms post stimulus onset with a fronto-central negative distribution, reportedly is not noticeable in children aged less than 10 years of age however can be observed in older children (Bishop, Hardiman, et al., 2007; Bruneau, Roux, Guerin, Barthelemy, & Lelord, 1997).

To conclude, the standard progression of the development of the visual P3 during the course of adolescence is a decrease in both amplitude and latency (Berman, Friedman, &

Cramer, 1990; Berman et al., 2006; Hill & Shen, 2002; Iacono, Carlson, Malone, & McGue, 2002). This is thought to reflect patterns of cortical changes occurring in the adolescent brain involving neuronal and synaptic alterations including the finalisation of frontal myelination (Berman, et al., 2006; Luna, et al., 2004; Luna & Sweeney, 2004; Sowell, Thompson, & Toga, 2004). There is also collective evidence for developmental changes with N2 components, with increasing age related to decreases in latency (van der Stelt, et al., 1998) and greater amplitudes in adults (DeFrance, Sands, Schweitzer, Ginsberg, & Sharma, 1997). Finally, both N1 and P1 are also affected by age-related changes with the N1 reported to be hardly visible in younger children compared to older children and the P1 presenting with greater amplitudes in childhood compared to adults (Bishop, Hardiman, et al., 2007; DeFrance, et al., 1997).

Event Related Potentials in childhood ADHD

Cognitive processes which involve response or stimulus expectation and preparation, selective attention, response inhibition and conflict monitoring (e.g., the capability to interrupt an activated response and to actively suppress responses) are vital to establish resourceful and goal-directed behaviour (Jonkman, 2006). Children with ADHD however have persistently been shown to have abnormal ERPs during tasks measuring these types of cognitive processes especially in frontal and parietal regions (Banaschewski et al., 2004; Brandeis et al., 1998; Brandeis, van Leeuwen, Steger, Imhof, & Steinhausen, 2002; Dimoska, Johnstone, Barry, & Clarke, 2003; Jonkman, Kemner, Verbaten, Koelega, Camfferman, vd Gaag, et al., 1997; Liotti et al., 2007; Pliszka, Liotti, & Woldorff, 2000b; Shen, Tsai, & Duann, 2011). .

Response (conflict) inhibition in children with ADHD: A review of Go/NoGo, Stop Signal and CPT-A-X tasks

Motor response inhibition is measured in Go/NoGo, Stop or inverted CPT-AX tasks. In the Go/NoGo task participants have to inhibit a motor response to infrequent NoGo trials in a string of frequent Go trials. The task measures selective attention and motor response inhibition. In the Stop task, a motor response that is already activated by the Stop signal has to be withheld, and thus measures the more difficult function of retraction of a motor response that was about to be executed (Rubia, Russell, et al., 2001). In the inverted CPT-AX, participants have to respond to frequent targets (e.g., letters) but inhibit their response to the letters A followed by X. The task therefore measures response inhibition in the same way as the Go/NoGo task. It is the low frequency of NoGo or Stop trials among a string of high frequent Go trials that elicits the

inhibitory effect. The more typical standard CPT requires participants to respond to non-frequent letters while ignoring the frequent ones, which requires selective/sustained attention. The traditional CPT will be discussed in a later section.

The two main ERP deflections that have been functionally linked to response inhibition processes in Go/NoGo tasks are the frontal negative NoGo-N2 and the fronto-central positive NoGo-P3 (Banaschewski, Brandeis, Heinrich, Albrecht, Brunner & Rothenberger, 2004). In NoGo trials relative to Go trials, increases in the amplitudes of the N2 wave have been specifically linked to response inhibition processes (Bekker, Kenemans, & Verbaten, 2004; Bruin & Wijers, 2002; Eimer, 1993; Falkenstein, et al., 1999; Jodo & Kayama, 1992; Nieuwenhuis, et al., 2003). However, a recent study by Smith, Johnstone and Barry (2007) suggest it is the P3 as opposed to the N2 which indicates the inhibition of a deliberate response and/or conflict between contra responses warranting the necessity for a key review of the existing understandings of the N2 and P3 components in inhibitory tasks (Smith, et al., 2007).

The ERP literature has elicited diverse results thus far in children with ADHD. For example, Overtoom, Kenemans & Verbaten et al., (2002) have reported markedly reduced N2 in children with ADHD alongside poorer response-inhibition performance compared to controls (Pliszka, Liotti, Woldorff, 2000). Fallgatter and colleagues (2004) have argued that there is a discrepancy between healthy and ADHD children in ERPs following both Go and NoGo stimuli. For example, in the NoGo ERPs a negative wave, maximal at frontocentral electrode sites is reported 200-400 ms after the onset of a stimulus (NoGo-N2) and suggests that this N2 component reflects inhibitory processes associated with NoGo trials (Brandeis, van Leeuwen, Rubia, Vitacco, Steger, Pascual-Marqui, & Steinhausen, 1998; van Veen & Carter, 2002; Fallgatter et al., 2004).

However, those studies employing the inverted CPT-A-X tasks which measure inhibition have contradicted these findings, and reported that children with ADHD did not significantly differ from that of the control group in N2 responses during NoGo trials (Overtoom et al., 1998; van Leeuwen, Steinhausen, Overtoom, Pascual-Marqui, van't Klooster, et al., 1998). Although, they did report that a small subgroup of children (n = 6) with comorbid ODD displayed smaller N2 amplitudes. Satterfield and colleagues (1994) reported significantly different N2 amplitudes between *delinquent* and *non-delinquent* boys with hyperactivity. The N2 amplitude in hyperactive and delinquent boys was observed to be significantly larger compared to the non-

delinquent and hyperactive boys (Satterfield & Schell, 1984). Larger N2 amplitudes have also been reported in children with ADHD implying a greater frontal association, in other words, more effort was applied to produce a correct response (Jodo & Kayama, 1992; Yong-Liang, Robaey, Karayanidis, Bourassa, Pelletier & Geoffroy, 2000). The variability in findings are often attributable to small between-study differences connected to the population groups such as age differences, diagnostic criteria (e.g., ADHD subtype, comorbidity, symptom severity), task, task difficulty (e.g., performance and utilisation of reward systems) and data analysis methods such as focused regional analysis founded on maximum amplitudes versus topographic techniques (Barry, Johnstone, et al., 2003a).

Another, reported distinction between Go and NoGo ERPs relates to the topography of the P3 which is localised to anterior regions, usually at Cz in NoGo relative to Go, which is maximum usually at Pz (Bokura, et al., 2001; Fallgatter, et al., 2004). This effect is thought to be associated with inhibitory response processes (Fallgatter, et al., 2004). Low resolution brain electromagnetic tomography (LORETA) software, which is a source localisation method, has associated the NoGo-evoked P3 wave to an activation of specific prefrontal brain regions, especially the anterior cingulate cortex (ACC) (Fallgatter, et al., 2004; Strik, et al., 1998) and can also be reflected in the FCz amplitude response. Fallgatter and colleagues (2004) have also reported a significant decrease in electrical activity (namely P3 amplitudes) in the NoGo condition of a CPT task in ADHD children relative to controls suggesting diminished NoGo activity in the ACC (Fallgatter, et al., 2004). Furthermore, Fassbender and Schweitzer (2006) argue that impairments in prefrontal and anterior cingulate cortex function reduce the ability to maximise subsidiary neural regions which are needed to optimally perform cognitive tasks (Fassbender & Schweitzer, 2006).

Other studies have reported reductions in P3 amplitudes relative to healthy control children in Go/NoGo tasks and investigations using a cued CPT target P3 (Benikos & Johnstone, 2009; Groom et al., 2010; Kratz, et al., 2011; Spronk, Jonkman, & Kemner, 2008; Wiersema, et al., 2006) in addition to cue-P3 amplitudes (Banaschewski et al., 2003; Brandeis et al., 2002; Doehnert, Brandeis, Imhof, Drechsler, & Steinhausen, 2010). The reductions of cue-P3 amplitudes have been interpreted in the context of attentional orienting or resource allocation suggesting a sub-optimal energetic state regulation (Banaschewski, et al., 2003; Doehnert, et al., 2010).

Other functional differences reported in ERP ADHD studies include reductions in the early components elicited by visual stimuli such as P1 and P2. This is apparent especially in responses to Go, NoGo and/or warning stimuli compared to controls (Smith, Johnstone & Barry, 2004; Johnstone, Barry, Markovska, Dimoska & Clarke, 2009). In ADHD children, atypical P1 responses have been revealed reflecting a decreased attentional priming effect on early sensory responses (Perchet, Revol, Fourneret, Mauguiere, & Garcia-Larrea, 2001). Reductions in P1 amplitudes in children with ADHD relative to typically developing children have been observed in response to standard and unexpected but not novel stimuli (Barry, Clarke, et al., 2003; Kemner et al., 1996). In hyperactive children and those with attentional difficulties, P2 amplitudes responses to standard stimuli were found to be larger (Callaway, Halliday, & Naylor, 1983; DeFrance, Smith, Schweitzer, Ginsberg, & Sands, 1996; Robaey, Breton, Dugas, & Renault, 1992; Wiersema, et al., 2006). The finding of smaller P1 components in ADHD relative to control children has also been observed during the Stop task (Shen, et al., 2011). Other studies employing the Stop task report reduced frontal N2 component along with poorer performance in the inhibitory measure (Dimoska, et al., 2003; Liotti, et al., 2007; Pliszka, et al., 2000b).

Other ERP studies have investigated ADHD alongside other comorbid conditions such as ODD or CD. For example, Wiersema and colleagues found that the ADHD group regardless of comorbidity with ODD/CD displayed a steeper increase in reaction time (RT) during the fast relative to slow ISI condition alongside the absence of an increase of the parietal P3 amplitude. The speed of response was further significantly associated with P3 (Wiersema, et al., 2006). During the fast condition, the ADHD group made a greater number of errors of commission and displayed smaller No-Go N2 (Wiersema, et al., 2006). However, when the authors controlled for ODD/CD these differences no longer remained significant. The reduced parietal P3's along with the steeper increase in RT in the ADHD group may be explained as an inability to apply the additional effort required to alter to a possible under-activated state (Wiersema, et al., 2006). Additionally, the larger P2 amplitudes observed in the ADHD group may reflect a disturbance in early automatic processing stages (Wiersema, et al., 2006). Banaschewski and colleagues (2004) examined groups of children with either hyperkinetic disorder (HD, $n = 15$), hyperkinetic disorder with conduct disorder (HCD, $n = 16$) or oppositional defiant disorder (ODD/CD, $n = 15$) and healthy children ($n = 18$) employing a cued CPT task to investigate motor response control by ERP parameters and performance (Banaschewski, et al., 2004). Their aim was to establish

whether behavioural parameters and ERP's would provide confirmation of an inhibition-specific deficit and whether any deviations were specific for HD/HCD (Banaschewski, et al., 2004). The findings showed that children with HCD diverged most from healthy control children on motor response control processes as measured their P3a response during the Go condition, they made more errors in terms of their performance and showed slow and variable RTs (Banaschewski, et al., 2004). The HD-only group displayed greater impairment during processing of the warning stimuli in preparation of motor responses (Banaschewski, et al., 2004). There were no differences in ERP responses specific for response inhibition (Banaschewski, et al., 2004). The results suggest that ADHD cannot fully be justified by an inhibition-specific deficit and allude to impairments also in response execution processes (Banaschewski, et al., 2004). In addition, the results implied that children with comorbid disorders suffer from a reduced ability to control their prepared motor responses, difficulty in switching attention timely and monitoring sensory input alongside the monitoring of their own actions and responses (Banaschewski, et al., 2004).

Continuous Performance Task (CPT)

Tests of continuous performance (e.g., CPT tasks which measure sustained attention) or oddball tasks, in which infrequently occurring targets have to be detected amongst frequently appearing non-targets, have shown that relative to normal controls, participants with ADHD exhibit smaller P3b components in response to targets (and also sometimes in response to non-targets) in addition to poorer performance on these tasks (Jonkman, Kemner, Verbaten, Koelega, Camfferman, vd Gaag, et al., 1997; Klorman, Brumaghim, Fitzpatrick, & Borgstedt, 1991). Although, the CPT is predominantly considered a test of sustained attention/vigilance, it also loads on selective attention functions and has been used extensively to study brain function using ERPs in various clinical populations including ADHD (Kirmizi-Alsan et al., 2006). Selective responses can be measured by ERPs by comparing the *to be attended to* (or target trials) to the *to be ignored* stimuli (non-target trials) (Gomarus, et al., 2006).

The CPT paradigm employed in this PhD thesis consists of a series of letters presented one at a time (e.g., B, C, D and G). It has three epoch types: Targets (which the participant is instructed to respond to when the same letter appears twice in a row by pressing two buttons with the index finger of each hand), Backgrounds (e.g., non-target letters) and Distractors (checkerboard patterns, which the participant is instructed to ignore). The target letters are described as "1-back" (that is repetitions of the previous letter) and are presented pseudo-

randomly. Non-target stimuli demand the updating of WM, as they bear target defining information while targets are described as consecutive stimulus repeats (Keage et al., 2008a). This task loads on sustained attention and selective attention, as well as working memory updating (although the WM load is minimal). The ERP literature has reported that children with ADHD have abnormally smaller P3 ERP amplitudes relative to healthy controls in both target and non-target conditions of the CPT (Banaschewski, et al., 2004; Jonkman, Kemner, Verbaten, Koelega, Camfferman, v.d. Gaag, et al., 1997; Liotti, et al., 2007; Seifert, Scheuerpflug, Zillessen, Fallgatter, & Warnke, 2003). Although this is dependent on the task paradigm, whether it is a standard event related CPT measuring sustained attention or an inverted CPT that measures inhibitory processes.

In ADHD, a common finding is impaired target processing, as exhibited by attenuated P3 amplitudes in response to targets (Loiselle, Stamm, Maitinsky, & Whipple, 1980; Verbaten et al., 1994). However, when the accuracy of detection is impaired, as is often the case in ADHD, and undetected targets are incorporated in the ERP, it has been postulated that the reduced P3 amplitudes may simply imitate the increased amount of target misses (with reduced P3s) as opposed to alterations in the detection process itself (van Leeuwen, Steinhausen, Overtoom, Pascual-Marqui, van't Klooster, et al., 1998). In simple CPTs effects on P3 during target processing may be confounded by effects on preparatory processing including orienting and response preparation (van Leeuwen, Steinhausen, Overtoom, Pascual-Marqui, van't Klooster, et al., 1998).

The non-target condition of the CPT (i.e., background letters) elicits specific ERP indices including N1 (involved in early discrimination), P150 (involved in the selection of material), and P3 (associated with working memory updating) (Clarke, Barry, McCarthy, & Selikowitz, 1998; Friedman, 1990; Keage, et al., 2008a; Vogel & Luck, 2000). In ADHD, after the presentation of stimuli that do not necessitate updating, both the N1 and P150 deflections have been found to be delayed over frontal scalp regions (Karayanidis et al., 2000; Keage, et al., 2008a).

Performance measures can also be acquired from the CPT including errors of commission (also known as false positives) and are thought to index impulsivity when executed speedily after the presentation of a stimulus and when they are anti-correlated with RT, suggesting a speed accuracy trade-off favouring speed (Alexander et al., 2008). However, they are thought to be a marker of inattention when delayed relative to stimulus onset and when they are associated with

overall slower RT (Alexander, et al., 2008; Barkley, 1997; Halperin, Wolf, Greenblatt, & Young, 1991). Of note, in the CPT only the background condition (i.e., non-targets) can draw out false positive responses (Alexander, et al., 2008).

Face (Affect) Processing

Human faces are one of the most significant visual stimuli to mankind in terms of our social interactions (Taylor, Batty, et al., 2004). Seminal research by Halgren and Marinkovic (1994) proposed an ERP model for the appraisal and response to emotional stimuli. In this model, there are 2 stages, "orienting" and "event integration" in distinguishing conscious and non-conscious emotion perception. *Orienting* can be described as the automatic interruption of ongoing processing so as to direct attention towards a new and considerably hostile event in order to mobilise cognitive and behavioural resources for flight or fight action (Halgren, Baudena, Heit, Clarke, Marinkovic, Chauvel, et al., 1994; Liddell, Williams, Rathjen, Shevrin, & Gordon, 2004). The orienting response is considered to be free from conscious consideration and is captured by the N2/P3a/slow wave complex peaking around 200, 280 and 350 ms post-stimulus onset (Halgren, Baudena, Heit, Clarke, Marinkovic, Chauvel, et al., 1994; Kenemans, Verbaten, Melis, & Slangen, 1992; Liddell, et al., 2004). A collection of evidence has demonstrated that the N2 is altered by emotional expressions and in particular face stimuli (Bentin, et al., 1996; Halgren, Baudena, Heit, Clarke, Marinkovic, Chauvel, et al., 1994; Halgren, Baudena, Heit, Clarke, Marinkovic, & Clarke, 1994; Liddell, et al., 2004; Sokolov & Boucsein, 2000). The P3a has been linked with the automatic features of the orienting response which are implicated in the perception of threatening and/or novel stimuli (Friedman, et al., 2001a; Johnston, Miller, & Burleson, 1986; Lagopoulos et al., 1998). The *event integration* aspect of the model is characterised by the N4/P3b and this stage of the time sequence (circa 430-600 milliseconds post-stimulus) is involved in the cognitive integration to generate conscious emotional experience (Liddell, et al., 2004). The N4 deflection is considered to be a marker of semantic processing (Kiefer & Spitzer, 2000; Liddell, et al., 2004). The P3b is generated in the response to the careful registration of the stimulus, created by the early orienting response and followed by the succeeding updating of the stimulus framework (Halgren, Baudena, Heit, Clarke, Marinkovic, Chauvel, et al., 1994; Liddell, et al., 2004). In contrast to the fairly sizeable adult ERP literature (and much smaller developmental literature in healthy

populations) in face processing, the neurophysiological correlates of emotional dysfunction in children/adolescents with ADHD is virtually unexplored. The following section will review only the most relevant face processing literature which has published using the same task paradigm as this PhD study.

Williams and colleagues (2006) used an ERP face recognition task to demonstrate that signals of potential threat as depicted in facial expression of fear are given precedence over neutral and positive (e.g., happy faces) signals (Williams, et al., 2006). In their study involving 219 healthy male participants the temporal sequence and source localisation of ERPs in response to facial expressions of fear (negative) and happiness (positive) were investigated in contrast to neutral faces (Williams, et al., 2006). The findings confirmed that fearful faces were persistently notable by increased positivity, linked with an active shift from temporal, frontal regions (first 120 ms) to more dispersed cortical sources (120 -220 ms) and again back to the medial fronto-centro region (220 – 450 ms) (Williams, et al., 2006). Faces depicting happiness, by contrast, elicited a separate increased wave of negativity, observed at a later time point of approximately 230-350 milliseconds, localised to the fusiform area of the temporal cortex (Williams, et al., 2006). Both fear and happiness generated enhanced right hemisphere activity and modulated the N170 component which is turn is linked to face processing (Williams, et al., 2006). The results indicate that fear signals seem to be given priority in neuronal processing schemes, over positive signals which may be held back until alertness for possible danger is resolved (Williams, et al., 2006). Furthermore, while fear may be processed by the use of parallel pathways instigated ahead of structural encoding, neural systems involved in the processing of positively valenced stimuli (e.g., happy faces) may be more localised and depend on structural encoding (Williams, et al., 2006).

ERPs have also been employed to investigate the time course of neural responses of perception of fear relative to neutral between conscious (overt) and non-conscious (covert) face processing in 20 healthy volunteers. Non-conscious fear perception (both discrimination and detection) elicited greater N2 responses at fronto-central sites (Fz, Cz), earlier P1 response (e.g., within 100 ms post-stimulus onset relative to neutral) and by contrast a more prominent N4 in relation to conscious perception (Williams et al., 2004). N2 may provide a temporal correlate of the early sensory processing of prominent facial configurations whereas the N4 may index the

conscious amalgamation of emotion stimuli in WM, subserved by larger cortical activity (Liddell, et al., 2004; Williams, et al., 2004).

Liddell and colleagues examined the sequential unconnected processing of fear perception during both subliminal (unconscious) and supraliminal (conscious) conditions using ERPs in 20 healthy adult participants (Liddell, et al., 2004). In the subliminal condition, N2 deflections to fearful faces were increased compared to neutral faces; a finding thought to reflect the orienting stage and automatic features of face processing (Liddell, et al., 2004). Conversely, P3b, which is associated in the merging of emotional features, was found to be enhanced to supraliminal perception of fear (Liddell, et al., 2004). The findings support the notion that systems for evaluating threat related signals may be instigated involuntarily and without the necessity for conscious recognition of these signals (Liddell, et al., 2004).

The neural basis of dysfunctional emotion processing in children and adults with ADHD remains relatively under researched (Gow, Matsudaira, Taylor, Rubia, Crawford, Ghebremeskel, Ibrahimovic, Vallée-Tourangeau, et al., 2009; Ibanez et al., 2011; Williams, Hermens, et al., 2008). Differences in emotion related ERP responses have been reported in adolescents with ADHD (n = 51) and typically developing controls (n = 51) (Williams, Hermens, et al., 2008). These differences were characterised by higher self-rated scores of both depression and anxiety as rated by the Depression, Anxiety and Stress scales (DASS), difficulties correctly identifying threat related faces (e.g., anger and fear) alongside differences in ERPs marked by a clear reduction in early stages of processing over occipital activity (circa 120 milliseconds), proceeded by an amplification of activity associated with structural encoding (120-220 ms), followed by a decrease and general slowing of activity over temporal areas known to sub-serving context processing (300-400 ms) (Williams, Hermens, et al., 2008). The ADHD patients displayed reductions in the P120 waves and increased N170 deflections to facial expressions (Williams, Hermens, et al., 2008). The abnormal, emotion related N170 found in the adolescent cohort with ADHD may be attributable to impaired ability in the face processing stage (Williams, Hermens, et al., 2008)

Other studies have also reported abnormalities in electrophysiological markers of emotion processing, for example Ibanez and colleagues (2011) found deficits in N170 emotion modulation in ADHD patients relative to controls, with reductions in N170 for positive stimuli in the ADHD group supporting the earlier work of Williams et al., (2008) who also showed a

dysfunction in the N170 in ADHD (Herrmann, Biehl, Jacob, & Deckert, 2010; Ibanez, et al., 2011). Hermann and colleagues investigated ERP responses to positive (i.e., happy faces), negative (i.e., fear, anger and sad faces) and neutral pictures in adults with and without ADHD (Herrmann, et al., 2010). The results demonstrated less reactivity to happy faces in ADHD relative to controls supporting the dysfunctional motivational-reward system theory in ADHD (Herrmann et al., 2009; Sonuga-Barke, 2003, 2005).

Essential Fatty Acids and EEG/ERPs

There are very few publications exploring the relationship between PUFA and assessments of brain function and thus the literature to date is extremely limited (Fontani, Corradeschi, Felici, Alfatti, Migliorini, et al., 2005; Gow, Matsudaira, Taylor, Rubia, Crawford, Ghebremeskel, Ibrahimovic, Vallée-Tourangeau, et al., 2009; McNamara, Able, et al., 2010; Sumich et al., 2009). Gow and colleagues (2009) reported for the first time the relationship between a cognitive bias ERP measurement and PUFA fractions in the blood samples of adolescent boys with ADHD (Gow, Matsudaira, Taylor, Rubia, Crawford, Ghebremeskel, Ibrahimovic, Vallee-Tourangeau, et al., 2009). ERP responses to the presentation of happy, sad and fearful faces were recorded and a blood sample taken to measure specific indices of PUFA. The findings demonstrated a positive association between EPA and a cognitive bias in orientation to overt expressions of happiness relative to both sad and fearful faces as indexed by midline frontal P3 amplitude. Furthermore, exploratory analyses reported a significant positive correlation between DHA and the right temporal N170 response to fear. The right temporal N170 response was also negatively correlated AA/DHA ratio to concealed expressions of fear. The results suggest that both EPA and DHA may be implicated in different features of affect processing in ADHD and provide insights into the current, inconsistent literature on PUFA intervention in ADHD and depression (Gow, Matsudaira, Taylor, Rubia, Crawford, Ghebremeskel, Ibrahimovic, Vallee-Tourangeau, et al., 2009).

A study by Boucher and colleagues (2011) examined the potential positive outcomes of prenatal omega-3 fatty acid intake to memory in a fish eating group of 154 Inuit children with a mean age of 11.3 years. The study had a prospective, longitudinal design and employed neurophysiologic (event related potential measures of a continuous visual recognition task) and neurobehavioral measures of memory (as measured by the Wechsler Intelligence Scales for Children, 4th edition and the California Learning Test Children's version (Boucher et al., 2011).

The results confirmed that children with higher concentrations of DHA as measured in cord plasma displayed a shorter N4 ERP latency deflection and larger late positive component (LPC). Both the N4 and LPC are thought to be involved in recognition memory processes. At the testing time, elevated DHA measures were related to enhanced N4 amplitude (Boucher, et al., 2011). Positive associations were also observed between cord DHA and behavioural performance of memory (Boucher, et al., 2011).

Fontani, Corradeschi, Felici, Alfatti, Migliorini and Lodi (2005) investigated whether omega-3 supplementation would have positive outcomes on cognitive and physiological parameters in healthy adults (Fontani, Corradeschi, Felici, Alfatti, Migliorini, et al., 2005). The results found a reduction in RT, i.e., faster performance following supplementation with omega-3 compared to placebo during both Go/NoGo and sustained attention tasks, along with a shift towards theta and alpha in the active group (i.e., those receiving omega-3 active capsules) (Fontani, Corradeschi, Felici, Alfatti, Migliorini, et al., 2005). Supplementation with omega-3 simultaneously had an effect on mood, e.g., self-rated measures of depression, anxiety and anger reduced while an increase in vigour was reported. Fontani and colleagues (2005) concluded that supplementation with omega-3 is linked to improvement of physiological and attentional functions, specifically involving complex cortical processing. However, a direct comparison between groups was not made so the results should be approached with caution (Fontani, Corradeschi, Felici, Alfatti, Migliorini, et al., 2005).

Finally, Sumich and colleagues (2009) reported for the first time differential associations between DHA and EPA with fast (alpha activity during eyes open and beta during eyes closed resting states) and slow (theta during both eyes open and eyes closed resting states) EEG activity respectively in children/adolescents with ADHD (Sumich, et al., 2009). In addition, alpha activity was found to be positively associated with performance for language fluency involving semantic memory while theta activity was negatively associated with verbal memory performance (Sumich, et al., 2009).

In consideration of the limited published literature in ADHD, LC-PUFA and EEG/ERPs this PhD study also examined the relationships between LC-PUFA and specific ERP components. The components (P2, N2, P3a, P3b, N4) in this study were selected as the most relevant, based on existing research documenting their purported involvement in tasks of motor response and interference inhibition, sustained and selective attention, and emotion processing.

The literature review provides categorical evidence for the involvement of the N2 (especially the frontal N2 in response to NoGo stimuli) and P3 in motor response and interference inhibition (as measured by the Go/NoGo, Stop signal and CPT-A-X tasks), and the P3 also in processes of WM, stimulus evaluation, contextual updating and effortful attention. In ADHD, both N2 and P3 deflections have been found to be significantly reduced compared to typically developing control children. Finally, there is some evidence, although limited, to suggest that omega-3 fatty acids are involved during cognitive and emotion processes. In conclusion, ADHD patients have shown abnormal ERP's in particular in frontal and parietal scalp regions during both Go/NoGo and tasks measuring sustained and selective attention. This is demonstrated in the main by reductions in N2 and P3 thought to be attributable to impaired inhibitory process and sub-optimal energetic state regulation respectively. During face processing tasks, the N170 is thought to reflect the orienting response in contrast to N2/P3a deflections which are thought to capture conscious emotion processing. There is some evidence to date implicating that fear is given precedence in neuronal emotion processing systems over neutral and happy faces in healthy participant groups. Emotion dysfunction in children/adolescents with ADHD is widely under-represented in the ERP literature and further research is needed. The relationship between PUFA and ERPs during cognitive and emotion processing tasks are not well documented. However, there is some suggestion that supplementation of omega-3 can improve physiological and attentional functions in healthy adults. To date, no-one has investigated relationships between LC-PUFAs and ERPs in children/adolescents with and without ADHD and therefore this PhD provides a novel contribution to the existing gap in the literature.

Chapter 6. Study Aims and Hypotheses

This aim of this PhD study was an investigation into LC-PUFA, event related potential assessments of brain function and behavioural measures in children and adolescents with and without ADHD. The first part of the study aimed to investigate whether differences existed between PUFA levels in blood samples of children/adolescents with and without ADHD. Furthermore, a battery of nine clinical questionnaires were employed to establish (1) differences in behaviour and ADHD symptoms and (2) whether the between group differences were associated with specific fatty acid indices. The second part of the study involved recording the neuronal electrical activity of children and adolescents with ADHD and healthy matched controls to test whether their corresponding event related potentials differed in magnitude and the relationship between those ERPs and key fatty acid indices. The ERPs tasks were chosen specifically as a representational measure of two executive function processes and one emotion processing function. The ERP tasks included (1) a task of motor and conflict response inhibition: the Go/NoGo task; (2) a test of selective/sustained attention: the continuous performance task (CPT), and (3) an emotion processing task.

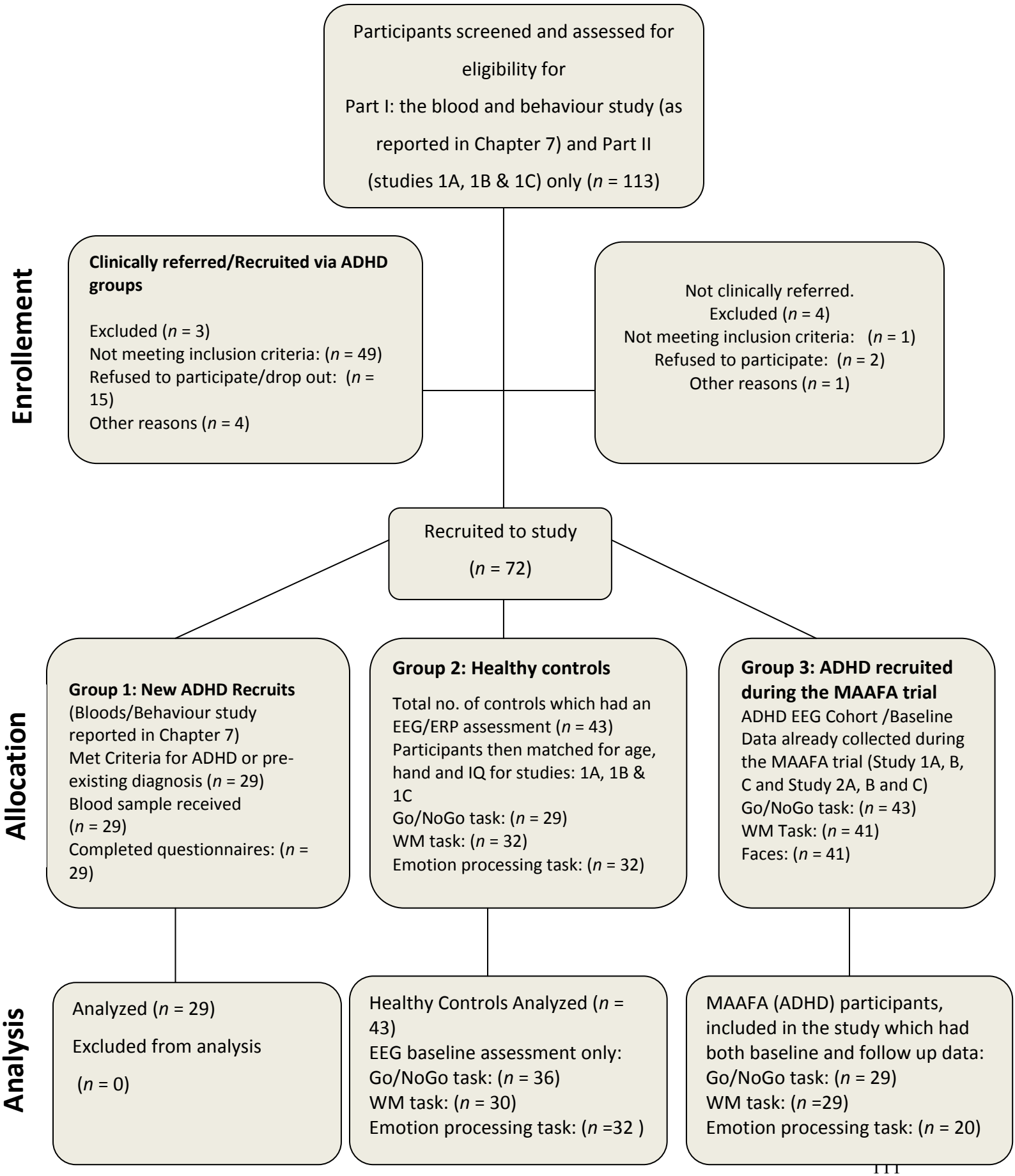


Figure 1. Participant Flow Chart illustrating recruitment into Part I (Behaviour and Blood Study), Part II (Studies 1A, 1B & 1C) and Part III (Studies 2A, 2B & 2C) of the PhD project

Questions and Hypotheses

Analyses regarding each study are documented in their respective Chapters (7, 8, 9, 10 & 11) and also in the Method section of this chapter. The main research questions for this PhD study are presented below:

Study 1A (see Chapter 7): Blood levels of LC-PUFA and behavioural questionnaires/clinical data in ADHD and healthy matched controls

1. Based on the findings of previous research, it was expected that children/adolescents with ADHD would have significantly lower levels of omega-3 fatty acids in plasma measures compared to healthy control (HC) children. Specifically, it was hypothesised that the ADHD group would have lower levels of the omega-3 series: ALA, DHA, DPA and EPA
2. There would be significant differences in scores of behaviour between ADHD and HC children across the battery of questionnaires, with ADHD children/adolescents scoring significantly higher than control children in measures of ADHD symptom severity and comorbid behaviours.
3. It was hypothesised that lower levels of PUFA would be associated with greater symptom severity of ADHD, as well as associated symptoms of aggression, depression/anxiety, impulsive and conduct-disorder traits, while higher levels of omega-3 fatty acids will be related to lower symptom severity. It also examined whether negative relationships existed between self rated scores of fatty acid deficiency and PUFA levels. Finally, exploratory analyses also sought to discover whether IQ scores would be significantly correlated with PUFA levels.

EEG/ERP studies (Chapters 8, 9, 10: Studies 1A, 1B & 1C)

Study 1A (Go/NoGo)

1. The aim of this study was to investigate neurophysiological correlates of prefrontal lobe mediated response control in children and adolescents with

ADHD. The key question was whether ADHD children differed from healthy controls in performance and in relation to the magnitude of electrical activity in the N2 and P3 AUC amplitude responses across the frontal-central and parietal regions. In line with previous findings, it was hypothesised that ERP differences will be characterised by smaller N2 and P3 in ADHD compared to healthy control children. Furthermore, based on previous research, it was predicted that children with ADHD make more errors of omission and commission compared to control children. Importantly, since the main interest of this thesis was the association between ERP assessments of brain function in ADHD and fatty acids, further analyses explored the relationship between fatty acid status in plasma choline phosphoglycerides in children and adolescents with and without ADHD and their ERP response. There were no previous studies in which to base firm a priori hypotheses but in consideration of related omega-3 research in impulse control and attention processes, it was hypothesised that P3 amplitude responses to Go stimuli would be significantly positively associated with omega-3 fatty acids indices in both ADHD and healthy control children. Finally, it was predicted that omega-3 fatty acids would be negatively correlated with both errors of omission and commission, i.e., the lower the omega-3 the greater the number of errors reflecting both executive and inhibitory processes respectively.

Study 1B (CPT)

2. This study sought to investigate potential differences in neural activation during task performance during a sustained attention task as measured by P3 amplitude responses in ADHD compared to controls. In consideration of previous research, it was predicted that differences will be characterised specifically by smaller mean AUC P3a and P3b amplitudes response in ADHD compared to control children in frontal-central and parietal sites which are in turn implicated in cognitive processes associated with sustained attention during both target and non-target conditions of the CPT. Furthermore, in line with previous research suggesting omega-3 are implicated in attention processes, it was predicted that PUFA status (as measured in terms of % of omega 3/6) will be positively correlated with brain function as indexed by the P3 family in ERP responses

during the CPT in both cases and controls. The early and late P3 family were chosen on the basis that they are the main waves associated in attention processes and furthermore have been found to be abnormal in ADHD. The final predication in relation to the performance data predicted negative associations between omega-3 fatty acids levels in the ADHD group and errors of commission and omission, that is, the lower the omega-3 – the higher the number of errors. In the healthy control group, negative relationships were anticipated, that is, the higher the omega-3 – the lower the number of commission and omission errors.

Study 1C (Emotion Processing task)

3. It was hypothesised that there would be differences in neuronal activation between cases and controls and furthermore that these differences would be characterised by less reactivity to emotional expressions of happy faces as indexed by P3 and N2 AUC amplitudes in the ADHD group and enhanced reactivity to fear in the healthy control group across frontal-central and parietal scalp regions. Furthermore, it was hypothesised that the healthy control group would demonstrate enhanced responses for the late N4/P3b inhibitory component. In relation, to the relationship between PUFA and ERP responses to facial expressions of emotion, it was predicted that (1) omega-3 levels would be negatively correlated with brain function as indexed by the N4/P3b complex and N4 wave to both positive and negative stimuli (i.e., facial expressions of fear, sad, happy and angry faces); (2) omega-3 PUFA, (in particular EPA) would be positively correlated with P3 responses to facial expressions of happiness (i.e., the higher the omega-3 the less the higher the response to happy faces); and finally (3) omega-6 would be positively associated with N4 responses to negative stimuli (i.e., facial expressions of fear, anger and sad faces) in both cases and controls.

MAAFA ADHD subgroup intervention analyses (Chapter 11, ERP studies 2A, 2B & 2C)

In light of the collective evidence documenting the potential benefit of omega-3/6 supplementation in ADHD, this final study sought to test whether 12 weeks of omega-3/6 supplementation would significantly enhance the performance (Studies 2A and 2B only) and neuronal activity of children as measured by ERP's with ADHD during the same three ERP tasks

reported in Studies 1A, 1B and 1C, measuring sustained attention, inhibition and emotion processing. The data reported in these three studies (2A, 2B and 2C) are from a subgroup of children with ADHD who took part in a larger randomised, placebo-controlled, double-blind, clinical trial (MAAFA) led by our research group. In all studies (2A, B, C), activation was assessed at baseline (0 months prior to supplementation) and then again at follow up (after 12 weeks of active or placebo supplementation).

1. The first prediction in relation to Study 2A was that ERP assessments of brain function would significantly differ between active and placebo groups following 12 weeks of supplementation in children/adolescents with ADHD. The hypothesis was based on the notion that omega-3 has a modulating role in impulse control in ADHD (Freeman, et al., 2006), along with the reported increase in P3 amplitudes in the Fontani study (2005) following supplementation in healthy individuals. It was therefore predicted differences would be indexed by increases in activation in both P3 and N2 responses during the Go/NoGo task. It was further predicted that the performance data (commission and omission errors) would be significantly negatively correlated with omega-3 in the active group compared to placebo at the follow up assessments. In other words, as omega-3 increased over 12 weeks, both commission and omission errors will decrease.

2. The second objective in relation to Study 2B was to ascertain whether a marker of attention, the P3, would significantly differ in the omega-3/6 supplementation group relative to placebo during the sustained attention task. The hypothesis was based on animal and human studies suggesting the omega-3 fatty acids have a modulating effect in cognition (Drover, Hoffman, Castaneda, Morale, & Birch, 2009; Hashimoto et al., 2011) and in particular sustained attention processes (McNamara, Able, et al., 2010). More specifically, it was predicted that an increase in P3 (which is an index of attention) would be present in the active group compared to placebo following 12 weeks of supplementation. As per the Go/NoGo task, it was further predicted that the performance data (commission and omission errors) would be significantly negatively correlated with omega-3 in the active group compared to placebo at the follow up

assessments. In other words, as omega-3 increased over 12 weeks in the active supplementation group, both commission and omission errors would decrease.

3. The final aim sought to test whether there would be significant differences in ERP amplitude responses to positive (i.e., happy faces) and negative (i.e., sad and fearful faces only) facial stimuli. This was based on previous research by our team which found an association between P3 amplitude responses and EPA to happy faces (Gow et al., 2009) alongside collective research showing that omega-3 may have a modulating effect in emotion processes including mood (Freeman, et al., 2006). It was predicted that these differences would be characterised by greater activation in N2 and N4 responses in the active group compared to the placebo group in children/adolescents with ADHD during an emotion processing task.

Overall dependent variables of interest include:

- Event related potential (ERPs) data previously collected in the context of a randomised, placebo-controlled, double-blind, clinical trial (MAAFA trial) in adolescents with ADHD during performance on three EEG/ERP tasks. In relation to the ERP measures collected during the MAAFA trial, assessments were carried out at baseline (i.e., prior to the supplementation of PUFAs or placebo) and then again at follow up (i.e., 3 months after supplementation of placebo or active omega-3/6 capsules)
- ERPs in healthy control children and adolescents across the same tasks at baseline only (the healthy controls were not supplemented with PUFAs or placebo).
- Blood test results of PUFA status in both participant groups (i.e., ADHD and healthy controls).
- Clinical symptoms as per self reports on ADHD behaviour including depression and anxiety, aggression, impulsiveness and conduct problems.

Summary and Rationale

These analyses represent a novel contribution to the field of fatty acids in relation to child and adolescent ADHD groups. For example, to date, nothing is known about the relationship between event-related brain potentials and fatty acids in children and adolescents with ADHD or healthy age matched controls. Previous research has left many unanswered questions concerning potential deficiencies and/or problems with the metabolism of PUFAs in ADHD. This PhD study aimed to provide indications into PUFA blood profiles of children/adolescents with ADHD, consequently establishing whether differences exist between groups (ADHD versus controls). The collective findings also aimed to provide insights into the relationship between PUFAs and cognitive and emotional function in ADHD and typically developing children as measured by ERPs.

Chapter Seven: Blood levels of LC-PUFA and Behavioural Questionnaires/Clinical Data in ADHD and Healthy Matched Controls

Introduction

ADHD is not a homogenous condition but a complex, multi-faceted, behavioural syndrome with over-lapping symptoms. Many aspects of a child's life can be negatively impacted by ADHD and impairments can be found across societal, familial, educational, occupational and health-care domains. The co-occurrence of additional types of psychopathology is very common because ADHD presents with great heterogeneity (Taylor, Dopfner, et al., 2004). Thus, symptoms of ADHD often overlap with symptoms of related disorders such as conduct disorder (CD) and oppositional defiant disorders (ODD), mood and emotional disorders (e.g., mood or pediatric bi-polar disorder), dyslexia (reading and writing difficulties), and motor-coordination disorder (also known as dyspraxia). For example, 25% of ADHD children meet the diagnostic criteria for anxiety disorders (Reiff & Stein, 2004; Ryan-Krause, 2010a). Emotional problems also frequently co-exist in ADHD although much less is known about the reasons for this. It is however postulated that these stem as a result of academic failures and difficulties in interpersonal relationships and may hence be secondary to the primary disorder (Taylor, Dopfner, et al., 2004).

It is well established that LC-PUFA's are vital for the optimal development of both the brain and retina (Eilander, et al., 2007; Innis, 2008; Lauritzen, et al., 2001; Ryan, et al., 2010). The dry weight of an adult brain comprises between 50-60% lipid, and of the lipid content at least 35% is made up of PUFAs (Haag, 2003; Innis, 2008). DHA and AA are the main PUFAs in neuronal membranes with DHA comprising at least 40% of the total PUFAs in the brain and 60% of the photoreceptor cell (Innis, 2007, 2008) . DHA alone makes up approximately 30% of the ethanolamine and serine phosphoglycerides of neuronally-enriched brain tissue (gray matter) (Brenna & Diau, 2007a; Diau, et al., 2005). Within brain tissues DHA preferentially accumulates in growth cones, astrocytes, synaptosomes, myelin, microsomal and mitochrondrial membranes (Bourre et al., 1992; Jones, Arai, & Rapoport, 1997). The balance of omega-3 and 6 fatty acids also impacts various features of serotonergic and catecholaminergic neurotransmission (Chalon, 2009; McNamara & Carlson, 2006a; McNamara, Jandacek, et al., 2010; Yavin, et al., 2009). EPA and DHA are associated with many important functions related to neural activity such as

cell membrane fluidity, neurotransmission, ion channel, enzyme regulation, gene expression and myelination (Lauritzen, et al., 2001; Sinn & Howe, 2008). The action of the phospholipases on membranes releases the fatty acids with varying consequences (Haag, 2003). For example, the omega-6: DGLA, AA and the omega-3, EPA play a role in the production of eicosanoids which in turn have pro- and anti-inflammatory, anti-thrombotic and vasodilatory properties (Haag, 2003). High levels of fatty acids lead to an increase in the fluidity of membranes which in turn increases the transport of serotonin into the endothelial cells (Block & Edwards, 1987; Freeman, et al., 2006; Hibbeln, et al., 1998).

Inadequate amounts of DHA *in-utero* are associated with impaired learning and attention in addition to emotional irregularities such as increased depression, anxiety and aggression in animal models (Fedorova & Salem, 2006; Mathieu, et al., 2008). There is some evidence implicating abnormalities in fatty acid blood profiles in children and adults with ADHD (Antalis, et al., 2006; Chen, et al., 2004; Colter, et al., 2008; Germano, et al., 2007; Mitchell, et al., 1987; Mitchell, et al., 1983). However, the exact nature of this association is not yet clear and further research is necessary to establish whether differences in blood levels between ADHD and controls are due to a deficiency in PUFAs, or an abnormality in metabolism. Supplementation trials with omega-3/6 fatty acids have reported some improvement in ADHD symptomology as measured by Parent / Teacher Conner's Rating scales or the Clinical Global Impression (CGI) scales (Johnson, et al., 2009; Richardson, 2004; Richardson & Montgomery, 2005; Richardson & Puri, 2002; Sinn & Bryan, 2007). In addition, low levels of omega-3 fatty acids may be associated with behavioural problems that are commonly related with ADHD and poor serotonergic function, such as aggression and depression (Edwards, et al., 1998; Gesch, et al., 2002; Logan, 2003; Nemets, et al., 2006; Osher, et al., 2006; Peet, et al., 1998; Sontrop & Campbell, 2006). In relation to the literature in ADHD and omega-3/6 fatty acids, a recent meta-analysis involving 10 trials and 699 children was conducted by Bloch and Qawasmi (2011). The results showed that omega-3 supplementation had a small but significant effect in improving ADHD symptomology. Specifically, it was found that higher doses of the omega-3 fatty acid, EPA, were significantly modestly correlated with supplement efficacy in the treatment of ADHD (Bloch & Qawasmi, 2011). The relative efficacy of omega-3 fatty acid supplementation was modest in contrast to currently available pharmacological interventions, e.g., psycho-stimulant medication for ADHD. However, taking into consideration its relatively mild side-effect profile

and evidence of modest efficacy, it may be rational to use omega-3 fatty acids to supplement traditional pharmacologic interventions or for children who resist or are resistant to psychopharmacologic options (Bloch & Qawasmi, 2011). Other studies also lend support to the potential beneficial effects of EPA in alleviating symptoms of low mood and depression (Bloch & Qawasmi, 2011; Freeman, et al., 2006; Jazayeri, et al., 2008; Peet & Horrobin, 2002b).

In healthy populations, lower levels of DHA have been linked to higher levels of cognitive impulsivity whereas inverse relationships have been reported between omega-6, ALA, and both motor and total impulsivity scores measures using the Barratt Impulsiveness Scale (BIS) (Conklin, Harris, et al., 2007). In addition, the same study reported inverse relationships between both omega-3 DHA and EPA and scores of neuroticism as measured by the NEO Five Factor Personality Inventory whereas DHA was significantly positively associated with measures of agreeableness using the same personality scale (Conklin, Harris, et al., 2007). Furthermore, relationships have also been observed between behavioural symptoms of ADHD as measured by the long version of the Conner's Parent Rating scales (CPRS) and blood levels of omega-3/6 fatty acids (Colter, et al., 2008). Specifically, DHA was significantly negatively associated with subscales measuring cognitive problems, restlessness, oppositional behaviour, problematic behaviour, hyperactivity, DSM-IV inattention and DSM-IV total. Negative associations were also observed between Total omega-3 and restlessness subscale scores. The omega-3/omega-6 ratio was inversely associated with restlessness, oppositional, and problematic behaviour scales and total omega-6 positively associated with problematic behaviour, oppositional, restlessness, DSM-IV total, ADHD index and DSM-IV inattentive subscales (Colter, et al., 2008). Although, the latter study had a small sample sizes (n = 23) and both studies in relation to the statistical analyses do not appear to have corrected for multiple testing.

To date, there are no publications in the literature employing an extensive battery of clinical behavioural questionnaires such as the one employed in this study to assess behavioural symptoms between LC-PUFA levels and ADHD symptoms alongside typically comorbid symptoms. In the main, previous studies such as those by Richardson and Montgomery (2005) and Sinn and Bryan (2007) have restricted their investigations of the association between LC-PUFA supplementation and clinical behaviour in children with ADHD and/or motor coordination difficulties by employing the Conner's Teacher and/or Parent rating scales as a primary outcome to examine post-supplementation differences compared to placebo. In a similar

fashion, Johnson and colleagues (2009) used the Parent version of the Conner's scale along with the CGI severity scale as their primary outcome to investigate whether scores on these scales would improve post-supplementation with omga-3/6 fatty acids. In all three of these studies blood samples were not obtained nor were the findings compared to a typically developing control group. Although some researchers such as Conklin et al (2007) and Colter et al (2008) have employed a personality measure along with Conner's scales and a measure of impulsivity, there were some noticeable limitations to both of these studies, namely a small sample size and neither study controlled for multiple testing implicating that their results could be due to Type 1 error. This is therefore first study to investigate the relationship between blood levels of LC-PUFA and clinical behaviour in both ADHD and healthy matched controls employing an extensive battery of clinical tests.

The aim of this part of the project was to assess LC-PUFA blood profiles of children/adolescents with ADHD as well as in healthy controls, consequently determining differences between cases and controls. The second part of the study aimed to assess the relationship between scores from an extensive battery of ADHD-related behavioural questionnaires and blood levels of PUFA within each group (ADHD and healthy controls) with the aim of providing an indication of the relationship of ADHD-associated behaviours and to essential fatty acids. For this purpose questionnaires directly assessing ADHD and impulsive behaviours were administered as well as behavioural questionnaires tapping into typical comorbid behaviours such as questionnaires of conduct problems, psychopathy, mood, anxiety, depression, anger and self-concept.

It was hypothesised that lower levels of PUFA would be associated with greater symptom severity of ADHD, as well as associated symptoms of aggression, depression/anxiety, impulsive and conduct-disorder traits, while higher levels of omega-3 fatty acids would be related to lower symptom severity. It also examined whether negative relationships existed between self rated scores of fatty acid deficiency and PUFA levels. Finally, exploratory analyses also sought to discover whether IQ scores were significantly correlated with PUFA levels.

Method

Eligibility Criteria

All children/adolescents were screened and interviewed for eligibility. Screening for ADHD included the completion of the Parent and Teacher Conners' Rating Scales (unless they had a pre-existing clinical diagnosis of ADHD and then in some instances Parent scores only were collected) and resulting scores of equal to or above 65. The children with ADHD had a standardised interview based on DSM-IV criteria to establish that ADHD criteria were met (ChIPS see Rooney, Fristad, Weller & Weller, 1999). The Kaufman Brief Intelligent test (K-BIT see Kaufman & Kaufman, 1993) ensured an IQ quota of above 70 was met.

The healthy controls were recruited from the London area. Clinical data/Behavioural questionnaires including the Beck's Youth Inventories (2nd edition: BYI-II; (Beck, Beck, Jolly & Steer, 2005), Buss-Perry Aggression Scale (Buss & Perry, 1992), Depression, Anxiety and Stress Scales (DASS) (Lovibond & Lovibond, 1995), Barratt Impulsivity Scale (BIS) (Patton, Stanford, & Barratt, 1995), Strengths and Difficulties Questionnaire (SDQ), Essential Fatty Acid Deficiency Questionnaire (EFADQ) (developed by Matsudaira, 2009 as part of her PhD project) was completed by all children and the scores assessed in respect of their essential fatty acid levels. The ADHD group alone additionally completed the following assessments: The Anti Social Screening Device (APSD) (Frick & Hare, 2001) and the Inventory of Callous Unemotional traits (ICU) for conduct disorder for Youth (Frick, 2004)

Procedure & Materials

Participants

Behavioural/clinical questionnaire data and blood samples were collected from 72 male children/adolescents split into 2 groups of participants: children and adolescents who either had a pre-existing clinical diagnosis of ADHD (n = 16) or met our research criteria for an ADHD diagnosis (n = 13) (see methods section for eligibility criteria) and healthy, age and sex matched, control children (n = 43) predominantly from the London area. Briefly, all participants were assessed to ensure they met criteria for ADHD (or did not if they were a healthy control) according to the DSM-IV. This included a diagnostic interview using the Children's Interview of Psychiatric Syndromes (ChIPS) and scores of the Conner's Parent and Teacher Rating Scale ADHD index t score which had to collectively exceed 65. The ChIPS interview determined which subtype of ADHD the participants had and this is illustrated in Figure 1. Seventeen out of 29 children were not taking medication for ADHD (55.2% unmedicated versus 44.8% medicated). Those that were taking stimulant medication were asked to take their dose at breakfast after the blood sample had been taken. Children were not recruited if they had a diagnosis of autism, learning disorders or serious mental health condition. Children were also not recruited if they had taken omega-3/6 supplements in the previous 6 months. None of the children in this study had received a formal diagnosis of any other comorbid disorder. It was originally planned to re-recruit / invite the ADHD participants that took part in the MAAFA trial. However this proved relatively unsuccessful for various reasons including: some of the adolescents were now over 16 and had left school, or moved address and changed contact details. Five participants from the MAAFA study who were still eligible were successfully re-recruited for this project. The rest of the ADHD participants came from local schools and adverts placed on registered ADHD charity website such as ADDISS[6]and The Studio (ADHD centre)[7].

[6] http://www.addiss.co.uk/

[7] http://www.studioadhdcentre.org.uk/

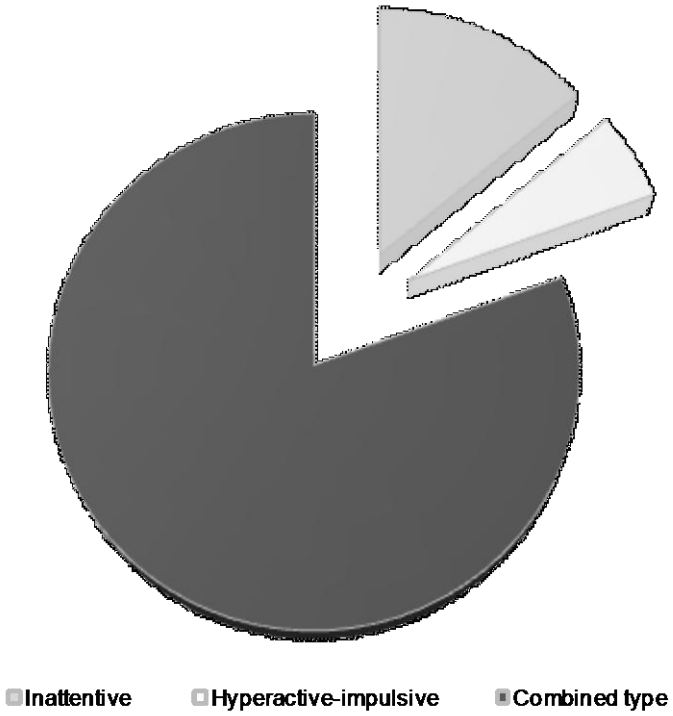


Figure 1. Diagnostic criteria according to Children's Interview of Psychiatric Syndromes (ChIPS)

Children and adolescents below the age of 16 years old were accompanied by an appropriate adult (parent or guardian) to the Institute of Psychiatry at The Maudsley Hospital where approximately 16 ml of blood was taken. Blood assessments required 8 hours fasting beforehand. The blood was taken by a qualified phlebotomist, transported by motor-cycle courier to the Science Centre at the London Metropolitan University where it was spun before storing at -80 degrees Celsius. Questionnaires were completed following a complimentary breakfast in a quiet, well-lit interview room. Each participant was fully briefed and all questionnaires were scored by hand.

Behavioural Task List

Beck's Youth Inventories (2nd edition: BYI-II)

- BYI-II - comprises of five self-report scales which assess a child's experience of depression, anxiety, anger, disruptive behaviour and self concept.
- BDI-Y - identifies depressive symptoms, reflects on negative thoughts about life, self and future includes items on sadness and physiological indicators of depression.
- BAI-Y - identifies fears in children/adolescents (e.g., fear of school, fear of getting hurt and fear of health problems). It also reflects on their worries and physiological symptoms associated with anxiety.
- BSCI-Y – examines self perceptions such as competence, potency, and positive self worth.
- BANI - assesses feelings of mistreatment, negative cognitions about others, angry feelings and physiological arousal.
- BDBI-Y – focus is on DSM-IV criteria for conduct and oppositional disorders (inc. disruptive behaviours).

Each inventory takes about 5-10 min to complete and contains 20 statements about thoughts, feelings and behaviours related with a particular emotional or social impairment. Children are required to describe the extent to which they feel the item is characteristic of them on a 4 point scale (0 = never, 1 = sometimes, 2 = often and 3 = always) (Beck, Beck, Jolly & Steer, 2005).

Buss-Perry aggression scale from carers

This aggression questionnaire, published originally in 1992 by Arnold Buss and Mark Perry, is a widely used measure assessing hostility and aggression. Twenty-nine statements ask the rater to score how uncharacteristic or characteristic the participant is using a 6 point scale. After scoring is completed, a brief report is provided and the result can be compared to both male and female averages (Buss & Perry, 1992).

Depression Anxiety Stress Scales from participants (DASS)

The DASS consists of three self-report scales which are designed to assess the negative emotional states of depression, anxiety and stress. There are 14 items on each of the three DASS scales. These are divided into subscales of 2-5 items with comparable content. The Depression scale assesses a variety of different negative emotions attached to dysphoria such as self-deprecation, lack of interest/involvement, and inactivity. The Anxiety scale measures levels of autonomic arousal, situational anxiety, and self-rated experience of anxiety. The Stress scale is sensitive to levels of chronic non-specific arousal and assesses agitations, irritability and impatience. Participants are asked to rate the extent to which they have experienced each state over the past week using a 4-point severity/frequency scales. Scores for Depression, Anxiety and Stress are established by summing the scores for each item (Lovibond & Lovibond, 1995).

Barratt Impulsive Scale

The Barratt Impulsivity Scale (BIS) is well established and one of the most widely used self-administered impulsivity tests. It was originally based on a unidimensional model of impulsiveness but added measures of sensation-seeking, extraversion and a lack of inhibitory behavioural controls. Further research led Barratt to categorise impulsivity into three main areas: motor control (i.e., acting without thinking), cognitive (ability to make decisions quickly), and non-planning (present orientation) (Patton, et al., 1995).

The APSD: Anti Social Screening Device for parent and teacher version

The APSD contains 3 forms teacher, parent and combined. There are 20 scaled items measuring 3 dimensions of behaviour: callous/unemotional traits (6 items); narcissism (7 items) and impulsivity (5 items). The remaining items are distributed accordingly, one adding towards the total score and the other towards the total of the parent edition. Items that are rated as “Not true at all”, “Sometimes true” and “Definitely true” form a combined score, in addition to individual scores for each of the 3 dimensions (Frick, 1988).

Frick, P. J. (1988). Callous/unemotional traits and conduct problems: A two-factor model of psychopathology in children. In D. J. Cooke, A. Forth, & R. D. Hare (Eds.), Psychopathology: Theory, research and implications for society (pp. 161-187). Dordrecht, Netherlands: Kluwer Academic Publishers.

ICU: Inventory of callous unemotional traits for conduct disorder for Youth

The Inventory of Callous-Unemotional Traits (ICU) was designed to provide a reliable, resourceful and valid evaluation of CU traits in youths. It consists of 24 items of relatively independent (uncaring/callousness and unemotional) behaviour that are rated on a four-point Likert scale from: 0 (Not at all true) to 3 (Definitely true) (Frick, Stickle, Dandreaux, Farrell, & Kimonis, 2005).

The Strengths and Difficulties Questionnaire (SDQ)

The SDQ is a short questionnaire that screens for the behaviour of 3 to 16 year olds. It comes in several versions to match the requirements of clinicians, researchers and those working in an educational setting. The SDQ consists of 25 items on various psychological attributes some of which are positive others negative. The 25 items are divided into 5 items of scales 1) emotional symptoms 2) conduct problems 3) hyperactive/inattention problems 4) peer relationship problems – these are summed together to generate a total "difficulties" score based on 20 items. The last item 5) is pro-social behaviour which also consists of 5 items. The same items are included for completion by the teachers, parents and self-completion, although the wording differs slightly, for young people (aged 11-16).

Conner's Teacher and Parent Rating Scales - Revised

Both versions (i.e., parent: CPRS and teacher: CTRS) are formulated for parents and teachers to rate the behaviour of children and adolescents aged 3-17. The subscales in the CTRS-R include: oppositional, cognitive problems/inattentive, hyperactivity, anxious shy, perfectionism and social problems. The remaining domains for ADHD symptom screening include; the Conners' ADHD index, Conners global index: emotional liability, Conners global index: total (related to symptoms of hyperactivity), DSM-IV hyperactive-impulsive and the DSM-IV total. The CPRS has the same subscales, with one additional category, 'psychosomatic'. These individual domains are established as a means for distinguishing children with ADHD from non-clinical children. They are also useful for screening children/adolescents in need of referral to a clinician (Conners, Sitarenios, Parker, & Epstein, 1998).

Results

Statistical Analyses

A series of independent-samples *t* tests were conducted to compare (i) the behavioural questionnaires scores and (ii) plasma fatty acid levels and behavioural measures between ADHD and control children. Given the large number of tests, the false discovery rate correction for multiple testing was employed for all analyses (Benjamini & Hochberg, 1995; this correction procedure is more conservative with the lower *p* values, but not as conservative as a Bonferroni correction).

Blood Analysis

Total lipids were extracted from 1 ml of red cell according to the Folch method[36]. The red cells were homogenized in chloroform and methanol (2:1 v/v) containing 0.01% butylated hydroxytoluene as an antioxidant, under nitrogen. Fatty acid methyl esters (FAMEs) were prepared by heating the extracted total lipid in 4ml of 15% acetyl chloride in methanol for 3h at 70°C, under nitrogen in a sealed vial. FAMEs were separated by a gas-lipid chromatograph (HRGC MEGA 2 series, Fisons Instruments, Italy) fitted with a capillary column (30m´0.32mm i.d., 0.25u film, BP20). Hydrogen was used as a carrier gas, and the injector, oven and detector temperatures were 235°C, 250°C and 178°C, respectively. FAMEs were identified by comparison with relative retention times of authentic standards and calculation of equivalent chain length values. Peak areas were quantified by a computer chromatography data system (EZChrom Chromatography Data System, Scientific Software Inc., San Ramon, CA, USA).

Three types of plasma were analysed: (1) plasma cholesterol esters (PCE), (2) plasma triglyceride (PTG) and (3) plasma phopshatidylcholine (PPC) and 2 types of red blood cell: Red blood cell phopshatidylcholine (RBC PC) and Red blood cell phosphatidylethanolamine (RBC PE). However, due to unforeseen contamination issues the RBC data is not reported in this thesis. Additionally, key trace elements (cooper, iron, magnesium, selenium and zinc) due to their involvement in fatty acid synthesis and absorption were examined for between-group differences.

Age and IQ

An independent samples *t* test was conducted to compare the IQ scores as measured by the Kaufman Intelligence test, second edition, between groups (ADHD and HC). There were no significant differences in verbal scores of IQ between ADHD and healthy controls, $t(70) = -1.88$, $p = .06$. There was a non-significant trend for non-verbal scores between ADHD and healthy control children, $t(69) = -1.99$, $p = .053$. There were no significant differences for overall / composite IQ scores between ADHD and healthy controls, $t(70) = -.833$, $p = .41$. The average IQ across both groups ($N = 72$) was $M = 118$, $SD = 20.53$. There were no significant differences between groups in age (ADHD: $M = 14.08$, $SD = 1.44$ versus HC: $M = 13.78$, $SD = 2.33$), $t(70) = .612$, $p = .54$. The means and standard deviations for IQ and all behavioural questionnaires are reported in Table 1 of this chapter.

Table 1. Mean differences for all behavioural questionnaire scores between ADHD and HC

	ADHD		Controls		
	M	*SD*	*M*	*SD*	
Kaufman Brief Intelligence Test					
Verbal scores	113.62	12.05	119.65	14.09	
Non-verbal scores	101.21	23.6	111.16	14.1	
Composite IQ scores	116	24.18	120.12	17.76	
Strengths and Difficulties Self-Report Scores					
Emotional Distress	3.04	2.44	2.21	1.86	
Behavioural Difficulties (Conduct Disorder)	4.30	2.45	1.85	1.80	**
Hyperactivity and Attentional Difficulties (ADHD)	6.07	2.36	3.12	2.28	**
Kind and Helpful Behaviours (ASD)	6.22	2.39	7.78	1.68	**
Diagnostic Predicitons					
Any Diagnosis	1.85	0.78	1.17	0.49	**
Emotional Disorder (Anxiety Disorder)	1.15	0.36	1.24	1.56	
Behavioural Disorder (Conduct Disorder)	1.67	0.83	1.14	0.47	**
Hyperactivity or concentration Disorder (ADHD)	1.41	0.57	1.07	0.26	**
Becks Youth Inventories (BYI-II) for children and adolescents					
BDI-Y (depressive symptoms)	52.55	8.83	47.79	6.86	**
BAI-Y (fear/anxiety)	52.97	11.10	48.07	7.54	*
BSCI-Y (anger)	54.10	11.37	44.95	7.11	**
BANI (disruptive behaviour)	60.10	13.92	48.14	12.01	**
BDBI-Y (self-concept)	45.10	11.02	50.07	8.62	*
Depression, Anxiety and Stress Scales					
Depression scale	10.13	9.91	4.86	7.46	*
Anxiety scale	8.27	8.17	4.88	5.08	
Stress scale	13.90	9.50	5.46	5.65	**
Buss-Perry Agression Questionniare					
Physical Agression	36.24	13.50	24.53	11.13	**
Verbal Agression	23.10	6.84	18.18	6.70	**
Anger	28.07	10.81	17.51	8.24	**
Hostility	26.62	11.74	21.19	7.87	*
Total Aggression score	114.41	35.64	82.37	28.72	**
Barratt Impulsiveness Scale					
Attentional Impulsiveness	20.53	4.06	15.30	3.29	**
Motor Impulsiveness	25.93	4.77	22.53	4.01	**
Non-Planning Impulsiveness	29.21	4.3	24.46	5.08	**
Total Impulsiveness Score	74.96	10.66	62.58	10.61	**

Note: * $p < .05$, ** $p < .01$

Blood data

Plasma Cholesterol Esters (PCE)

For all plasma analyses, a series of independent-samples *t* tests were conducted to compare the plasma fatty acid levels between ADHD and control children. The means and standard deviations are presented in Table 2. Given the large number of tests, the false discovery rate correction for multiple testing was employed for all analyses (Benjamini & Hochberg, 1995). None of the correlations remained significant following adjustment (those correlations which reached significance or trend level prior to correction are highlighted in bold in Table 2); the largest non-significant differences were observed between the ADHD and control groups for c22:4n6 (a metabolite of AA), $t(41) = 2.03$, $p = .048$ which was higher in the ADHD group and for c22:6n-3 (DHA), $t(69) = -2.63$, $p = .011$ which was higher in the control group.

Plasma triglyceride (PTG)

The means and standard deviations are presented in Table 2. There were no significant differences between the ADHD and control groups for any of the omega-3/6 fatty acid levels. The largest non-significant trend level differences were observed for c20:4n6 (AA), $t(68) = 1.82$, $p = .074$ and for c20:5n-3 (EPA), $t(68) = -1.78$, $p = .079$, with higher levels observed in the ADHD group compared to controls.

Plasma choline phosphoglycerides (PPC)

The means for ADHD and control children are listed in Table 2. There was a significant difference between the ADHD and control groups for c20:3n-3 (eicosatrienoic acid), $t(68.45) = -3.74$, $p = .001$; there was also a significant difference for c22:5n-3 (docosapentaenoic acid: DPA), $t(67.07) = -2.07$, $p = .043$, which however, did not survive correction for multiple testing, both were higher in the control group, see Table 2.

Trace Elements (Copper, Iron, Magnesium, Selenium and Zinc)

The means for ADHD and control children are shown in Table 2. None of the differences reached significance, the largest trend level difference was observed for copper for which the ADHD group had a higher mean score ($M = 15.80$) than controls ($M = 14.49$), $t(67) = 1.92$, $p = .058$.

Table 2. Mean % Levels of Total Fatty Acid Composition and Trace Elements for ADHD (n = 29) and HC groups (n = 43)

	ADHD		Healthy Control	
	M	*SD*	*M*	*SD*
Plasma phopshatidylcholine (PPC)				
Omega 6				
c18: 2n-6 (LA)	25.02	2.76	24.49	3.56
c18: 3n6	0.11	0.07	0.10	0.06
c20: 2n6	0.28	0.08	0.30	0.08
c20: 3n6	3.55	0.82	3.70	0.94
c20: 4n6 (AA)	10.79	1.85	11.54	2.23
c22: 4n6	0.35	1.85	0.39	0.09
c22: 5n6	0.23	0.08	0.26	0.09
Total n6	40.34	3.30	40.79	3.42
Omega 3				
c18: 3n3 (ALA)	0.37	0.17	0.34	0.12
c20: 3n3	**0.10**	0.03	**0.13**	0.04
c20: 5n3 (EPA)	1.04	0.49	0.99	0.44
c22: 5n3	**0.94**	0.17	**1.06**	0.30
c22: 6n3 (DHA)	3.18	1.00	3.64	0.95
Total n3	5.62	1.49	6.15	1.42
Plasma cholesterol esters (PCE)				
Omega 6				
c18: 2n-6 (LA)	52.40	3.40	52.01	4.04
c18: 3n6	0.85	0.36	0.77	0.38
c20: 2n6	0.02	0.01	0.02	0.01
c20: 3n6	0.66	0.16	0.65	0.15
c20: 4n6 (AA)	6.62	1.22	7.10	1.44
c22: 4n6	**0.04**	0.02	**0.03**	0.01
c22: 5n6	0.04	0.02	0.04	0.02
Total n6	60.61	3.15	60.59	3.27
Omega 3				
c18: 3n3 (ALA)	0.63	0.16	0.58	0.15
c20: 3n3	0.08	0.04	0.09	0.04
c20: 5n3 (EPA)	0.67	0.36	0.69	0.32
c22: 5n3	0.06	0.03	0.06	0.03
c22: 6n3 (DHA)	**0.44**	0.13	**0.51**	0.11
Total n3	1.88	0.48	1.93	0.44
Plasma triglyceride (PTG)				
Omega 6				
c18: 2n-6 (LA)	17.06	4.52	18.19	4.45
c18: 3n6	0.45	0.17	0.40	0.24
c20: 2n6	0.14	0.04	0.14	0.04
c20: 3n6	0.32	0.09	0.32	0.11
c20: 4n6 (AA)	**1.54**	0.55	**1.32**	0.46
c22: 2n6	0.05	0.04	0.06	0.03
c22: 4n6	0.19	0.06	0.18	0.07
c22: 5n6	0.15	0.05	0.15	0.05
Total n6	19.90	4.93	20.76	4.80
Omega 3				
c18: 3n3 (ALA)	1.18	0.57	1.29	0.48
c20: 3n3	0.14	0.07	0.13	0.09
c20: 5n3 (EPA)	**0.35**	0.18	**0.29**	0.11
c22: 5n3	0.39	0.17	0.39	0.13
c22: 6n3 (DHA)	0.63	0.45	0.58	0.19
Total n3	2.69	1.00	2.68	0.59
Trace elements				
Copper (Cu)	**15.80**	3.02	**14.49**	2.60
Iron (Fe)	27.43	12.16	26.49	11.78
Magnesium (Mg)	780.48	86.33	802.27	76.93
Selenium (Se)	7.79	1.04	7.95	0.75
Zinc (Zn)	11.49	2.15	11.93	1.81

Note: * $p < .05$, ** $p < .01$. Those in bold were significant or reached trend level prior to the FDR correction

Behavioural data comparison between ADHD and controls

A series of independent samples *t* tests were conducted to assess for differences in the behavioural questionnaire scores between groups (i.e., SDQ, BYI, CPRS, CTRS, Buss-Perry, BIS, DASS, EFADQ).

ADHD and impulsiveness related questionnaires

Strengths and Difficulties Questionnaire (SDQ)

The means for ADHD and control children are reported in Table 1. As expected and following the FDR correction for multiple testing, there were significant differences between groups for scores of behavioural difficulties (CD), $t(66) = 4.74$, $p = .001$ and hyperactivity and attentional difficulties (ADHD), $t(66) = 5.14$, $p = .001$, with higher mean scores in the ADHD relative to controls. ADHD children had significantly lower mean scores for kind and helpful behaviour (measuring ASD traits) compared to controls, $t(66) = -3.16$, $p = .002$. The ADHD group also had higher mean scores for "risk of any diagnosis", $t(66) = 4.45$, $p = .001$, conduct disorder, $t(66) = 3.27$, $p = .002$ and ADHD, $t(66) = 3.26$, $p = .002$. There were no significant differences for scores of emotional distress, $t(66) = 1.56$, $p = .123$.

Conners' Parent Rating Scales (CPRS)

The means for ADHD and control children are presented in Table 3. As expected, following the FDR correction, there were significantly higher mean scores for the ADHD group compared to controls in all subscales, $p < .001$.

Conners Teaching Rating Scales (CTRS)

The means for ADHD and control children are presented in Table 3. As expected, following the FDR correction there were significantly higher mean scores for the ADHD group compared to controls, $p < .001$.

Table 3. Mean scores for Conners' Parent Rating Scales

Parent Rating Scales	**ADHD** (*N* = 29)		**Controls** (*N* = 43)		
	M	*SD*	*M*	*SD*	
Oppositional	73.21	14.20	48.40	8.68	**
Cognitive Problems/Inattention	71.07	6.17	46.91	5.77	**
Hyperactivity	81.48	11.15	50.42	7.59	**
Anxious-Shy	69.55	14.43	51.37	7.93	**
Perfectionism	60.31	13.50	47.30	6.85	**
Social Problems	63.24	13.18	49.02	7.87	**
Psychosomatic	67.10	14.47	50.26	8.98	**
Conners' ADHD Index	77.03	8.78	47.53	5.62	**
Conners' Global Index (CGI): Restless-Impulsive	79.79	8.80	48.72	6.49	**
CGI: Emotional Lability	75.00	14.99	48.12	8.95	**
CGI: Total	79.55	9.44	48.12	7.05	**
DSM-IV Inattentive	74.55	7.67	46.81	5.43	**
DSM-IV Hyperactive-Impulsive	80.24	14.98	48.72	6.43	**
DSM-IV: Total	82.93	7.90	47.53	5.56	**
Teacher Rating Scales					
	ADHD (*N* = 25)		**Controls** (*N* = 43)		
	M	*SD*	*M*	*SD*	
Oppositional	79.12	13.22	50.91	10.18	**
Cognitive Problems/Inattention	73.04	12.74	49.91	9.51	**
Hyperactivity	79.28	11.19	49.56	7.82	**
Anxious-Shy	72.72	12.55	52.12	9.17	**
Perfectionism	58.69	12.97	46.60	6.00	**
Social Problems	66.64	15.28	50.00	8.78	**
Conners' ADHD Index	79.77	10.33	48.40	7.54	**
Conners' Global Index (CGI): Restless-Impulsive	80.32	9.60	49.47	8.41	**
CGI: Emotional Lability	82.64	12.13	49.63	9.13	**
CGI: Total	82.92	10.21	49.42	8.51	**
DSM-IV Inattentive	76.04	11.49	49.72	8.04	**
DSM-IV Hyperactive-Impulsive	79.44	11.12	48.47	8.32	**
DSM-IV: Total	81.52	9.47	49.95	9.34	**

Note: * $p < .05$, ** $p < .01$

Buss-Perry Aggression scores

The means for each of the subscales of the Buss-Perry Aggression scores for ADHD and control children are presented in Table 1 of this chapter. As expected, following correction, there were significant differences between all measures of self-rated scores of aggression between ADHD and controls, with a higher mean difference for ADHD. Specifically, scores of physical aggression, $t(70) = 4.01$, $p < .001$, verbal aggression, $t(70) = 3.03$, $p < .001$, anger, $t(70) = 4.69$, $p < .004$, hostility, $t(70) = 2.35$, $p < .04$ and finally total aggression scores, $t(70) = 4.21$, $p < .001$, were all significantly higher for the ADHD compared to the control group.

Barratt Impulsiveness scores (BIS)

The means for each of the BIS for ADHD and control children are presented in Table 1 of this chapter. As expected, there were significant differences in all self-reported scores of impulsivity between ADHD and controls, with a higher mean difference for ADHD. Specifically, scores of attentional impulsiveness, $t(69) = 5.97$, $p < .001$, motor-impulsiveness, $t(69) = 3.23$, $p < .003$, non-planning impulsiveness, $t(69) = 4.08$, $p < .001$ and finally total impulsiveness score, $t(69) = 5.97$, $p < .001$ were all significantly higher compared to controls.

Depression, Anxiety and Essential Fatty Acid Related Questionnaires

Becks Youth Inventories

The means for ADHD and control children are listed below in Table 1 of this chapter. Following the FDR correction, there were significant differences between groups for scores of self-concept, $t(70) = -2.14$, $p = .036$, which were higher in the control group relative to ADHD. Children/adolescents with ADHD had significantly higher scores of depressive symptoms, $t(70) = 2.57$, $p = .012$, fear/anxiety, $t(70) = 2.23$, $p = .029$, anger, $t(42.73) = 3.85$, $p = .001$ and disruptive behaviour $t(70) = 3.89$, $p = .001$ relative to controls.

Depression, Anxiety and Stress scales

The means for ADHD and control children are listed below in Table 1 of this chapter. Following the FDR correction, there were significant differences between groups for scores of depression, $t(48.76) = 2.45$, $p = .018$, which were higher in the control group relative to ADHD. Children/adolescents with ADHD also had significantly higher scores of stress symptoms, $t(41.37) = 4.29$, $p = .001$, relative to controls. A trend finding was observed for scores of anxiety, $p = .053$ which were higher in the ADHD group compared to controls.

Essential Fatty Acid Deficiency Questionnaire (EFADQ)

The means for each of the EFADQ scores for ADHD and control children are presented in Table 4 below. Following the FDR correction, there were no significant differences between ADHD and controls in any of the fatty acid deficiency scales. Prior to the FDR correction, a significant difference in self-reported scores of sleep problems was observed between ADHD and controls, with a higher mean difference for ADHD, $t(70) = 2.50$, $p = .015$.

Table 4. Essential Fatty Acid Deficiency Questionnaire Self-Report

	ADHD (*N* = 29)		**Controls** (*N* = 43)	
	M	*SD*	*M*	*SD*
Dryness	2.76	2.76	2.49	2.24
Hyper-secretory	2.45	2.08	1.65	1.77
Skin	1.38	2.09	1.02	1.56
Somatic	3.93	2.84	3.23	2.99
Sleep	3.1	2.06	2.02	1.59
Total	13.65	8.66	10.46	7.61

Relationships between behavioural data and blood measures of LC-PUFA within each diagnostic group

Key fatty acid indices from the omega-3 and omega-6 series, namely (1) c18:3n-3 (ALA), (2) c20:5n-3 (EPA), (3) c22:5n-3 (DPA), (4) c226n-3 (DHA) and (5) Total n-3 and (6) c18:2n6 (LA), (7) c18:3n6 (GLA), (8) c20: 4n6 (AA) and (9) Total n6 respectively, were selected to establish relationships between blood levels of LC-PUFA in the plasma choline phosphoglycerides only (as this is the main fraction and the most representative of fractions such as AA, EPA and DHA) and IQ scores of verbal, non-verbal and composite as well as behavioural questionnaires. All correlations were conducted within each diagnostic group. For all correlational analyses the FDR was employed to correct for multiple testing.

IQ scores and PUFA levels

The selected variables were first examined individually to determine whether they were normally distributed. Out of the 9 fatty acid indices and 3 IQ measures, only 4 were not normally distributed as determined by the Kolmogorov-Smirnov test, $p < .05$, namely c18:3n6, c20:5n-3 and measures of non-verbal and composite scores. For correlations involving these 4 variables, Spearman coefficients for non-parametric data were also calculated.

The data file was split to compare the 2 groups, ADHD and controls. Following the FDR adjustment for multiple testing none of the relationships survived correction. Prior to the FDR correction, in the ADHD group only, a negative correlation was observed between c20:5n-3 (EPA) and verbal IQ: $r = -.559$, $n = 29$, $p = .002$. However following correction this became significant at trend level only, $p = .054$. None of the other correlations survived correction, although of note was the correlation between c18:3n-3 (ALA) and non-verbal IQ scores, $r = -.414$, $n = 29$, $p = .026$. There were no significant relationships between fatty acid indices and IQ in the control group.

SDQ scores and PUFA levels

Tests of normality as determined by the Kolmogorov-Smirnov test showed that the SDQ scores for (i) emotional distress, (ii) behavioural difficulties, (iii) hyperactivity and attentional difficulties and (iv) kind and helpful behaviour were not normally distributed, $p < .05$. Therefore, Spearman coefficients for non-parametric data were calculated. There were no significant correlations between any of the SDQ scores and fatty acid indices, $p > .005$ for any of the groups.

CPRS and PUFA levels

The data was split using the compare group's option into ADHD and control groups. Tests of normality as determined by the Kolmogorov-Smirnov test showed that for the ADHD group ($n = 29$) 8 (Hyperactivity domain, Anxious-Shy domain, Social problems domain, Psychosomatic domain, CGI: Emotional lability, CGI:Total, DSM-IV: Hyperactive-Impulsive and DSM-IV Total) out of the 14 CPRS were not normally distributed, $p < .05$. The following subscales: Oppositional domain, Cognitive problems/Inattention, Perfectionism, Conners' ADHD Index: Total, CGI: Restless-Impulsive and DSM-IV Inattentive did meet the criteria for parametric data. In the control group, 13 of the 14 subscales did not meet the criteria for parametric data; $p > .05$, the exception was scores for DSM-IV total, $p > .05$. Therefore both Pearson coefficients for parametric data and Spearman rho coefficients for non-parametric data were calculated for ADHD and controls.

For the ADHD Group, following the FDR correction for multiple testing, none of the relationships survived. However, prior to correction, Pearson coefficients for parametric data revealed a large significant negative correlation between c22:6n-3 (DHA) and Cognitive problems/inattentive domain CPRS subscale, r = -.535, n = 29, p = .003. Modest to large correlations were observed between c22:6n-3 (DHA) and CGI: Restless-Impulsive, r = -.502, n = 29, p = .005. A modest non-significant correlation was also observed between Total n-3 and CGI; Restless-Impulsive, r = -.477, n = 29, p = .009.

Several large non-significant relationships are of note. The first a negative correlation between Total n-3 and Cognitive problems/inattentive domain**,** r = -.447, n = 29, p = .015. The second, a modest non-significant negative correlation between c20:5n-3 (EPA) and CGI: Restless-Impulsive, r = -.422, n = 29, p = .022, and finally with CGI: Total scores, r = -.418, n = 29, p = .024. Modest to large non-significant correlations were observed between c22:6n-3 (DHA) and CGI: Total scores, r = -.422, n = 29, p = .023 as well as DSM-IV Inattentive domain, r = -.437, n = 29, p = .018.

Spearman's rho for non-parametric data for the ADHD group

There were no significant correlations for the non-parametric CPRS subscales and fatty acid indices in the ADHD group, $p > .005$.

Pearson coefficients for the HC Group

None of the correlations survived the FDR correction for multiple tests. The largest non-significant correlation was between Total n6 and DSM-IV subscale, $r = -.348$, $n = 42$, $p = .024$.

Spearman's rho for non-parametric data for the HC group

None of the correlations survived the FDR correction for multiple tests. Prior to the FDR correction, Total n6 was positively associated with Conners' ADHD Index, $r = .428$, $n = 42$, $p = .005$.

The largest non-significant correlations of note are reported as follows. Three omega-3 fatty acids indices were negatively correlated with the Anxious-Shy domain; the first Total n-3, $r = -.471$, $n = 42$, $p = .002$, the second c22:6n-3 (DHA), $r = -.455$, $n = 42$, $p = .002$ and finally c20:5n-3 (EPA), $r = -.307$, $n = 42$, $p = .048$. Total n6 was positively associated with CGI: Restless-Impulsive, $r = .305$, $n = 42$, $p = .050$, CGI: Total scores, $r = .348$, $n = 42$, $p = .024$, and DSM-IV Inattentive subscales, $r = .395$, $n = 42$, $p = .010$.

CTRS and PUFA levels

The data was split using the compare group's option into ADHD and control groups. Tests of normality as determined by the Kolmogorov-Smirnov test showed that for the ADHD group ($n = 25$) 6 (Oppositional domain, Hyperactivity domain, CGI: Emotional lability, CGI: Total, DSM-IV: Hyperactive-Impulsive and DSM-IV Total) out of the 13 CPRS were not normally distributed, $p < .05$. The following subscales: Cognitive problems/Inattention, Anxious-shy domain, Perfectionism, Social Problems domain, Conners' ADHD Index, CGI: Restless-Impulsive and DSM-IV Inattentive did meet the criteria for parametric data. In the control group, none of the 14 subscales met the criteria for parametric data; $p > .05$. Therefore both Pearson for parametric and Spearman rho coefficients for non-parametric were calculated for ADHD and controls. The FDR correction for multiple testing was applied as per before to minimise Type 1 error.

Pearson coefficients for the ADHD Group

None of the other correlations survived correction for multiple testing. Prior to the FDR correction, a negative relationship was observed between DPA (c22:5n3) and Conners' social problems domain, $r = .529$, $n = 25$, $p = .005$.

Spearman's rho for non-parametric data for the ADHD group

None of the relationships survived the FDR correction. The largest non-significant correlation was between DSM-IV hyperactive-impulsive and DPA (c22:5n3), $r = -.412$, $n = 25$, $p = .041$.

Spearman's rho for non-parametric data for the HC group

Following correction, there were no significant relationships between any of the CTRS and PUFA levels. Prior to the FDR correction, a significant positive correlation was observed between c18:3n6 (GLA) and cognitive problems/inattentive domain, $r = .458$, $n = 42$, $p = .002$. and c18:3n6 (GLA) and DSM-IV Inattentive, $r = .410$, $n = 42$, $p = .007$.

Buss-Perry Aggression scores and PUFA levels

Tests of normality as determined by the Kolmogorov-Smirnov test showed that all Buss-Perry subscale measures of (i) physical aggression, (ii) verbal aggression, (iii) anger, (iv) hostility and (v) total aggression scores were normally distributed for the ADHD group. Therefore for this group Pearson correlations for parametric tests were employed. In the control group, only total aggression scores met the criteria for parametric tests, $p > .05$. Therefore, Spearman rho for non-parametric tests was conducted on the remaining subscales. None of the correlations reached significance in either the ADHD or control employing either Pearson or Spearman correlational analysis.

The largest non-significant positive correlation in the ADHD group was between c20:4n6 (AA) and scores of anger, $r = .346$, $n = 29$, $p = .066$. In the control group, the largest non-significant negative correlation was between Total n-6 and scores of physical aggression, $r = -.294$, $n = 42$, $p = .059$.

Barratt Impulsiveness scores (BIS) and PUFA levels

Tests of normality as determined by the Kolmogorov-Smirnov test showed that in the ADHD group 4 out of 5 of the BIS subscales (namely attentional impulsiveness, non-planning impulsiveness and total impulsiveness scores) met the criteria for parametric testing with the exception of motor-impulsiveness. In the control group only 2 subscale measures namely non-planning impulsiveness and total impulsiveness scores met the criteria for normal distribution. Therefore both Pearson correlations and Spearman were employed and reported accordingly. None of the correlations reached significance following the FDR correction.

Of note, were 2 correlations of interest in the ADHD group, the first largest a non-significant negative correlation between c20:5n-3 (EPA) and total impulsiveness, r = -.488, n = 28, p = .008. The second, a non-significant negative correlation between total n-3 and total impulsiveness, r = -.473, n = 28, p = .011. In the control group, no correlations reached significance.

Becks scores and PUFA levels

Tests of normality as determined by the Kolmogorov-Smirnov test showed that all the Becks scores for (i) self concept, (ii) anxiety and (iii) depression (iv) anger and (v) disruptive behaviour met the criteria for parametric tests, $p > .05$ in the ADHD group. In the control group only scores for disruptive behaviour were not normally distributed, $p < .05$. None of the correlations, in either the ADHD or HC group reached significance employing either Pearson or Spearman correlational analysis following the FDR correction. The largest non-significant positive correlation prior to correction was observed between anxiety and c18:3n-3 (ALA), $r = .388$, $n = 43$, $p = .011$.

DASS scores and PUFA levels

Tests of normality as determined by the Kolmogorov-Smirnov test showed that the DASS scores for (i) depression, (ii) anxiety and (iii) stress did not meet the criteria for parametric tests, $p > .05$, in either ADHD and control groups. Therefore, Spearman rho coefficients for non-parametric data were calculated. There were no significant relationships in either the ADHD or control group between any of the fatty acids indices and DASS scores, $p > .05$.

EFADQ and PUFA levels

Tests of normality as determined by the Kolmogorov-Smirnov test showed that the EFADQ scores for (i) dryness, (ii) hypersecretory and (iii) skin problems (iv) skin problems (v) sleep problems and (vi) total deficiency scores did not meet the criteria for parametric tests, $p > .05$, in either ADHD and control groups. Therefore, Spearman rho coefficients for non-parametric data were calculated.

None of the correlations reached significant following the FDR correction. In the ADHD group, of note, are the three largest non-significant negative correlations all of which involved the measure sleep problems. The first, was a negative correlation between sleep problems and c20:4n6 (AA), $r = -.415$, $n = 29$, $p = .025$, the second a negative correlation between sleep problems and c22:6n-3 (DHA), $r = -.424$, $n = 29$, $p = .022$ and finally sleep problems was also negatively associated with total n-3, $r = -.456$, $n = 29$, $p = .013$.

In the control group, there was a modest non-significant positive correlation between c22:6n-3 (DHA) and hypersecretory problems, r =-.386, n = 42, p = .012.

Questionnaires for ADHD group only. Inventory of callous and unemotional traits (ICU)

The ICU data were only collected in the ADHD group only (n = 29) as measure traits related to comorbid conduct disorder. The Kolmogorov-Smirnov test revealed that scores were normally distributed and therefore met the criteria for parametric data. Pearson coefficients were calculated to assess relationships between the ICU scores and 9 fatty acid indices. Following adjustment for multiple testing using the FDR, there was a significant negative correlation between EPA (c20:5n-3) and ICU traits, r = -.597, n = 29, p = .009. There was also significant negative relationship between total n-3 and ICU scores, r = -.498, n = 29, p = .027. Finally, the relationship between DHA (c22:6n-3) and ICU scores, r = -.436, n = 29, p = .054 was significant at trend level only. The largest corrected non-significant positive correlation was observed between total n6 and total ICU traits, r = .375, n = 29, p = .081.

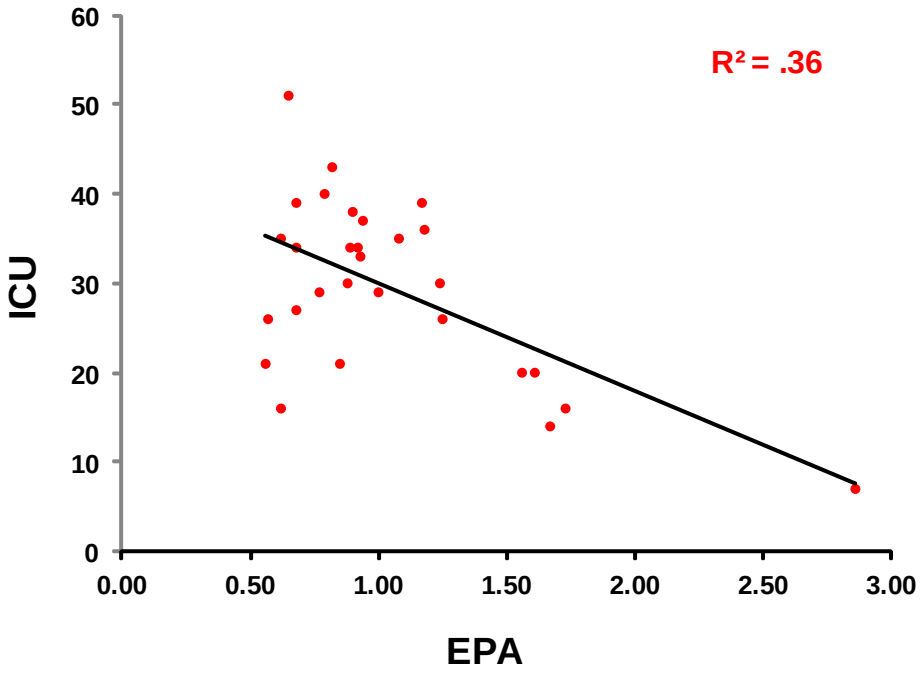


Figure 2. The relationship between blood levels of omega-3, EPA and mean scores for callous and unemotional traits in the ADHD group only (n = 29)

ICU scores were also significantly positively associated with CPRS subscale for oppositional behaviour (which measures rule breaking, problems with authority and feasibility for annoyance), $r = .464$, $n = 29$, $p = .011$.

Anti-social processing screening device (APSD), youth version for callous unemotional total traits

The APSD data were only collected in the ADHD group ($n = 29$). The Kolmogorov-Smirnov test revealed that scores were normally distributed and therefore met the criteria for parametric data. Pearson coefficients were calculated to assess relationships between the APSD total score and 9 fatty acid indices. There were no significant relationships between any of the fatty acid indices and the APSD traits. The largest non-significant negative correlation involved total n-3 and APSD, $r = -.365$, $n = 29$, $p = .051$.

Brief Discussion & Conclusion

The objective of the study reported here was to investigate (i) whether differences in blood levels of LC-PUFA existed between children and adolescents with and without ADHD, (ii) whether LC-PUFA levels significantly correlated with scores of essential fatty acid deficiency (EFADQ) and (iii) whether LC-PUFA levels were associated with IQ and behavioural measures of ADHD symptoms including impulsivity, aggression, depression/anxiety, conduct disorder and callous and unemotional traits. The rationale for this study was based on previous literature suggesting abnormal fatty acid levels in young people with ADHD compared to controls. Specifically, in this study, it was anticipated that the ADHD group would have lower levels of omega-3 fatty acid indices and higher levels of omega-6 compared to HC children. Furthermore, that lower levels of omega-3 fatty acids would be associated with greater symptom severity of aggression, depression/anxiety, impulsive and conduct disorder traits. In turn, it was hypothesised that inverse relationships would also be found, that is, higher levels of omega-6 fatty acids would be related to lower symptom severity.

No differences were observed between any of the fatty acid plasma indices between the ADHD and control groups following correction for multiple testing. As expected, there were significant differences in behavioural scores of aggression, depression/anxiety, impulsive and conduct disorder traits between ADHD and control children. There were however no significant relationships between clinical behavioural measures of aggression, depression, anxiety, impulsivity or self-concept and LC-PUFA levels in ADHD or control groups. Furthermore, there were no significant differences between fatty acid levels and IQ. In addition, self-reported fatty acid deficiency scores were not significantly associated with any of the blood fatty acid indices.

However, a significant relationship was observed between callous-unemotional traits as measured by the ICU and EPA, specifically the lower the EPA – the higher the scores for callous and unemotional traits. The same pattern was observed between the total omega-3 ratio and CU traits which were negatively correlated. ICU scores were also significantly positively correlated with the Conner's parent rating subscale for oppositional behaviour in the ADHD group. Furthermore, there were two trend associations, the first between low levels of DHA and higher CU traits and the second a positive association between omega-6 and CU traits. In support of the

latter, a trend finding was also observed between self-rated scores of anti-social behaviour (as measured by the APSD) and low omega-3.

Blood levels of LC-PUFA

The blood results of this study are essentially negative and do not support previous literature suggesting abnormal fatty acid status in ADHD compared to control children (Antalis, et al., 2006; Chen, et al., 2004; Colter, et al., 2008; Mitchell, et al., 1987; Stevens, et al., 1996). However, the findings do support research reporting no differences or abnormal alterations in PUFA status between ADHD and HC children (Mitchell, et al., 1983; Spahis et al., 2008). This is the first study to compare LC-PUFA status between children/adolescents with and without ADHD in the U.K. and the majority of these children were from the London area. The data suggests that there are no significant differences between groups in dietary intake of omega-3/6 fatty acids and no evidence to suggest metabolic alterations as previously reported in the literature. Of note, the majority of studies so far reporting differences in PUFA status are from the United States, although differences have also been observed in 1 study with children from Taiwan (Chen, et al., 2004) and 1 study with children from Canada (Colter, et al., 2008). Diet differs according to geographical location and the relationship between fish intake and behaviours associated with ADHD such as depression has been well-documented (Hibbeln, 2009; Hibbeln, et al., 2007; Hibbeln, Nieminen, Blasbalg, Riggs, & Lands, 2006; Tanskanen, Hibbeln, Hintikka, et al., 2001). Ethnicity which is not always reported in comparable studies, is another factor which can influence diet and in our ADHD group 17.2% were black or black British, and consumed fish at least once a week. In two similar studies, the first by Antalis and colleagues (2006), 92% of participants were described as Caucasian and 8% as Asian. The second study by Stevens and colleagues (1995) described 100% of the control children and 92.4% of the ADHD group as white. Other fatty acids studies, reviewed in Chapter 2, such as those by Colter and colleagues (2008) and another by Stevens et al. (2003) have omitted to report ethnicity (for a review see Chapter 2).

The present study had a relatively small sample size of ADHD children (n = 29) and larger sizes would be recommended in the future. However, the sample size was sufficient enough to ascertain differences in percentage fatty acid levels between groups. Comparable published studies have recruited fewer children e.g., Colter et al., (2008) recruited a sample size of 23 (11

ADHD and 12 controls) and Antalis et al., (2006) recruited just 12 in each group (ADHD and controls). Of note, is that the aforementioned studies highlighting significant differences in blood fatty acid concentrations between ADHD and HC do not seem to have corrected or adjusted for multiple testing and instead seemingly reported the raw data employing a significance level of 0.05. At an alpha level of .05, and without correction, the raw data revealed *significant* differences between groups in plasma cholesterol esters (PCE) levels of DHA which were higher in the HC and for c22:4n6 (a metabolite of AA) which was higher in the ADHD group. In plasma triglycerides (PTG), the ADHD group had higher mean levels of AA and EPA (which was also reported in a study by Germano et al., 2007), significant at trend level only, relative to control children. In plasma choline phosphoglycerides (PPC), both the omega-3 c20:3n3 (eicosatrienoic acid) and c22:5n3 (DPA) were significantly higher in the HC compared to ADHD. It could be argued that although the False Discovery Rate (FDR) employed in this study although not as conservative as a Bonferroni correction, may be too stringent to apply to sensitive measures such as percentage levels of PUFA concentrations in blood samples. This is especially relevant in respect of the predicted hypotheses, which were directional and therefore 1 tailed, implying an alpha level of .05 could have been accepted.

Study Limitations

One of the main limitations of the present study was the unforeseen contamination of the red blood cell samples. This was a major disadvantage as red blood cell samples are in the main the most indicative of the stable fatty acids status over time. However, in respect of the different plasma measures, PCE reflects the cholesterol in humans and is generally esterified with linoleic acid in the main. PTG reflects the circulating adipose tissue at any given time and the concentration of fatty acids depends on the mobilisation or sequestration of fat in the adipose tissue and of course the intake of triglycerides from the diet (e.g., amounts of butter, milk, fats, fish oils consumed etc). Therefore, depending on the time of day and eating activity it will vary. It is generally never rich in either AA or DHA or EPA. Variations in fatty acids will exist in human triglycerides according to geographical location and diet. For example, the Greenland Inuit and Japanese populations will have much more EPA and DHA in their triglycerides than people from London (Holub, 2002). Plasma choline phosphoglycerides are the main fraction containing AA, EPA and DHA. They are formulated in the liver from incoming sources and various metabolic activities such as anabolism or catabolism. The phosphatidylcholine (PC) is

distributed in the lipoproteins with the richest for AA and DHA being high-density lipoprotein (HDL), followed by low-density lipoprotein (LDL), and then very-low-density lipoprotein (VLDL). The latter, VLDL, functions as a main transporter for lipids in the body and is responsible for the transportation of phospholipids, cholesterol esters, cholesterol and triglycerides. In plasma PC, unadjusted levels of DHA and AA were lower in ADHD compared to controls a finding that supports the earlier and influential work of Mitchell and colleagues (1987). Furthermore, out of the 3 plasma measures analysed in this study and for the reasons aforementioned, PC is arguably the most representative of stable fatty acid levels and was selected on that basis[8].

Relationship between LC-PUFA and Behavioural Measures

The results of the LC-PUFA and behavioural correlations demonstrated that in eight out of nine of the behavioural questionnaires, significant associations did not survive correction for multiple testing. Overall, this study reports the absence of significant relationships between measures of IQ, scores on the Strength and Difficulties Questionnaire, scores on both the Conner's Teacher and Parent Rating Scales, scores of aggression as measured by the Buss Perry, scores of impulsivity as measured by the Barratt Impulsiveness Scale, scores on the Becks Youth Inventory (measuring depressive symptoms, fear/anxiety, anger, disruptive behaviour and self-concept), scores on the DASS measuring depression, anxiety and stress. Furthermore scores of fatty acid deficiency did not significantly correlate with PUFA levels demonstrating that blood levels of fatty acids are not related to the behavioural measures. The only behavioural questionnaire scores which were significantly associated with PUFA levels was the Inventory of Callous and Unemotional traits (ICU) which measured the presence of conduct disorder related callous and unemotional traits. In this sample, just under half (44.4%) of boys with ADHD were at medium or high risk of CD according to the SDQ. ICU scores were also significantly positively associated with CPRS subscale for oppositional behaviour which further measures CD type behaviour (e.g., rule breaking, problems with authority and feasibility for annoyance). Furthermore, there were two associations, the first a negative association between low levels of total omega-3 and higher CU traits and the second (a trend finding), also a negative association,

[8] Written correspondence with Professor Michael Angus Crawford, July 2011

between low levels of DHA and higher CU traits. In support of the latter, a trend finding was also observed between self-rated scores of anti-social behaviour (as measured by the APSD) and low omega-3.

Callous and unemotional (CU) traits are widely under-researched in children diagnosed with ADHD despite research suggesting that children with ADHD are 11 times more likely to have conduct problems and/or comorbid ODD (Angold, Costello, & Erkanli, 1999; Costello, Mustillo, Erkanli, Keeler, & Angold, 2003). Typically, CU traits are described as individual variation in guilt, remorse and empathy and are considered to be significant feature of aggression and psychopathy in youth (Frick, Cornell, Barry, Bodin, & Dane, 2003; Frick & White, 2008). In a previous study, both teacher ratings of CU and ADHD behaviour predicted greater disciplinary action, increased peer rejection and higher adventure-seeking behaviour in 154 children compared to ADHD only (Barry et al., 2000). Another study demonstrated that children with ADHD, conduct problems and CU traits did not respond favourably to behaviour modification therapy compared to children with ADHD and conduct problems alone (Waschbusch, Craig, Pelham, & King, 2007). Therefore, emerging evidence suggests that the developmental course of children with both CU traits and ADHD is meaningfully altered. The findings of this PhD demonstrate that EPA and behaviour symptoms of ADHD is particularly strong with CU traits and thus may suggest a link between ADHD, CU and omega-3 fatty acids, in particular with EPA. There is already evidence in the literature that anti-social behaviours are related to fatty acids. For example, in human cohorts, one of the most compelling studies to date demonstrating the mediating effects of fatty acids in reducing conduct problems is the work of Gesch et al (2002). Their RCT demonstrated that supplementing young offenders in prison with vitamins, minerals and fatty acids (circa 180 mg of EPA and DHA daily) reduced anti-social and aggressive behaviours by approximately 37% compared to placebo. Another prison study in Holland, also significantly lowered incidents of disruptive, dangerous behaviour and/or violations of prison rules over a 3 month period employing nutritional supplements (vitamins, minerals and essential fatty acids) compared to placebo (Zaalberg, et al., 2010). Associations have also been reported between nutritional status and anti-social and delinquent behaviours (Raine, Mellingen, Liu, Venables, & Mednick, 2003) with fatty acids reducing parent and teacher ratings of aggression in school aged children (Hamazaki & Hirayama, 2004). There is hence a link in the literature between aggressive and anti-social behaviours and essential fatty

acids levels. The results of this study further extend this evidence by demonstrating that the association between anti-social behaviours and EPA may be particularly close with CU traits.

The orbitofrontal cortex (OFC) is well known for its role in emotion processing and has reciprocal links with the nucleus accumbens, hypothalamus, amygdala and hippocampus (Bechara, 2004; Kringelbach & Rolls, 2004). Omega-3 fatty acids are rich in orbito prefrontal and medial temporal cortices and, in turn, these regions have been implicated in various aspects of psychopathy (Connor, Neuringer, & Lin, 1990; Cotter, Hudson, & Landau, 2005; Gur et al., 2000). Furthermore, abnormalities in fatty acid compositions have been found in post-mortem OFC of schizophrenic patients (Horrobin, Manku, Hillman, Iain, & Glen, 1991; McNamara et al., 2007). It has been hypothesised that inadequate levels of EPA and DHA during critical period of neurogenesis may result in autonomic dysregulation and underdevelopment of neurotransmitter systems, leading to the manifestation of aggressive and anti-social type behaviours (Hibbeln, Ferguson, & Blasbalg, 2006). There appears thus to be a link between EPA, its effects on the orbitofrontal cortex and psychopathic behavioural traits.

In typically developing adolescent boys, DHA supplementation has been found to increase prefrontal cortex activation compared to placebo, suggesting that dietary intakes of omega-3, DHA plays a role in the modulation of functional cortical activity (McNamara, Able, et al., 2010).

The findings of this PhD study confirm the association between antisocial behaviours and EPA and further extend this association by demonstrating that fatty acids levels are particularly associated with CU traits rather than with anti-social behaviours per se or ADHD behaviours per se. Previous research by Gow et al. (2009) reported negative associations between (1) N170 amplitude responses and DHA levels and (2) N170 amplitude responses and happy faces during an ERP emotion processing task in a small group of adolescent boys with ADHD. An inverse relationship was also found between EPA and P3 amplitude responses to happy faces relative to both fear and anger, in other words, the higher the EPA the greater the orientation to facial expressions of happiness (Gow, Matsudaira, Taylor, Rubia, Crawford, Ghebremeskel, Ibrahimovic, Vallee-Tourangeau, et al., 2009). Clinical populations with ADHD, conduct disorder and/or psychopathy are known to be impaired in face processing as evidenced by both behavioural and imaging studies (Davidson, Putnam, & Larson, 2000a; Davidson & Slagter, 2000; Loney, Frick, Clements, Ellis, & Kerlin, 2003; Marsh et al., 2008). Furthermore, callous-

unemotional traits in both adults and adolescent populations are linked to impairments in emotion processing (Jones, Laurens, Herba, Barker, & Viding, 2009). For example, Jones et al. (2009) reported a negative association between reduced activation in right amygdala activity to fearful faces and higher CU traits. In consideration of the link between high EPA and enhanced P3 responses to happy faces in ADHD as reported previously by Gow and colleagues (2009), alongside the evidence that higher CU traits are negatively associated with impaired emotion processing as reported by hypoactivity to fearful faces, it may be that lower EPA is a potential risk factor for the development of psychopathy. The link between perinatal deficiencies in omega-3 and the later development of psychopathy has also been discussed by McNamara and colleagues (2006). Their theory was primarily based on the necessity of omega-3 for healthy brain development and the well documented alterations in cortical development and maturation of the brain following dietary induced deficiencies in omega-3 fatty acids in animal models (McNamara & Carlson, 2006b).

In conclusion, this study suggests that lower omega-3 blood levels of total omega-3 and EPA is associated with higher scores of traits related to immoral and anti-social behaviour in children with ADHD. Furthermore, two further associations were observed between low levels of DHA and higher CU traits, low omega-3 and high CU traits and low omega-3 and high scores of anti-social behaviour. Further research with larger samples sizes of children with ADHD and with comorbid CD or in children with CD/CU traits alone are needed to disentangle this relationship further. In addition, supplementation trials could investigate whether omega-3 may be a safe, efficacious intervention in reducing symptoms of CD and CU traits in those patients at risk from delinquent, violent and criminal behaviours.

Chapter Eight: Study 1A

Conflict/Response Inhibition (Go/NoGo) Task in ADHD and Healthy Matched Controls

Introduction

Cognitive processes which involve response expectation and preparation, selective attention, response inhibition and conflict monitoring (e.g., the capability to interrupt an activated response and to actively suppress responses) are vital to establish resourceful and goal-directed behaviour (Jonkman, 2006). Children with ADHD however have persistently been shown to have abnormal ERP waveforms during tasks measuring these types of cognitive processes, most prominent during tasks of motor response inhibition and selective attention in frontal and parietal brain regions relative to controls (Banaschewski, et al., 2004; Brandeis, et al., 1998; Brandeis, van Leeuwen, et al., 2002; Dimoska, et al., 2003; Fallgatter, et al., 2004; Jonkman, Kemner, Verbaten, Koelega, Camfferman, vd Gaag, et al., 1997; Liotti, et al., 2007; Pliszka, et al., 2000b; Shen, et al., 2011).

The association between LC-PUFA and brain development has progressively increased since the role of omega-3 PUFA in brain structure and function became apparent in the 1970's through the work of Crawford and his colleagues (Crawford, et al., 1976). Crawford and Sinclair (1972) were the first to demonstrate that arachidonic (AA, c20:4n6) and docosahexaenoic (DHA, c22:6n-3) acids were essential for mammalian brain development (Sinclair & Crawford, 1972a, 1972b). Since then, the behavioural effects of omega-3 deficiency has been well established in animal models and thought to be mediated by serotonergic and dopaminergic systems which are in turn implicated in the modulation of attention, motivation and emotion (Chalon, 2006). Feeding rats chronically deficient omega-3 diets alters several neurotransmission systems including the dopaminergic and serotonergic systems and results in a 40-60% reduction in dopamine in the frontal cortex during early development (Chalon, 2006). Repletion of both omega-3/6 fatty acids into the diet during lactating results in restoration of the brain fatty acid composition and several parameters of neurotransmission. However, evidence suggests that despite a reversal diet given during weaning, neurochemical factors were unable to fully recover resulting in only partial recovery of biochemical parameters (Chalon, 2006). Therefore, it can be

postulated that chronic omega-3 deficiency in animal models results in irreversible damage in particular brain functions which are potentially associated with the emergence of crucial neurodevelopmental procedures during the lactating period (Chalon, 2006). Additional research has also demonstrated the effect of maternal omga-3 deficiency to neuronal migration in the developing rat brain (Yavin, et al., 2009). Maternal nutritional deficiencies during neurogenesis and angiogenesis have long been associated with impairment in behaviour in both animal models (Garcia-Calatayud et al., 2005; Salem et al., 2001; Wainwright, 2002) and in humans (Al, van Houwelingen, & Hornstra, 2000; Alessandri et al., 2004; McNamara & Carlson, 2006b). It is further postulated that deficiencies in LC-PUFA during the early critical periods of prenatal and child development result in a predisposition towards both depressive and aggressive behaviours. This is likely to be due to impaired connectivity, dendritic arborisation, neuronal migration, timed apoptosis resulting in irrevocable disturbance in the neuronal pathways that modulate behaviour (Hibbeln, Ferguson, et al., 2006).

It has been hypothesised that perinatal deficits in brain DHA may in turn play a role in the deficits observed in ADHD (McNamara & Carlson, 2006b). Studies supplementing preterm infants with DHA have resulted in improvements in processes associated with visual attention thus implicating the importance of DHA in the maturation of brain regions associated in attention including the dorsolateral prefrontal cortex (Carlson & Werkman, 1996; McNamara & Carlson, 2006a; Werkman & Carlson, 1996). There are very few publications exploring the relationship between PUFA and assessments of brain function and thus the literature to date is extremely limited (Fontani, Corradeschi, Felici, Alfatti, Migliorini, et al., 2005; Gow, Matsudaira, Taylor, Rubia, Crawford, Ghebremeskel, Ibrahimovic, Vallée-Tourangeau, et al., 2009; McNamara, Able, et al., 2010; Sumich, et al., 2009). There is just one study in the literature to date which has investigated the effects of omega-3 supplementation to cognitive and physiological parameters in healthy adults using tasks measuring inhibition (Go/NoGo) and sustained attention (Fontani, Corradeschi, Felici, Alfatti, Migliorini, et al., 2005). The results reported an increase in amplitude for the P3 post-stimulus during both Go and NoGo trials and also in the negative wave (CNV) preceding the target after omega-3 supplementation (Fontani, Corradeschi, Felici, Alfatti, Migliorini, et al., 2005). A reduction in RT, i.e., faster performance following supplementation with omega-3 was also observed during both Go/NoGo and sustained attention tasks, along with a shift towards theta and alpha in the active group (i.e., those receiving omega-3 active capsules)

(Fontani, Corradeschi, Felici, Alfatti, Migliorini, et al., 2005). Supplementation with omega-3 simultaneously had an effect on mood, e.g., self-rated measures of depression, anxiety and anger reduced while an increase in vigour was reported. Fontani and colleagues (2005) concluded that supplementation with omega-3 was linked to improvement in physiological and attentional functions, specifically involving complex cortical processing (Fontani, Corradeschi, Felici, Alfatti, Migliorini, et al., 2005). However, of note, is that the researchers in this group did not compare the active group directly with the placebo group but instead looked at the groups separately and compared baseline to endpoint only.

As reviewed in Chapter 5, motor response inhibition is classically measured in Go/NoGo or Stop tasks. In the Go/NoGo task participants have to inhibit a motor response to infrequent NoGo trials in a string of frequent Go trials. Furthermore, it is the low frequency of NoGo trials among a string of high frequent Go trials that elicits the inhibitory effect. To recap, the two main ERP deflections that have been functionally linked to response inhibition processes in Go/NoGo tasks are the frontal negative NoGo-N2 and the fronto-central positive NoGo-P3 (Banaschewski, Brandeis, Heinrich, Albrecht, Brunner & Rothenberger, 2004). In NoGo trials relative to Go trials, increases in the amplitudes of the N2 wave have been specifically linked to response inhibition processes (Bekker, et al., 2004; Bruin & Wijers, 2002; Eimer, 1993; Falkenstein, et al., 1999; Jodo & Kayama, 1992; Nieuwenhuis, et al., 2003). However, others such as Smith, Johnstone and Barry (2007) suggest that it is the P3 as opposed to the N2 which indicates the inhibition of a deliberate response and/or conflict between contra responses warranting the necessity for a key review of the existing understandings of the N2 and P3 components in inhibitory tasks (Smith, et al., 2007).

The ERP literature has elicited diverse results thus far in children with ADHD. For example, Overtoom, Kenemans & Verbaten et al., (2002) have reported markedly reduced N2 in children with ADHD alongside poorer response-inhibition performance compared to controls (Pliszka, Liotti, Woldorff, 2000). Fallgatter and colleagues (2004) have argued that during NoGo trials a negative wave, maximal at fronto-central electrode sites is reported 200-400 milliseconds after the onset of a stimulus (NoGo-N2), reflecting the inhibitory processes associated with NoGo trials (Brandeis, van Leeuwen, Rubia, Vitacco, Steger, Pascual-Marqui, & Steinhausen, 1998; van Veen & Carter, 2002; Fallgatter et al., 2004). Studies employing the Stop task report have also reported reduced frontal N2 component along with poorer performance in the

inhibitory measure (Dimoska, et al., 2003; Liotti, et al., 2007; Pliszka, et al., 2000b). For a review please refer back to Chapter 5. Other studies such as the inverted CPT-A-X tasks, also measuring inhibition, contradict these findings, and have reported no significant difference between children with ADHD and controls N2 responses during NoGo trials (Overtoom, et al., 1998; van Leeuwen, Steinhausen, Overtoom, Pascual-Marqui, van't Klooster, et al., 1998). Larger N2 amplitudes have also been reported in children with ADHD implying a greater frontal association, in other words, more effort is applied in order to produce a correct response (Jodo & Kayama, 1992; Yong-Liang, Robaey, Karayanidis, Bourassa, Pelletier & Geoffroy, 2000). Differences observed during Go and NoGo ERPs are thought to relate to the topography of the P3 which is localised to anterior regions, usually at Cz during NoGo trials relative to Go trials, which in turn are maximum frequently at Pz (Bokura, et al., 2001; Fallgatter, et al., 2004). This effect is thought to be associated with inhibitory response processes (Fallgatter, et al., 2004). Source localisation methods such as low resolution brain electromagnetic tomography (LORETA) software has associated the NoGo-evoked P3 wave to an activation of specific prefrontal brain regions, especially the anterior cingulate cortex (ACC) (Fallgatter, et al., 2004; Strik, et al., 1998) and can also be reflected in the FCz amplitude response. Decreases in activation of P3 amplitudes in the NoGo condition of a CPT task have also been reported in ADHD children relative to controls suggesting diminished NoGo activity in the ACC (Fallgatter, et al., 2004). Furthermore, Fassbender and Schweitzer (2006) argue that impairments in prefrontal and anterior cingulate cortex function reduce the ability to maximise subsidiary neural regions which are needed to optimally perform cognitive tasks (Fassbender & Schweitzer, 2006).

Other functional differences reported in ERP ADHD studies include reductions in the early components elicited by visual stimuli such as P2. This is apparent especially in responses to Go, NoGo and/or warning stimuli compared to controls (Smith, Johnstone & Barry, 2004; Johnstone, Barry, Markovska, Dimoska & Clarke, 2009). In hyperactive children and those with attentional difficulties, P2 amplitudes responses to standard stimuli were found to be larger (Callaway, et al., 1983; DeFrance, et al., 1996; Robaey, et al., 1992; Wiersema, et al., 2006).

Of interest, is that some neurophysiological studies in healthy control populations have provided evidence for the neuronal efficiency hypothesis (Neubauer, Grabner, Fink, & Neuper, 2005). This theory suggests that intelligence is linked to a more efficient utilisation of the cortex

during tasks requiring cognitive demands which is reflected by a decrease in activation (Neubauer & Fink, 2009; Neubauer, et al., 2005). This pattern of decreased activation has also been found to be more pervasive in males than females (Neubauer & Fink, 2009)

The aim of this study was to investigate neurophysiological correlates of prefrontal lobe mediated response control in children and adolescents with ADHD. The key question was whether ADHD children differed from controls in performance and in relation to the magnitude of electrical activity in the N2 and P3 AUC amplitude responses across the frontal-central and parietal regions. In line with previous findings, it was hypothesised that ERP differences will be characterised by smaller N2 and P3 in ADHD compared to control children (Banaschewski, et al., 2004; Fallgatter, et al., 2004; Overtoom, et al., 2002; Pliszka, et al., 2000b). Furthermore, based on previous research, it was predicted that children with ADHD make more errors of omission and commission compared to control children (Fisher, Aharon-Peretz, & Pratt, 2011; Halperin, et al., 1991; Rubia, Smith, Brammer, et al., 2007; Willcutt, et al., 2005). Importantly, since the main interest of this thesis was the association between ERP assessments of brain function in ADHD and fatty acids, further analyses explored the relationship between fatty acid status in plasma choline phosphoglycerides in children and adolescents with and without ADHD and their ERP response. There were no previous studies in which to base firm a priori hypotheses but in consideration of related omega-3 research in impulse control and attention processes (Freeman, et al., 2006; McNamara, Able, et al., 2010; Schuchardt, Huss, Stauss-Grabo, & Hahn, 2010), it was hypothesised that P3 amplitude responses to Go stimuli would be significantly positively associated with omega-3 fatty acids indices in both ADHD and control children. Finally, it was predicted that omega-3 fatty acids would be negatively correlated with both errors of omission and commission, i.e., the lower the omega-3 the greater the number of errors reflecting both executive and inhibitory processes respectively.

Method

Participants

The EEG/ERP data for the ADHD children had previously been collected during the MAAFA trial. During the MAAFA trial, a total of 76 male children/adolescents with symptoms of ADHD were drawn from various special educational settings (e.g., boarding schools, mainstream schools with provision for children with emotional and behavioural difficulties) in and around the London area and were screened to ensure they met the criteria for ADHD according to the DSM-IV. As per before, this included a short semi-structured interview, (Children's Interview for Psychiatric Syndromes, *ChIPS*) based on DSM-IV criteria (ChIPS see Rooney, Fristad, Weller & Weller, 1999) and that both Parent and Teacher Connor Rating Scales (CPRS/CTRS) were equal to or above 65 (> 95th percentile). The EEG/ERP data was age matched to the healthy control group. Intelligence quotient (IQ) for all participants had to be higher than 70 on the prorated IQ as assessed using the Kaufman Brief Intelligence test (K-BIT).

Data from a total of 73 male adolescents were included in this study. This was made up of baseline data from 43 children/adolescents that had been recorded during the Maudsley Adolescence ADHD Fatty Acid (MAAFA) trial and met criteria for ADHD and 29 age and gender matched controls.

In relation to the recruitment of the healthy age and sex matched controls, participants were screened in the same way as the ADHD group but to ensure they did not meet criteria for ADHD. Teachers were advised of the recruitment criteria and pre-screened children in terms of suitability for each group e.g., ensured that the healthy controls were not known to have behavioural difficulties or special educational needs. As well as completing the battery of clinical questionnaires, and providing a 16 ml blood sample, the healthy controls were tested on all EEG/ERP tasks so as to provide control data for the pre-existing EEG/ERP data collected during the MAAFA trial.

Exclusion criteria for all participants were:

- Participants who had taken omega-3/6 supplements in the previous 6 months.
- A personal history of diabetes or other metabolic disorder influencing fatty acid metabolism.
- Participants who were not in school during the assessment.
- Non-English speakers.
- Serious or chronic disease, low blood coagulation function (e.g. haemophilia, hepatic dysfunction, low vitamin K).
- Uder these medication: alpha tocopherol, selected anticoagulants (aspirin, warfarin, heparin), cyclosporine, clopidogrel, etretinate and topical steroids, cholesterol lowering medications (atorvastatin, lovastatin, and simvastatin), non-steroidal anti-inflammatory drugs (NSAIDs), dalteparin, dipyrdamole, enoxaparin and ticlopedine, participants who had pancreatic insufficiency or abnormal blood data.
- Participants with neurological problems or substance use were also excluded.
- Comorbid disorders such as a diagnosis of autism or learning disorder were also excluded; however comorbid ODD or CD was permitted.

Age, IQ, Handedness and Medication

The mean age for the ADHD group was 13.85 years (M = 13.85, SD = .93) versus 14.27 years for the HC group (M = 14.27, SD = 1.08). There were no significant differences in age between groups, $t(72) = -1.81$, $p > .05$. In the HC group, 38 males were right handed, 1 was left handed and 2 were ambidextrous. In the ADHD group, 28 were right handed, and 1 was left-handed. A Chi-square test for independence indicated no significant association between handedness between ADHD and control children, $\chi^2 (3, 73) = 6.29$, $p = .10$. Thirty two of the ADHD participants were medication naïve and the remaining 13 underwent a 48 hour wash out period for stimulant medication, in line with current EEG practice.

There were significant differences in composite (overall) IQ scores with higher mean scores in the control group (M = 120.89, SD = 13.34), compared to ADHD (M = 96.51, SD = 12.70), $t(57) = -7.19$, $p > .001$. It should be considered that the selection of groups recruited in this study was not random (as in most case-control studies) and therefore covarying for IQ would violate the ANCOVA assumptions arguably altering the group effect in potentially problematical ways arguably resulting in

spurious findings (Bridgett & Walker, 2006; Dennis et al., 2009; Miller & Chapman, 2001). However, to address potential confounds of IQ, all significant between group measures were correlated with IQ in each group.

Procedure & Materials

Children and adolescents below the age of 16 years old were accompanied by an appropriate adult (parent, teacher or guardian) to the Institute of Psychiatry at The Maudsley Hospital where a blood sample was taken by a qualified phlebotomist. Blood assessments required 8 hours fasting beforehand, although water was permitted, and this was explained fully to the parent and child prior to the appointment. The blood was then transported by motor-cycle courier to the Science Centre at the London Metropolitan University where it was spun before storing at -80 degrees Celsius. Following the blood sample all participants were given a complimentary breakfast at the Maudsley restaurant. They were then taken to a quiet, well lit testing room to complete all the self-reported questionnaires. Instructions were given for each questionnaire and assistance during completion where necessary. All participants in the healthy control group were given also an EEG/ERP assessment. On occasion, data collection took place over 2 visits, with the child assessment and screening sometimes taking place at school. Participants were given £20.00 for their participation and all associated travel expenses were refunded to the parent/guardian. During the MAAFA trial, any children taking stimulant medication for ADHD were required a wash-out for 48 hours prior to the EEG recording.

Blood Analysis

Total lipids were extracted from 1 ml of plasma according to the Folch method[36]. The red cells were homogenized in chloroform and methanol (2:1 v/v) containing 0.01% butylated hydroxytoluene as an antioxidant, under nitrogen. Fatty acid methyl esters (FAMEs) were prepared by heating the extracted total lipid in 4ml of 15% acetyl chloride in methanol for 3h at 70°C, under nitrogen in a sealed vial. FAMEs were separated by a gas-lipid chromatograph (HRGC MEGA 2 series, Fisons Instruments, Italy) fitted with a capillary column (60 m x 0.25 mm x 0.25 μm, BPX70). Hydrogen was used as a carrier gas, and the injector, oven and detector temperatures were 235°C, 200°C and 250°C, respectively. FAMEs were identified by comparison with relative retention times of authentic standards and calculation of equivalent

chain length values. Peak areas were quantified by a computer chromatography data system (EZChrom Chromatography Data System, Scientific Software Inc., San Ramon, CA, USA). This study reports blood data from plasma phopshatidylcholine (PPC) measures only.

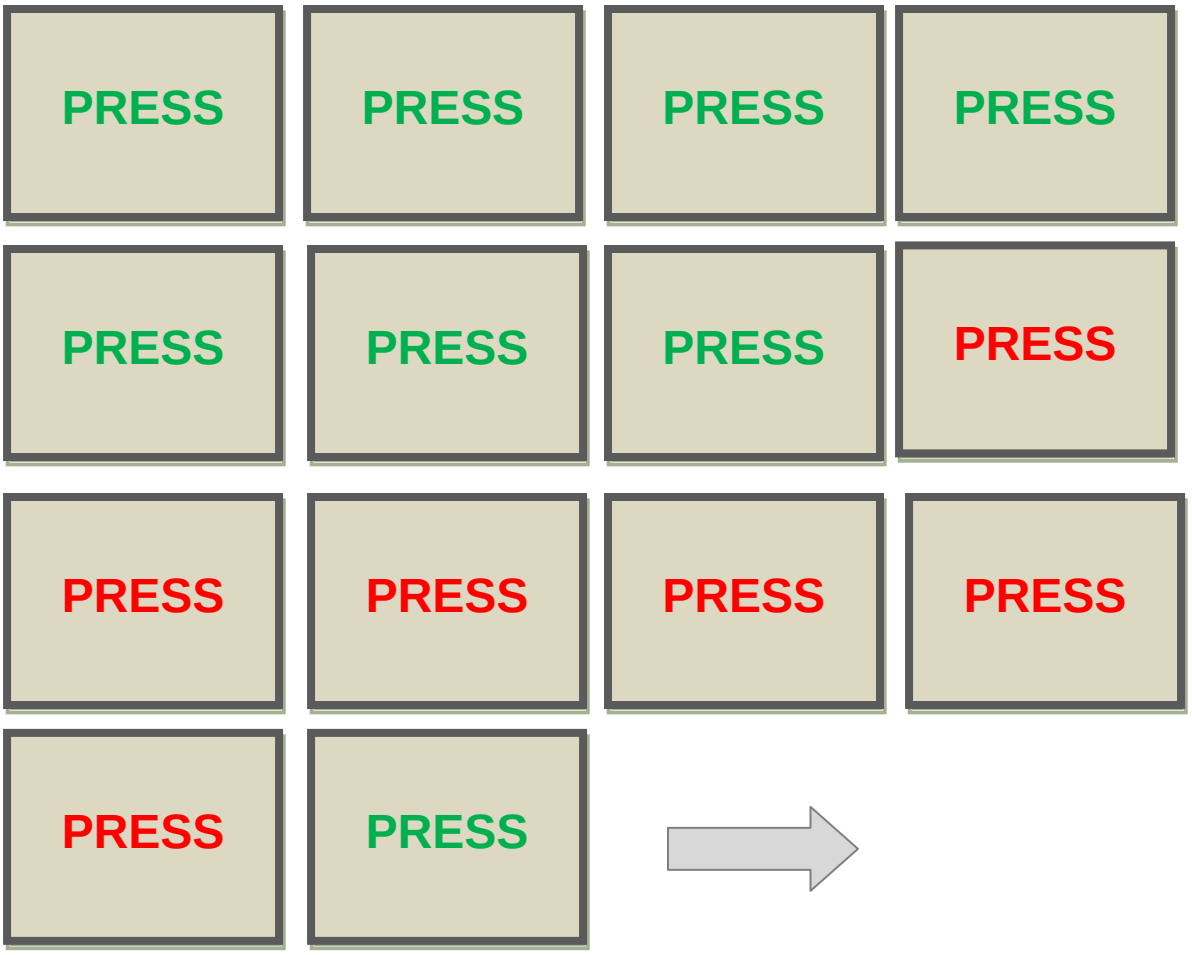
ERP recording

EEG data were collected from 26 electrodes using an adapted 10-20 system following an internationally standardized protocol LabNeuroTM (Brain Resource, 2010). Participants sat in a light and sound attenuated room with an ambient temperature of 24˚C. A NeuroScan Quik cap and NuAmps amplifier (sampling rate = 500 Hz) were employed to collect EEG data from electrode sites. Data was recorded relative to a virtual ground, but referenced offline to linked mastoids. Horizontal eye movements were recorded with electrodes placed 1.5cm lateral to the outer canthus of each eye. Vertical eye movements were recorded with electrodes placed 3mm above the middle of the left eyebrow and 1.5cm below the middle of the left bottom eye-lid. Skin resistance was < 5 kOhms. A continuous acquisition system was employed and data was EOG corrected offline (Gratton, Coles, & Donchin, 1983). A low pass filter with attenuation of 40dB per decade above 100 Hz was employed prior to digitision.

ERP area under the curve

Average ERPs were calculated for event types corresponding to a stimulus type in each paradigm. For each channel, the individual single-trial epochs were filtered with a low-pass Tukey filter function that attenuates frequencies above 25 Hz. A cosine ramp from 1 down to 0.5 between 25Hz and 35Hz is used as an envelope on the FFT data in the Tukey filter. The single trials were then averaged to form conventional ERPs. The averages of the pre-stimulus period -300 to 0 ms were subtracted from the ERP data. The signal was then down sampled by a factor of 4 (leading to 8 ms samples). The amplitude of the waveform (in microvolts) relative to the zero baseline is calculated for single time points in 8ms, then multiplied by a factor of 8 to achieve a measure of the area under the curve in a unit of microvolt-milliseconds. The AUC is the integral of the curve over a specified time range. Using the AUC measure, the space between the ERP waveform and baseline was divided into multiple 50ms time-windows that can be mapped onto ERP component time-windows. P2 is mapped onto time unit 5 (200-250 ms), N2 on to time unit 6 (250-300 ms), P3a onto time unit 7 (300-350 ms) and P3b (350-400 ms) on time unit 8. Units are in microvolts multiplied by ms.

Go/NoGo (response conflict inhibition) task



The word, 'PRESS' was flashed alternately in green and red colours. The Go stimuli ("PRESS" in green letters appeared in the centre of a black screen) and the NoGo stimuli ("PRESS" in red in the centre of a black screen) were presented to participants for 500 milliseconds (ms) with an interstimulus interval (ISI) of 1143 ms, with the aim of building up a prepotent-response tendency. Seven blocks of 6 No/Go each (i.e., in total 42 NoGo stimuli = 25% or trials) were interspersed between 7 blocks of Go stimuli (i.e., 3 blocks containing 12, two 18, one 24 and one 30 Go stimuli (i.e., in total 126 stimuli, 75% of trials). All participants received standardised visual and auditory instructions to tap a response box by pressing left and right buttons simultaneously upon the appearance of the Go stimuli and to withhold response upon the appearance of the NoGo stimuli. All participants completed a quick practice test prior to performance to ensure that the task instructions were understood. In total the test lasted 7 minutes. The dependent variables of the Go process of the task were mean reaction time and

omission errors to Go stimuli. The dependent variables for the inhibitory process were commission errors (responses to NoGo stimuli).

Results

Behavioural Data (Reaction times and error rates)

The data did not meet criteria for parametric tests (viz, Homogeneity of Variance and Normality) for any of the behaviour measures of reaction time, commission errors (i.e., to NoGo stimuli) or omission errors (i.e., to Go stimuli). Omission errors consisted of Go stimuli which participants did not respond to, whereas commission errors occurred whenever participants responded erroneously to NoGo stimuli which they were expected to inhibit.

A series of Mann Whitney U tests for non-parametric data were conducted. There were no significant differences found in reaction time between the children and adolescents in the ADHD group, $U = 555.00$, $Z = -.78$, $p > .05$. The ADHD group made significantly more omission errors than the control group, $U = 379.00$, $Z = -2.89$, $p > .01$, whereas both groups did not differ significantly for commission errors, $U = 495.00$, $Z = -1.49$, $p < .05$. The data are plotted in Figure 1 below.

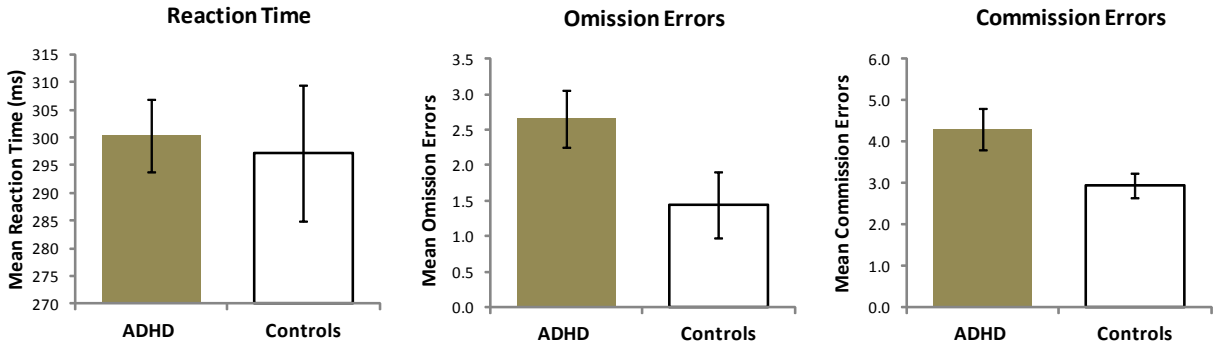


Figure 1. Mean reaction time including errors (left panel), mean omission errors (left middle panel), and mean commission errors (right middle panel) for ADHD ($n = 43$) and control children/adolescents ($n = 29$), along with standard errors for the Go/NoGo task.

Electrophysiological data

For statistical purposes, a series of 2 x 4 x 2 analysis of variance (ANOVAs) for repeated measures were carried out on the data. The between subjects factor was *group* (ADHD versus control children), while *condition* (Go versus NoGo) and *electrode position* (Fz, FCz, Cz, and Pz) were the within subjects factors. The dependent measures were each time point AUC amplitude range (time point 5 to 8). The time points correspond to either positive and negative deflections or waveforms: time point 5/P2: 200-250 ms, time point 6/N2/N250 complex: 250-300 ms, time point 7/P3a: 300-350 ms and time point 8/P3b: 350-400 ms. The mean values (μV) and standard errors for each corresponding AUC amplitude responses for both ADHD and control children are plotted in Figures 2 to 5. Significant main effects of electrode site or condition were not further followed up with post-hoc tests. However, all group or interaction findings were followed up with post-hoc tests and pairwise comparisons. Bonferoni corrected for multiple testing.

Time point 5 (P2 deflection)

The mean values (μV) and standard errors for the P2 amplitude response for electrode position (Fz, FCz, Cz and Pz) for ADHD and control children are plotted in Figure 2. The grand average curves of the AUC ERPs of children/adolescents with ADHD and healthy controls across all AUC timepoints (P2, N2, P3a and P3b) for Go and NoGo at electrode positions: Fz, FCz, Cz and Pz are illustrated in Figure 2.

Mauchly's test indicated that the assumption of sphericity had been violated for electrode site, χ^2 (5) = 106.14, $p < .05$ and for the interaction between condition and electrode site, χ^2 (5) = 62.19, $p < .05$. This was corrected for using Greenhouse-Geisser estimates of sphericity. The corrected results are reported as follows.

There was no significant main effect for the between-subjects factor group (ADHD versus control), $F < .1$. There were significant main effects for the within-subject factors, electrode site (Fz, FCz, Cz and Pz), $F(1.58, 111.89) = 41.87$, $p < .001$ this was driven by greater activation at Pz ($M = 395.02$, $SE = 32.32$), and condition (Go versus NoGo), F (1, 71) = 9.13, $p < .01$ which was driven by greater activation during the Go condition compared to NoGo. There was a significant interaction between electrode site and condition, F (1.98, 140.37) = 32.28, $p < .001$.

Post-hoc tests showed that this was due to a significantly greater activity at Pz electrode site in the Go condition compared to the NoGo condition, $p < .001$. No other interactions reached significance, $F < 1$.

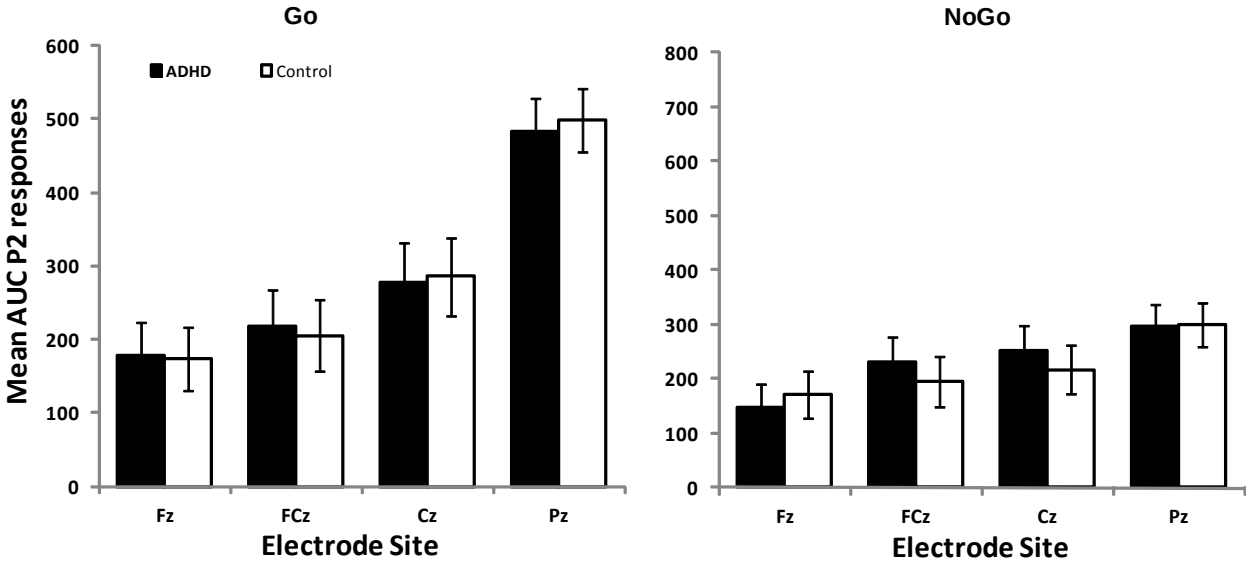


Figure 2. Mean AUC P2 amplitude responses for ADHD and Control participants in the Go (left panel)/NoGo (right panel) task across frontal (Fz), frontal-central (FCz) central (Cz), and parietal (Pz) electrode sites.

Time point 6 (N2 deflection)

The mean values (μV) and standard errors for the N2 amplitude response for electrode position (Fz. FCz, Cz and Pz) for ADHD and control children are plotted in Figure 3.

Mauchly's test indicated that the assumption of sphericity had been violated, χ^2 (5) = 95.47, $p < .05$ for electrode site and the interaction between condition and electrode site, χ^2 (5) = 62.68, $p < .05$. This was corrected for using Greenhouse-Geisser estimates of sphericity. The corrected results are reported as follows. At time point 6 (N2), the main effect of the between-subjects factor group (ADHD versus control) was not significant, $p > .05$. Significant main effects were found for the within-subject factors, condition (Go versus NoGo), $F(1, 70) = 9.69$, $p < .01$, as per before this was driven by greater positive going activity at Pz ($M = 520.73$, $SE = 33.74$), and electrode site (Fz, FCz, Cz and Pz), $F(1.67, 116.78) = 155.70$, $p < .001$ again driven by greater positive going activity in the Go condition. There was a significant interaction between electrode site and condition, $F(2.00, 139.99) = 62.04$, $p < .001$. Post-hoc tests confirmed that, as per before, there was significantly greater activity at Pz electrode site in the Go condition compared to the NoGo condition, $p < .001$. There was a trend finding for the 3 way interaction between condition, electrode site and group, $p = .079$. Post-hoc tests confirmed that this was driven by significantly greater activation during the Go condition at Pz for both ADHD (Go: $M = 693.28$, $SE = 46.28$ versus NoGo: $M = 327.53$, $SE = 48.09$) and healthy control (Go: $M = 685.41$, $SE = 56.47$ versus NoGo: $M = 376.67$, $SE = 58.56$) groups. The group interaction arose because Pz was larger in controls for Go trials, but larger for ADHD during NoGo. No other interactions reached significance, $F < 1$.

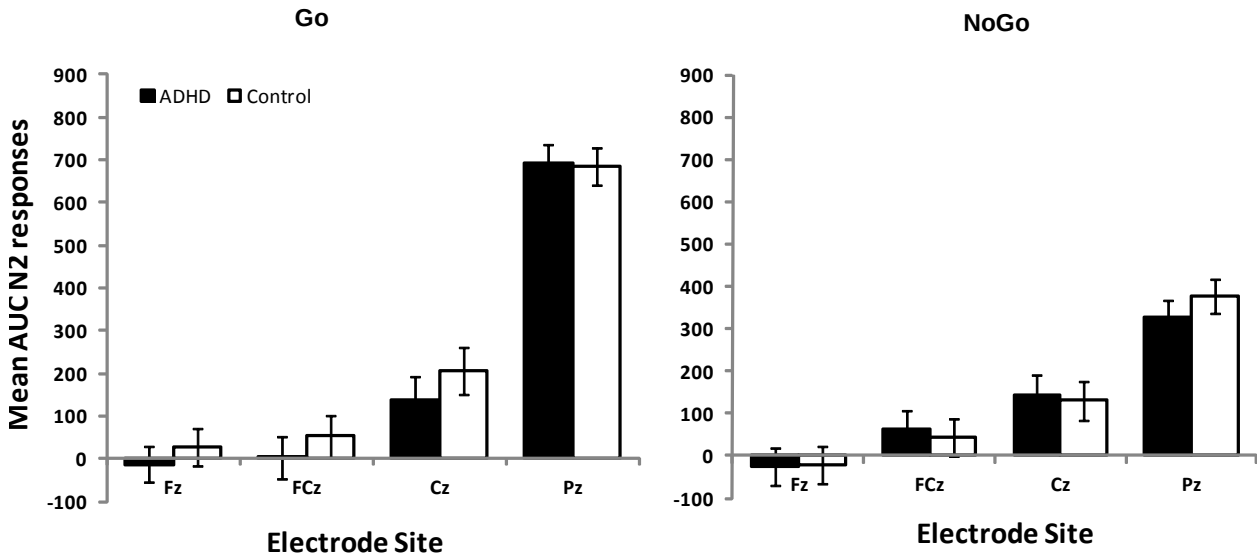


Figure 3. Mean N2 amplitude responses for ADHD and Control participants in the Go (left panel)/NoGo (right panel) task across frontal (Fz), frontal-central (FCz), central (Cz), and parietal (Pz) electrode sites.

Time point 7 (P3a deflection)

The mean values (μV) and standard errors for the AUC P3a amplitude responses for electrode position (Fz, FCz, Cz and Pz) for ADHD and control children are plotted in Figure 4. Mauchly's test indicated that the assumption of sphericity had been violated, χ^2 (5) = 111.79, p < .05 for electrode site and the interaction between condition and electrode site, χ^2 (5) = 65.47, p < .05. This was corrected for using Greenhouse-Geisser estimates of sphericity. The corrected results are reported as follows. For the P3a response amplitude (time point 7), the main effect of the between-subjects factor group (ADHD versus control) was not significant, F <1. The within-subjects factor condition (Go versus NoGo) was not significant F < 1. The main effect of the within-subjects factor, electrode position (Fz, FCz, Cz, Pz) was significant, F(1.57, 110.42) = 493.43, p < .001, and driven by greater positive going activity at Pz, (M = 445.00, SE = 28.11). There was a significant interaction between electrode position and condition, F (3, 210) = 35.17, p < .001. Post-hoc tests showed that this was due to significantly greater positive activity at Pz electrode site in the Go condition compared to the NoGo condition, p < .001. The 3-way interaction electrode position, condition and group was also significant, F(3, 210) = 4.41, p < .01. Post-hoc tests showed that this was due to a trend level in increased positivity at Fz, in the Go condition in the HC group compared to ADHD, p = .081. No other interactions reached significance, F < 1.

The waveform data shows a visible shift towards an increase in positivity which is greatest at the Pz electrode site in the Go condition, see Figure 6.

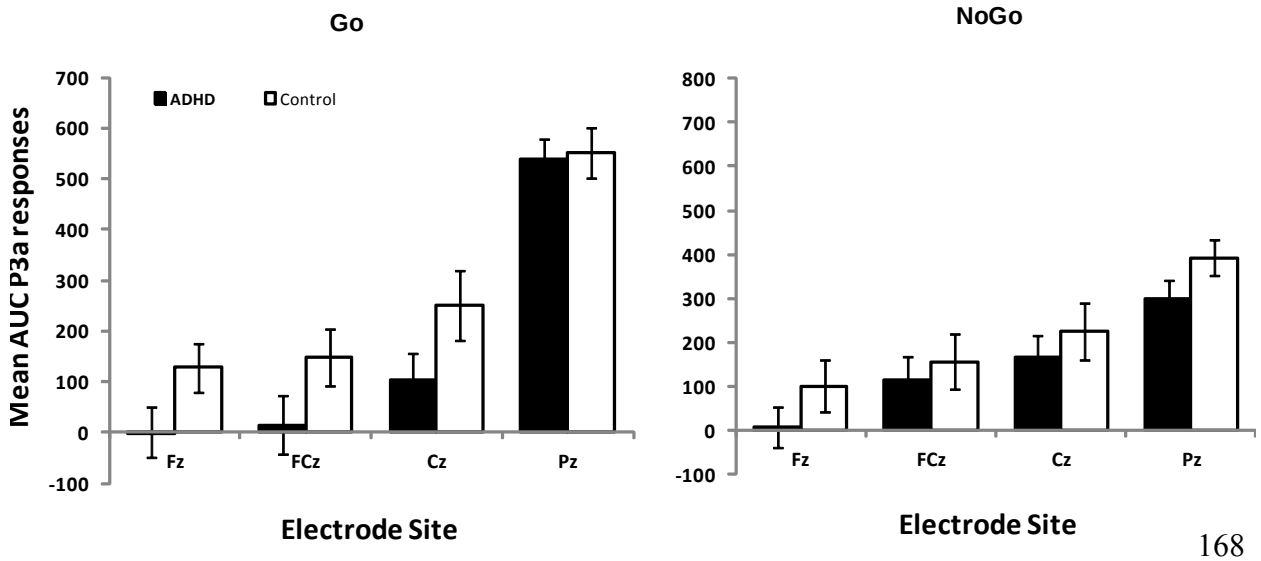


Figure 4. Mean P3a amplitude responses for ADHD and Control participants in the Go (left panel)/NoGo (right panel) task across frontal (Fz), frontal-central (FCz), central (Cz), and parietal (Pz) electrode sites.

Time point 8 (P3b deflection)

The mean values (μV) and standard errors for the mean AUC P3b amplitude responses for electrode position (Fz. FCz, Cz and Pz) for ADHD and control children are plotted in Figure 5. Mauchly's test indicated that the assumption of sphericity had been violated, χ^2 (5) = 97.116, p < .05 for electrode site and for the interaction between condition and electrode site, χ^2 (5) = 50.32, p < .05. This was corrected for using Greenhouse-Geisser estimates of sphericity. The corrected results are reported as follows. For the P3b amplitude, there was no significant effect for the main effect of group (ADHD versus control), p > .05. For the within-subject factors, significant main effects were found for electrode site (Fz, FCz, Cz and Pz), F(1.64, 116.72) = 34.63, p < .001 with a higher mean activation again at Pz (M = 249.62, SE = 27.29). There was a trend finding for the main effect of condition (Go versus NoGo), F(1, 71) = 3.52, p = .065, driven by greater positive going activation during NoGo trials (M = 159.16, SE = 28.32) compared to Go (M = 104.37, SE = 31.03). There were significant interactions between electrode site and condition, F(2.17, 154.12) = 21.55, p < .001. Post-hoc tests showed that this was due to significant differences between all electrode sites (Fz, FCz, and Cz) and condition (Go versus NoGo), p < .05. The greatest mean difference was at FCz which was higher in the NoGo condition (M = 155.44, SE = 31.66) relative to Go (M = 24.41, SE = 35.93). There was a 3-way interaction between electrode site, condition and group, F(3, 213) = 2.89, p < .05. Post-hoc tests showed that this was due to a trend level finding for group (p = .077) observed at Cz in the Go condition with a higher mean difference in activity for controls (M = 165.75, SE = 59.08) compared to ADHD (M = 27.40, SE = 49.35). There were significant differences also in the ADHD group only between all electrode sites (Fz, FCz, Cz and Pz) and condition (Go versus NoGo), p < .05.

No other interactions reached significance, F < 1.

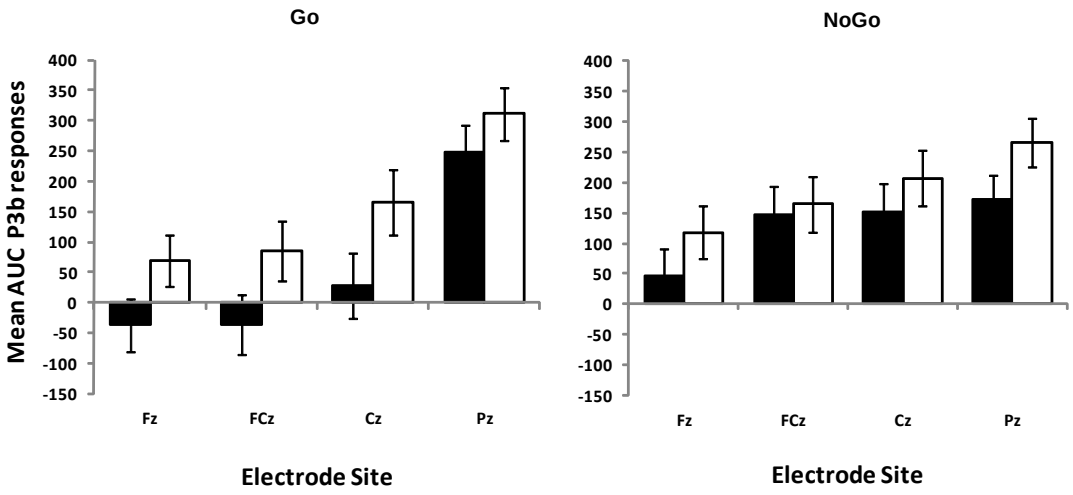


Figure 5. Mean P3b amplitude response for ADHD and Control participants in the Go (left panel)/NoGo (right panel) task across frontal (Fz), frontal-central (FCz), central (Cz), and parietal (Pz) electrode sites.

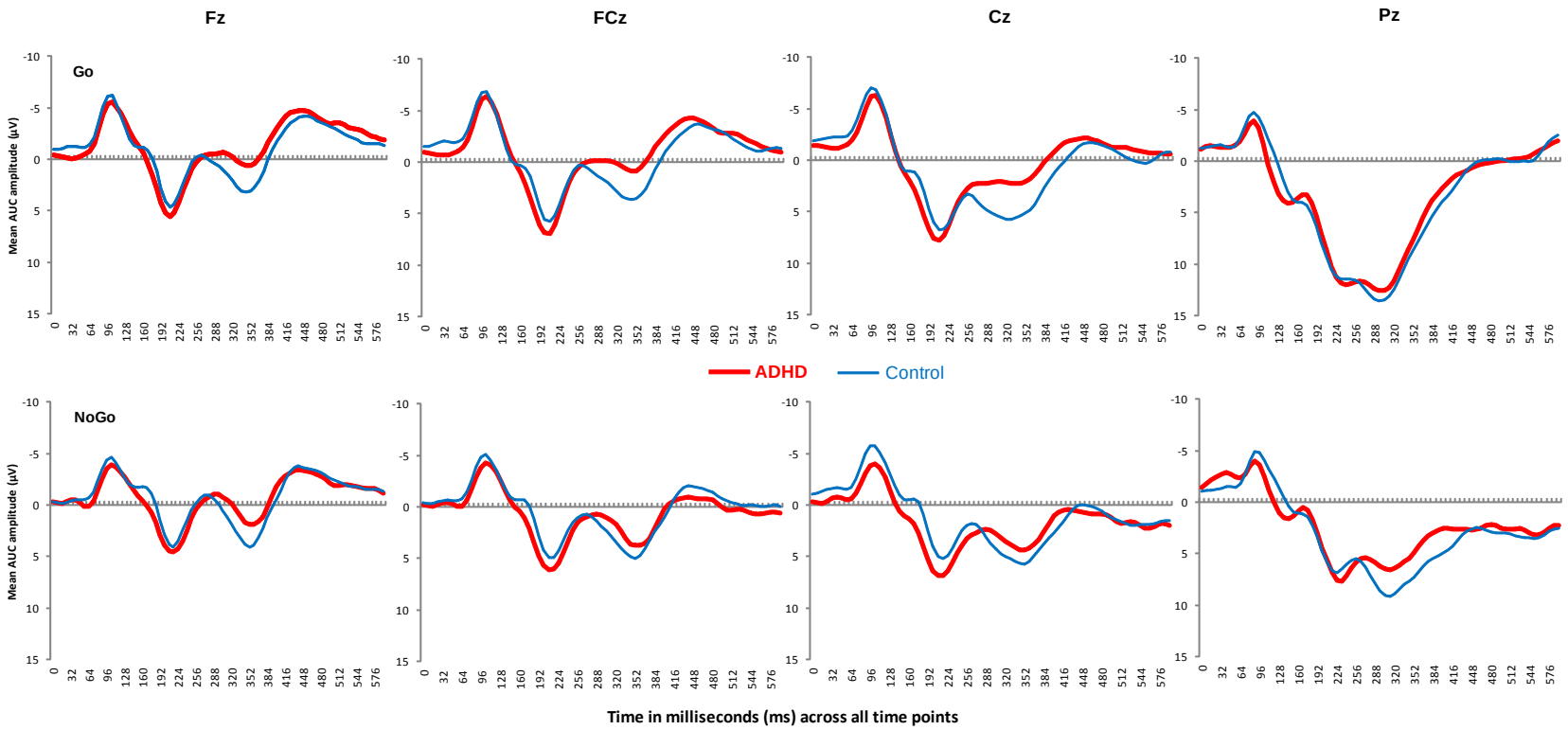


Figure 6. Grand average curves of the AUC ERPs of children/adolescents with ADHD (bold line) and healthy controls (thin line) across all timepoints (P2, N2, P3a and P3b) for Go (top panel) and NoGo (bottom panel) at electrode positions: Fz, FCz, Cz and Pz.

IQ and correlations for significant results

To test for potential confounds of IQ, all significant between-group findings were correlated with IQ. The Kolmogorov-Smirnov test revealed that scores were normally distributed for the ERP responses for both P3a and P3b responses at Fz and Cz to targets (Go condition) in both the ADHD and the healthy control (HC) group and therefore met the criteria for parametric data. Omissions errors were not normally distributed and therefore Spearman Rho for non-parametric data was applied. Pearson correlations showed that P3a responses at Fz to Go stimuli were significantly positively associated with composite IQ in the HC group, $r(29)$, .404, $p = .03$. The relationship however was not significant in the ADHD group, $p > .05$. There were no significant relationships between P3b responses at Cz to Go stimuli and composite IQ in the HC or ADHD groups, $p > .05$. Finally, omission errors were not significantly related to IQ scores in either the HC or ADHD groups, $p > .05$.

LC-PUFA Blood Data between Children/Adolescents with ADHD and Healthy Control Children

The means and standard deviations are presented in Table 1. For all plasma choline phosphoglycerides (PPC) analyses, a series of independent-samples *t* tests were conducted to compare the plasma fatty acid levels between ADHD and control children for Go/NoGo task. As per previously reported in chapter 7, the key fatty acid indices chosen were from the omega-3 and omega-6 series, namely (1) c18:3n-3 (ALA), (2) c20:5n-3 (EPA), (3) c22:5n-3 (DPA), (4) c226n-3 (DHA) and (5) Total n-3 and (6) c18:2n6 (LA), (7) c18:3n6 (GLA), (8) c20: 4n6 (AA) and (9) total n-6 respectively. Given the large number of tests, the false discovery rate correction for multiple testing was employed for all analyses (Benjamini & Hochberg, 1995; this correction procedure is more conservative with the lower *p* values, but not as conservative as a Bonferroni correction). The relationships that survived correction only are reported below.

Go/NoGo task

There were significant differences between the ADHD and control groups for nine out of the thirteen fatty acids indices any of the omega-3/6 fatty acid levels. From the omega-6 series there were significant differences for c20:2n6, $t(63) = -3.19$, $p = .003$ which were higher in controls, c20:3n6, $t(65) = -3.66$, $p = .001$ which were higher in controls, c20:4n6 (AA), $t(65) = -$

9.70, p = .001 which were higher in controls, c22:4n6 (a metabolite of AA), $t(65) = -5.61$, p = .001 which were higher in controls, and total n-6, $t(65) = -4.67$, p = .001 which were higher in controls.

From the omega-3 series, there were significant differences between ADHD and control children for c18:3n3 (ALA), $t(64) = -4.20$, p = .002 which were higher in controls, c20:5n3 (EPA), $t(64) = -5.74$, p = .003 which were higher in controls, c22:5n3 (DPA), $t(64) = -6.43$, p = .004, which were higher in controls, c22:6n3 (DHA) $t(66) = -10.09$, p = .006 which were higher in controls and total n-3 $t(64) = -10.85$, p = .01 which were higher in controls.

***Table 1*. Mean LC-PUFA fractions in plasma choline phosphoglycerides (PPC) in ADHD and HC groups for the Go/NoGo task**

Go/NoGo Task (ADHD: n = 29, HC: n = 38)					
	ADHD		**Healthy Control**		
	M	*SD*	*M*	*SD*	
Omega 6					
c18: 2n-6 (LA)	25.22	3.74	24.76	3.44	
c18: 3n6	0.11	0.07	0.10	0.06	
c20: 2n6	0.26	0.05	0.31	0.08	**
c20: 3n6	2.90	0.68	3.67	0.96	**
c20: 4n6 (AA)	7.58	1.08	11.63	2.25	**
c22: 4n6	0.29	0.06	0.39	0.08	**
c22: 5n6	0.31	0.24	0.25	0.08	
Total n6	36.65	4.43	41.11	3.39	**
Omega 3					
c18: 3n3 (ALA)	0.22	0.08	0.34	0.13	**
c20: 3n3	-	-	0.13	0.04	
c20: 5n3 (EPA)	0.51	0.20	0.99	0.44	**
c22: 5n3	0.60	0.25	1.06	0.31	
c22: 6n3 (DHA)	1.83	0.39	3.71	0.92	**
Total n3	3.18	0.51	6.23	1.42	**

Note: * p < .05, ** p < .01

PUFA fractions and ERP measures

The Kolmogorov-Smirnov test revealed that scores were normally distributed for all ERP measures and therefore met the criteria for parametric data. Pearson coefficients were calculated to assess relationships between both Go (Targets) and NoGo (Backgrounds) conditions and 7 key fatty acid indices. The strength of the associations between the fatty acid indices (omega-6: ALA, c18:3n6, AA, c20:4n6 and Total n-6 and omega-3: ALA: c18:3n3, EPA: c20:5n3, DHA: c22:6n3 and Total n-3) and earlier and later P3 (P3a and P3b) amplitudes measures at four electrode sites (Fz, FCz, Cz, Pz) were assessed using Pearson correlation coefficients. The performance data however were not normally distributed (e.g., behaviour measures of reaction time, errors of commission and errors of omission) and therefore Spearman Rho coefficients were conducted for non-parametric data. All analyses were subjected to correction for multiple testing employing the Benjamini and Hochberg False Discovery Rate (FDR).

Performance data (omission and commission errors only)

In the control group, the omega-3, EPA, was significantly negatively correlated with errors of commission, $r(29)$, -.399, $p = .032$ only. No other relationships reached significance, $p > .05$.

P3a and PUFA fractions

In the ADHD group for P3a amplitude responses to Go stimuli (Targets) there was a significant negative relationship between electrode site Cz and Total n-3, $r(40)$, -.491, $p = .001$ (adjusted $p = .056$). There were also significant negative relationships between Pz and c22:6n3 (DHA), $r(40)$, -.471, $p = .002$ (adjusted $p = .037$) and Pz and Total n-3, $r(40)$, -.492, $p = .001$ (adjusted $p = .028$).

None of the relationships in the control group survived correction. The two largest non-significant correlations were observed in the NoGo condition (Backgrounds) at frontal electrode sites (Fz and FCz) and EPA. The first correlation, EPA was negatively correlated with the Fz electrode site, r (31), -.512, $p = .003$ and the second correlation was observed also between EPA and P3a responses to Backgrounds at FCz electrode site, $r(31)$, -.491, $p = .005$.

P3b and PUFA fractions

In the ADHD group for P3b amplitude responses to Go stimuli (Targets) there was a significant negative relationship between electrode site Cz (which was reduced relative to controls during the Go condition) and Total n-3, $r(40)$, -.531, $p = .001$ (adjusted $p = .056$). There were also significant relationships between P3b amplitudes responses at Cz and c22:6n3 (DHA), $r(40)$, -.488, $p = .001$(adjusted $p = .018$). There were also significant negative relationships between P3b amplitudes responses at Pz and c22:6n3 (DHA), $r(40)$, -.563, $p = .001$ (adjusted $p = .014$), and between P3b amplitudes responses at Pz and Total n-3, r (40), -.530, $p = .001$ (adjusted $p = .028$). At FCz, there was a significant negative relationship between Total omega-3 and P3b amplitude responses to Targets, $r(40)$, -.418, $p = .007$ which only survived correction at trend level, adjusted $p = .065$.

In the control group, for responses to NoGo stimuli (backgrounds) there were significant negative relationships between c20:5n3 (EPA) and FCz, $r(31)$, -.550, $p = .001$ (adjusted was significant at trend level only, $p = .056$), and c20:5n3 (EPA) and Fz, $r(31)$, -.506, $p = .001$ (adjusted was significant at trend level only, $p = .074$). None of the other relationships survived correction.

Brief Discussion

The results of the performance data showed that the children and adolescents in the ADHD group made significantly more omission errors relative to controls, but differences in commission errors, although higher in the ADHD group, did not reach significance. The findings suggest that deficits were more pronounced for sustained and selective attention, which are thought to be reflected by high omission errors (Brodeur & Pond, 2001; Halperin, et al., 1991) rather than in the inhibition process. The ERP findings were in line with the behavioural findings. Contrary to our prediction, the electrophysiological findings showed no significant differences in N2 or P3 ERP deflections or at any of the other time points between ADHD and controls. Furthermore, there were no specific ERP deficits for the inhibitory process of the task. There were, however, significant interactions between group, condition and electrode demonstrating that ADHD children had significantly lower P3a deflection at Cz for Go trials as well as lower P3b waves at Fz during Go trials suggesting that deficits in ERPs in ADHD were specific over fronto-central brain regions during the Go process of the task. There were significant differences as predicted in key omega-3 and omega-6 fatty acids indices. There was an overall pattern with persistently higher levels of both omega-3/6 in the healthy control group compared to ADHD. In addition, there were several significant inverse relationships between P3 ERP responses to Go trials (targets) and omega-3 fatty acids in the ADHD group. Specifically, both total omega-3 and DHA were negatively related to early and late P3 amplitude responses at both centro and parietal scalp regions to Go trials. In the healthy control group, there were also some inverse relationships at trend level only between P3b and EPA at both frontal and frontocentral regions, during the NoGo process of the task. Finally, there was a significant negative relationship between EPA and errors of commission in the healthy control group, in line with the notion that more impulsive performance is associated with lower levels of omega-3 in controls.

Group differences in performance and ERP measures

In relation to performance, the ADHD group made more omission errors compared to the healthy control children which is in line with previous studies and reflects poor attention processes (Brodeur & Pond, 2001; Fallgatter, et al., 2004; Fisher, et al., 2011; van Leeuwen, Steinhausen, Overtoom, Pascual-Marqui, van't Klooster, et al., 1998). The absence of a group

difference in commission errors is likely to be due to the block design of the trial which in turn had a lower inhibitory load compared to other Go/NoGo task designs (for example, during which Go and NoGo stimuli are presented in random order with 50% probability of each occurring) consequently making performance easier. Although, the number of commission errors did not reach statistical significance, children with ADHD had a greater mean number of commission errors compared to control children.

There were no significant differences in any of the ERP measures. However, there was a significant finding for the 3-way interaction between group, condition and electrode site at both time points 7 and 8, i.e., P3a and P3b deflections respectively. This was due to increased positivity in the healthy control group during Go trials at frontocentral sites (Fz and Cz respectively) relative to ADHD, suggesting differences in the executive processes involving selective attention, response selection and motor response execution (Eagle, Bari, & Robbins, 2008). Responses to Go trials reflect the executive process of the task involved in attention allocation and response selection (Rubia, Russell, et al., 2001). The series of Go trials appear more frequently than NoGo, allowing a prepotency to develop towards response execution (Johnstone, et al., 2007). Furthermore, the finding of enhanced responses to Go in healthy control children lends some support to previous findings (Fallgatter, et al., 2004). A study by Liotti et al. (2005) also found enhanced P3a waves in control children appears which they suggested reflect more resourceful monitoring or successful execution during processes associated with response inhibition. Furthermore, that the observed reduction in amplitudes of ADHD children is likely to signify a shortfall in this inhibitory process (Liotti, Pliszka, Perez, Kothmann, & Woldorff, 2005). Finally, the enhanced responses to Go trials coupled with the findings of fewer omission errors indicate overall better performance in executive processing in the healthy controls compared to ADHD. The results of this study do not support enhanced P3 to NoGo trials in healthy controls which as discussed by Smith et al. (2007) are thought to reflect inhibition or conflict during inhibitory tasks. This is likely to be due to task design which will be discussed further in the limitations section. A final note is that midline P3a responses in healthy controls were significantly associated with composite IQ scores which suggest the possibility that this group difference may be associated with the lower IQ in ADHD cases rather than the ADHD pathology itself. However, this was the only wave which correlated with IQ.

Across all participants, there were, as expected, significant ERP differences between the Go and NoGo conditions capturing the executive (Go) and inhibitory (NoGo) processes of the task respectively. For the earlier deflections (e.g., time points: 5: P2 and, 6: N2), and also for time point 7 (P3a) significant interactions were observed between electrode site (Fz, FCz, Cz and Pz) and condition (Go versus NoGo), with consistently greater activity at parietal sites for Go stimuli compared to NoGo in all cases. This finding for greater activation for Go, maximum at Pz is a common finding during tasks of conflict response inhibition and is thought to be associated with inhibitory processes (Fallgatter, et al., 2004). At the later P3b wave, the significant interaction between electrode site and condition confirmed that the greatest mean difference was at fronto-central (FCz) electrode sites for the NoGo condition relative to Go, this effect is commonly found in NoGo tasks and is referred to as the NoGo-P3 (Banaschewski, et al., 2004).

Overall, the findings of this study do not support previous research suggesting abnormal N2 and P3 ERP responses in ADHD compared to control children during NoGo trials in Go and Stop tasks (Fallgatter, et al., 2004; Johnstone & Barry, 1996; Karayanidis, et al., 2000; Pliszka, Liotti, & Woldorff, 2000a; Smith, Johnstone, & Barry, 2004). The absence of between groups differences however is consistent with previous research reporting no significant differences in N2 waves between ADHD and control children (Overtoom, et al., 1998; van Leeuwen, Steinhausen, Overtoom, Pascual-Marqui, van't Klooster, et al., 1998). It is important also to note that this task was a block design and thus the inhibitory load was smaller than in other previous event-related potential tasks. NoGo trials were presented together in blocks and thus only the first NoGo in a block had a high inhibitory load while all subsequent NoGo trials may have caused adaptation to the inhibitory process. In addition, it was a mix between motor and interference inhibition and the literature in interference inhibition in ADHD is controversial with the latter producing less impairment in ADHD compared to during tasks measuring motor inhibition alone (Homack & Riccio, 2004; Rubia et al., 2006a). For example, the N2 wave indicates the inhibition of a deliberate response in Go/NoGo tasks and in our study, the task had a low load on inhibition, this is likely to be the reason no group differences were apparent. Another potential reason for the negative findings in ERP measures of inhibition could be that the children with ADHD were selected from a community sample. Clinically referred cases with ADHD, which have been recruited in most of the cited previous ERP studies, are likely to have

more severe symptoms of ADHD compared to community ADHD patients (Sprafkin, Gadow, Weiss, Schneider, & Nolan, 2007).

Blood measures of LC-PUFA and associations between performance and ERPs

The LC-PUFA levels in plasma choline phosphoglycerides measures were contrasted between the children with ADHD recruited during the MAAFA trial and the healthy control group. There were significant differences as predicted in key omega-3 and omega-6 fatty acids indices. Overall there was a pattern of persistently higher levels of both omega-3/6 in the healthy control group compared to ADHD. These findings support previous research suggesting abnormal levels of fatty acids in ADHD (Antalis, et al., 2006; Stevens, et al., 1996; Stevens, et al., 1995; Young, et al., 2004). These findings warrant further investigation to explore whether the differences reflect dietary intakes of fatty acids or alterations in fatty acid metabolism.

This study reports for the first time, that EPA was negatively associated with the inhibitory measure of the task, namely commission errors, in healthy controls only. This suggests that higher levels of EPA are related to lower errors capturing inhibitory processes in the HC group. Although, the ADHD group had a higher mean number of commission errors compared to the HC group, this did not reach significance. The fact that the HC made less commission errors and that this was negatively associated with EPA suggests a potential beneficial role in the regulation of inhibitory control. However, further research would be necessary to support these preliminary findings.

In relation to the PUFA fractions and ERP amplitude measures, significant negative relationships were observed at centroparietal scalp regions with both total omega-3 and DHA in the ADHD group. These relationships suggest that as omega-3 increases P3a amplitude responses to targets (Go stimuli) decrease. Also, in the ADHD group, P3b responses to Go stimuli were similarly negatively associated with omega-3 indices namely total omega-3 and DHA across fronto-central, central and parietal scalp regions (FCz, Cz and Pz), suggesting that as omega-3 measures increased P3b amplitude responses decreased. The frontocentral regions were found to be reduced in activation in ADHD and furthermore associated with fatty acid measures. However, the 3 way interaction between group, electrode site and condition confirmed that group was only significant following correction at trend level only. Of interest, Go stimuli are associated with response selection and selective attention and the associated frontocentral

ERP waves are in turn related to anterior cingulate cortex and supplementary motor area (SMA) function and reported to be impaired in ADHD (Rubia, Russell, et al., 2001). It is well documented that children with ADHD have difficulty with attention networks involving response preparation and arousal (e.g., during the preliminary phase of information processing) (Engert & Pruessner, 2008; Nigg, 2005). It may be that during the succession of Go trials in the present study, which required both alertness and response preparation during stimulus execution (i.e., a repetitive succession of button presses), that omega-3 levels were unable to modulate attentional processes in this group hence resulting in lower activation. It should be noted that several omega-3 fatty acid indices including DHA were lower in ADHD compared to healthy control children. ADHD is a disorder associated with a dysregulation of noradrenergic function and faulty neurotransmitter systems, especially, of the dopaminergic system involving specifically the dorsolateral prefrontal cortex networks (Arnsten, 2011). These catecholamine networks are also known to be altered, in some cases permanently, as a result of omega-3 deficiency in utero (McNamara & Carlson, 2006b). It is well documented that the psychostimulant Methylphenidate can reverse some of these neuropsychological deficits observed in ADHD patients whilst in healthy adults improve reaction time, working memory and vigilance by directly impacting on dopamine and noradrenaline release in the prefrontal cortex (Cooper et al., 2005; Elliott et al., 1997; Engert & Pruessner, 2008; Greenhill, Beyer, et al., 2002; Mehta et al., 2000). In a similar fashion, omega-3 modifies the activity and production of neurotransmitters and signal transduction (Yehuda, Rabinovitz, Carasso, & Mostofsky, 2002). In addition, an association between FADS2 and ADHD has also been found which in turn has implications for the absorption and synthesis of key HUFA in the omega-3 series such as EPA and DHA from the parent precursor (Brookes, et al., 2006b). As discussed in Chapter 2, the fatty acid desaturase 2 (FADS2) encodes essential enzymes – delta 5 and delta 6 desaturase - for absorption into plasma phospholipids and erythrocyte membranes however the activity of these enzymes is susceptible to metabolic, nutritional and hormonal regulation (Rzehak et al., 2009). The minor allele of the FADS2 gene is associated also with decreased activity resulting in diminished amounts of PUFA but elevated amounts of the un-metabolised precursors (Steer, Hibbeln, Golding, & Davey Smith, 2012). It may be that the omega-3 indices related to decreased activation in our ADHD cohort may be linked to inefficient metabolic pathways as a result of the suppression of the conversion of omega-3 due to the excessive intake of the other (i.e., omega-6), resulting in a

build of omega-3 precursors which are then rerouted for breakdown to the oxidation pathway (Brookes, et al., 2006b). Future research should investigate the fatty acid desaturase genes in addition to the neuropsychological and imaging profile of children with ADHD relative to healthy control children to fully extrapolate these findings further.

An alternative theory could be that enhanced omega-3 is associated with more efficient and hence decreased activation. This is in line with previous published research which describes a neuronal efficiency hypothesis in males suggesting that reduction in neuronal activation is linked with a more efficient utilisation of the cortex (Neubauer & Fink, 2009). This would be supported by the fact that the same association has been observed in healthy controls. Higher omega-3 may be associated with less need to recruit frontal brain regions to achieve the same inhibitory capacity.

There were no significant relationships in the control group between P3 responses and fatty acids following the FDR correction. The largest non-significant relationships were two negative associations between EPA and P3 amplitude responses across frontal-central (Fz and FCz) scalp regions, suggesting that as EPA increases responses to NoGo (Backgrounds) stimuli decreased. This may be associated with better efficiency, in other words, individuals with higher omega-3 need to spend less effort in activating inhibitory regions in order to achieve the same performance (Neubauer & Fink, 2009). These brain EPA relationships support the association finding observed in the performance data (that is, higher omega-3 was related to lower number of commission errors) and suggest that EPA may play in role in the regulation of inhibitory control.

However, the correlations were inversely related in all cases which were unexpected. This part of the study's hypothesis was based on the rationale that both the P3 family and omega-3 are implicated in attentional processes. Although, it should be noted that these analyses are largely exploratory as the literature in this area is virtually unexplored.

Study Limitations

As mentioned, task differences across studies play a role and it may be that this particular task was too easy for all the participants. For example, the task was relatively short 7 minutes (compared to other similar tasks of between 10 and 13 minutes), the Go stimuli were also presented in blocks for 500 ms while other tasks present the Go and NoGo stimuli for a shorter length of time, e.g., 200 ms. Also, as previously mentioned, the presentation of the NoGo stimuli is relevant. In our study they appeared in blocks of 6 following each other compared to other

studies where they are presented in pseudo-random order, usually after several Go trials. This way a predominant response tendency is built up. The blocked presentation may have produced an adaptation effect for all NoGo trials in the block with the exception of the first one and produced a relatively lower load on inhibitory control. Despite, the task differences, this battery is standardised and has been used in many published studies (Williams, Gatt, et al., 2008; Williams, Hermens, et al., 2010). It is designed also to assess switching between those automatic responses which elicit very early ERPs, against suppression of them during the NoGo trials.

Although IQ differed between groups, there is a strong argument against co-varying for IQ based on robust literature (Miller & Chapman, 2001). The main principle is that these children were not randomly sampled into groups but carefully selected on a case control basis. Controlling for any IQ differences between ADHD and HC groups may eradicate any variance which is due to ADHD in the measures under examination increasing the risk of Type II error i.e., erroneously concluding there are no differences between groups, when in fact, there are (Bridgett & Walker, 2006). Also, it would violate the ANCOVA assumption that expects that participants are randomly selected which was not the case in this study or any case-control studies. Furthermore, upon inspection, the significant findings were not significantly correlated with IQ, with the exception of the one positive association between P3a ERP responses at Fz to Go trials which suggests this particular significant finding may be linked with IQ. Although correlations do not imply causation and results should be interpreted with caution.

A final limitation concerns the diagnosis of the ADHD group within a community sample. For example, in this study, although all the children in the ADHD group met the criteria for ADHD according to the DSM-IV, the majority were recruited from schools which had a provision for children with emotional, and behavioural difficulties and many of the group did not have a previously given clinical diagnosis of ADHD. However, *t* scores of over 65 on both the Teacher and Parent Conner's Rating Scale Long version along with a structured interview as per the one employed in this study are considered sufficient to indicate the presence of ADHD. However, clinically referred cases with ADHD may present more severe symptoms than a community sample (Sprafkin, et al., 2007).

Conclusion

In conclusion, this study demonstrated that there were no significant differences in neural activity of children with and without ADHD during the inhibitory part of the task. However, ADHD patients had significantly reduced ERP deflections over P3a and P3b across fronto-central regions during the Go process of the task. The omega-3 PUFA fractions were related to the performance measures of the task in controls - with EPA negatively associated with the inhibitory measure of the task - namely commission errors.

Interestingly, in the ADHD group omega-3 PUFA fractions were negatively associated with both P3a and P3b amplitude responses to Go trials across central regions, which were impaired in ADHD relative to controls, as well as over parietal scalp regions. This finding was unexpected and suggests that higher omega-3 is related to decreased brain responses to Go trials in the ADHD group. In the control group, EPA was negatively correlated to NoGo stimuli across frontal scalp regions at trend level only, implying that as omega-3 increases neural electrical activation decreases. Both of these negative associations in controls and in ADHD cases may suggest greater efficiency of neuronal processing with increased levels of EPA. Further research is required potentially with larger sample sizes to explore these preliminary findings further.

Chapter Nine: Study 1B.

Sustained Attention (CPT) Task in ADHD and Healthy Matched Controls

Introduction

Children with ADHD have consistently been found to be impaired in performance during selective and sustained attention tasks, most commonly measured in the continuous performance task (CPT) (Losier, et al., 1996a; Rubia, Smith, Brammer, et al., 2007; Sonuga-Barke, et al., 2010; Willcutt, et al., 2005). The CPT task, originally a long, complex and prolonged clinical assessment, was designed by Rosvold, Mirsky, Sarason, Bransome and Beck (1956) to specifically measure *vigilance* or *sustained attention*; however shorter and simpler versions have since been developed (Robertson, Manly, Andrade, Baddeley, & Yiend, 1997). Sustained attention can be broadly summarised as the ability to concentrate on a specific stimulus over a period of time while excluding the distraction of other irrelevant stimuli. It requires the ability to maintain performance (as measured by the behavioural response) during incessant activities which rely on the capacity to detect a stimulus, vigilance and the ability to resist distraction (Pasini, et al., 2007; Shalev, et al., 2011).

Typically, CPTs necessitate the recognition of low probability items such as the letter X or successions of targets for example A-X randomly presented among high frequent alphabetical strings (Corkum & Siegel, 1993). The more difficult A-X version of the CPT is called a cued CPT and presents a cue such as the letter A (a warning stimulus) followed by the target stimulus (e.g., X) or a non-target stimulus (non-X). These stimuli can be controlled by increasing the probability of either the target or the non-target item after the cue (Dias, Foxe, & Javitt, 2003). Performance measures can also be acquired from the CPT including errors of omission (i.e., the failure to respond to target trials) which are considered to be an estimate of poor sustained attention and errors of commission (incorrect hits to non-target trials, also known as false positives) which are thought to index impulsivity when executed speedily after the presentation of a stimulus and when they are anti-correlated with RT, suggesting a speed accuracy trade-off favouring speed (Alexander, et al., 2008; Halperin, et al., 1991; Rubia, Smith, Brammer, et al., 2007). However, they are thought to be a marker of inattention when delayed relative to stimulus onset and when they are associated with overall slower RT (Alexander, et al., 2008; Barkley, 1997; Halperin, et al., 1991). Of note, in the CPT only the background condition (i.e., non-

targets) can draw out false positive responses (Alexander, et al., 2008). Children with ADHD during CPT tasks have typically more errors of commission, omission and display more variable reaction times (Holcomb et al., 1986; Castellanos & Tannock, 2002; Rubia et al., 2001, 2007, Willcutt et al., 2005; Levy & Hobbes, 1997; Strandburg, Marsh, Brown, Asarnow, Higa et al., 1996). Most CPTs have a small load on working memory which in turn is considered a core EF domain and has been frequently found to be impaired in ADHD, alongside inhibition (Best & Miller, 2010; Castellanos & Tannock, 2002a; Keage, et al., 2006; Martinussen, et al., 2005; Miyake, et al., 2000; Shallice, et al., 2002; Stevens, et al., 2002; Willcutt, et al., 2005; Willcutt, et al., 2001).

Although, the CPT is predominantly considered a test of sustained attention/vigilance, it also loads on selective attention functions and has been used extensively to study brain function using ERPs in various clinical populations including ADHD (Kirmizi-Alsan, et al., 2006). Selective responses can be measured by ERPs by comparing the *to be attended to* (or target trials) to the *to be ignored* stimuli (non-target trials) (Gomarus, et al., 2006). The CPT paradigm employed in this PhD thesis consists of a series of letters presented one at a time (e.g., B, C, D and G). It has three epoch types: Targets (which the participant is instructed to respond to when the same letter appears twice in a row by pressing two buttons with the index finger of each hand), Backgrounds (e.g., non-target letters) and Distractors (checkerboard patterns, which the participant is instructed to ignore). The target letters are described as "1-back" (that is repetitions of the previous letter) and are presented pseudo-randomly. Non-target stimuli demand the updating of WM, as they bear target defining information while targets are described as consecutive stimulus repeats (Keage, et al., 2008a). This task loads on sustained attention and selective attention, as well as working memory updating (although the WM load is minimal).

The ERP literature has reported that children with ADHD have abnormally smaller P3 ERP amplitudes relative to healthy controls in both target and non-target conditions of the CPT (Banaschewski, et al., 2004; Jonkman, Kemner, Verbaten, Koelega, Camfferman, v.d. Gaag, et al., 1997; Liotti, et al., 2007; Seifert, et al., 2003). Although this is dependent on the task paradigm, whether it is a standard event related CPT measuring sustained attention or an inverted CPT that measures inhibitory processes (where participants have to inhibit targets and respond to non-targets). A common finding also in ADHD is impaired target processing, as exhibited by attenuated P3 amplitudes in response to targets (Loiselle, et al., 1980; Verbaten, et al., 1994).

However, when the accuracy of detection is impaired, as is often the case in ADHD, and undetected targets are incorporated in the ERP, it has been postulated that the reduced P3 amplitudes may simply imitate the increased amount of target misses (with reduced P3s) as opposed to alterations in the detection process itself (van Leeuwen, Steinhausen, Overtoom, Pascual-Marqui, van't Klooster, et al., 1998). In simple CPTs, effects on P3 during target processing may be confounded by effects on preparatory processing including orienting and response preparation (van Leeuwen, Steinhausen, Overtoom, Pascual-Marqui, van't Klooster, et al., 1998). The non-target condition of the CPT (i.e., background letters) elicits specific ERP indices including N1 (involved in early discrimination), P150 (involved in the selection of material), and P3 (associated with working memory updating) (Clarke, et al., 1998; Friedman, 1990; Keage, et al., 2008a; Vogel & Luck, 2000). In ADHD, after the presentation of stimuli that do not necessitate updating, both the N1 and P150 deflections have been found to be delayed over frontal scalp regions (Karayanidis, et al., 2000; Keage, et al., 2008a).

There are several lines of enquiry which suggest LC-PUFA have an important role in cognitive development throughout childhood and in the regulation of cognitive brain function (Marszalek & Lodish, 2005; Ryan, et al., 2010) and are furthermore implicated in the mediation of neurotransmitter function including serotonergic responsivity, signal transduction and phospholipid turnover (Condray et al., 2008; Haag, 2003; Yehuda, Rabinovitz, & Mostofsky, 1999). However, there are very few publications exploring the relationship between LC-PUFA and neurocognitive development (e.g., neuropsychological measures of brain function including executive function processes in children/adolescents groups and thus the literature to date is extremely limited.

In ADHD, Sinn and colleagues (2008) also employed neuropsychological measures of cognition and reported significant advancements in the capability to switch and control attention (as measured by the Creature Counting Task) in the same group of children (aged 7 to 12 years) with ADHD receiving omega-3/6 polyunsaturated fatty acids at 15 weeks compared to placebo. Following a cross-over from the placebo into the active group at 16 weeks (from week 16 -30) a significant improvement in this measure was also observed. The authors noted that improvements in cognitive performance were also supported by parent rated improvements in attention (Sinn, Bryan & Wilson, 2008).

In healthy school children (aged 8-10 years), McNamara and colleagues (2010) have explored the mediating effects of DHA supplementation on brain activity during a sustained attention task using fMRI. The boys were randomly allocated to receive 1 or 2 measures of DHA (400 mg versus 1200 mg) or placebo. Cortical brain activity was recorded at two time points: (1) at the start of the study prior to supplementation and (2) at endpoint, 8 weeks later. DHA erythrocyte membrane composition had increased at 8 weeks in those receiving the 400 mg dose by 47% and by 70% in those receiving the 1200 mg dose compared to the placebo group (McNamara, Able, et al., 2010). There were no significant differences in performance measures (e.g., RT, commission errors) between active and placebo groups at the study end point. DHA was however significantly negatively correlated with RT at both baseline and end point. The two active groups both displayed greater alterations in activation of the dorsolateral prefrontal cortex (DLPFC) relative to placebo (McNamara, Able, et al., 2010). Furthermore, there were greater decreases in activation from baseline to end point in the occipital and cerebral cortex in both low and high dose groups compared to placebo (McNamara, Able, et al., 2010). The 1200 mg dose group in comparison to the 400 mg dose group resulted in greater decreases in activation in the bi-lateral cerebellum (McNamara, Able, et al., 2010). At both baseline and endpoint, the blood measures of DHA were positively associated with activation in the DLPFC and negatively associated with RT (McNamara, Able, et al., 2010). This is the first study to demonstrate that dietary intake of DHA and respective increases of RBC measures are in turn associated with changes in cortical attention networks in healthy boys (McNamara, Able, et al., 2010).

Another study by Ryan and colleagues (2010) also in healthy, pre-school children (n = 175) assessed the efficacy of DHA versus placebo (high oleic sunflower oil) on cognitive functions including measures of sustained attention, memory, vocabulary acquisition and impulsivity for 4 months. The results of this study demonstrated no statistically significant benefit of DHA supplementation for each of the cognitive tests between active and placebo groups in this cohort of healthy school-children (Ryan, et al., 2010).

This study sought to investigate potential differences in neural activation during task performance during a sustained attention task as measured by P3 amplitude responses in ADHD compared to controls. In consideration of previous research, it was predicted that differences will be characterised specifically by smaller mean AUC P3a and P3b amplitudes response in ADHD compared to control children in frontal-central and parietal sites which are in turn implicated in

cognitive processes associated with sustained attention during both target and non-target conditions of the CPT (Banaschewski, et al., 2004; Jonkman, Kemner, Verbaten, Koelega, Camfferman, v.d. Gaag, et al., 1997; Liotti, et al., 2007; Seifert, et al., 2003). Furthermore, in line with previous research suggesting omega-3 are implicated in attention processes (McNamara, Able, et al., 2010), it was predicted that PUFA status (as measured in terms of % of omega 3/6) will be positively correlated with brain function as indexed by the P3 family in ERP responses during the CPT in both cases and controls. The early and late P3 family were chosen on the basis that they are the main waves associated in attention processes and furthermore have been found to be abnormal in ADHD (Barry, Johnstone, & Clarke, 2003b; Brandeis, Banaschewski, et al., 2002; Jonkman, Kemner, Verbaten, Koelega, Camfferman, v.d. Gaag, et al., 1997). The final predication in relation to the performance data predicted negative associations between omega-3 fatty acids levels in the ADHD group and errors of commission and omission, that is, the lower the omega-3 – the higher the number of errors. In the healthy control group, negative relationships were anticipated, that is, the higher the omega-3 – the lower the number of commission and omission errors.

Method

Participants

The EEG/ERP data for the ADHD children had previously been collected during the MAAFA trial. During the MAAFA trial, a total of 76 male children/adolescents were drawn from various special educational settings (e.g., boarding schools, mainstream schools with provision for children with emotional and behavioural difficulties) in and around the London area and were screened to ensure they met the criteria for ADHD according to the DSM-IV. As per before, this included a short semi-structured interview, (Children's Interview for Psychiatric Syndromes, *ChIPS*) based on DSM-IV criteria (ChIPS see Rooney, Fristad, Weller & Weller, 1999) and that both Parent and Teacher Connor Rating Scales (CPRS/CTRS) were equal to or above 65 (> 95^{th} percentile). Data was available for a total of 73 male adolescents. This was made up of baseline data from 41 children/adolescents that had been recorded during the

Maudsley Adolescence ADHD Fatty Acid (MAAFA) trial and met criteria for ADHD and 32 in the control group.

In relation to the recruitment of the healthy age and gender matched controls, participants were screened in the same way as the ADHD group to ensure that their IQ was higher than 70 using the K-BIT and that they did not meet criteria for ADHD according to the DSM-IV. This included the ChIPS interview and ensuring scores of the Conner's Parent and Teacher Rating Scale ADHD index *t* score were collectively lower than 65. Their teachers were advised of the recruitment criteria and pre-screened children in terms of suitability for each group e.g., ensured that the healthy controls were not known to have behavioural difficulties or special educational needs. As well as completing the battery of clinical questionnaires, and providing a 16 ml blood sample, the healthy controls were tested on all EEG/ERP tasks so as to provide control data for the pre-existing EEG/ERP data collected during the MAAFA trial.

Exclusion criteria are as per Chapter 8 as will not be repeated here.

Age, IQ, Handedness and Medication

The EEG/ERP data was age matched to the healthy control group. Intelligence quotient (IQ) for all participants had to be higher than 70 on the prorated IQ as assessed using the Kaufman Brief Intelligence test (K-BIT). There was a significant difference between ADHD (M = 97.90, SD = 11.47) and the control group (M = 119.83, SD = 12.53) in composite IQ, $t(75) = -8.01$, $p < .001$ as measured by the Kaufman Brief Intelligent test. There were no significant differences in age between ADHD (M = 14.02, SD = .91) and control children (M = 14.42, SD = 1.07), $p > .05$. It should be considered that the selection of groups recruited in this study was not random (as in most case-control studies) and therefore covarying for IQ would violate the ANCOVA assumptions arguably altering the group effect in potentially problematical ways arguably resulting in spurious findings (Bridgett & Walker, 2006; Dennis, et al., 2009; Miller & Chapman, 2001).

In the ADHD group, 38 males were right handed, 1 was left handed and 2 were ambidextrous. In the control group, 28 were right handed, and 4 were left-handed. A Chi-square test for independence indicated no significant association between handedness between ADHD and control children, χ^2 (2, 73) = .228, p = .13. Thirty two of the ADHD participants were medication naïve and the remaining 9 underwent a 48 hour wash out period for stimulant medication, in line with current EEG practice.

Procedure & Materials

Children and adolescents below the age of 16 years old were accompanied by an appropriate adult (parent, teacher or guardian) to the Institute of Psychiatry at The Maudsley Hospital where approximately 16 ml of blood was taken by a qualified phlebotomist from in total 29 participants and 43 healthy controls. Blood assessments required 8 hours fasting beforehand, although water was permitted, and this was explained fully to the parent and child prior to the appointment. The blood was taken by a qualified phlebotomist, transported by motor-cycle courier to the Science Centre at the London Metropolitan University where it was spun before storing at -80 degrees Celsius. Following the blood sample all participants were given a complimentary breakfast at the Maudsley restaurant. They were then taken to a quiet, well lit testing room to complete all the self-reported questionnaires. Instructions were given for each questionnaire and assistance during completion where necessary. All participants in the healthy control group were given also an EEG/ERP assessment. On occasion, data collection took place over 2 visits, with the child assessment and screening sometimes taking place at school. Participants were given £20.00 for their participation and all associated travel expenses were refunded to the parent/guardian. During the MAAFA trial, any children taking stimulant medication for ADHD were required a wash-out for 48 hours prior to the EEG recording.

Blood Analysis

Total lipids were extracted according to the Folch method as reported in Chapter 8 and therefore will not be repeated here. This study reports blood data from plasma phopshatidylcholine (PPC) measures only.

ERP recording

EEG data were collected from 26 electrodes using an adapted 10-20 system following an internationally standardized protocol LabNeuroTM (Brain Resource, 2010). Participants sat in a light and sound attenuated room with an ambient temperature of 24˚C. A NeuroScan Quik cap and NuAmps amplifier (sampling rate = 500 Hz) were employed to collect EEG data from electrode sites. Data was recorded relative to a virtual ground, but referenced offline to linked mastoids. Horizontal eye movements were recorded with electrodes placed 1.5cm lateral to the outer canthus of each eye. Vertical eye movements were recorded with electrodes placed 3mm above the middle of the

left eyebrow and 1.5cm below the middle of the left bottom eye-lid. Skin resistance was < 5 kOhms. A continuous acquisition system was employed and data was EOG corrected offline (Gratton, Coles, & Donchin, 1983). A low pass filter with attenuation of 40dB per decade above 100 Hz was employed prior to digitization.

ERP area under the curve

Average ERPs were calculated for event types corresponding to a stimulus type in each paradigm. For each channel, the individual single-trial epochs were filtered with a low-pass Tukey filter function that attenuates frequencies above 25 Hz. A cosine ramp from 1 down to 0.5 between 25Hz and 35Hz is used as an envelope on the FFT data in the Tukey filter. The single trials were then averaged to form conventional ERPs. The averages of the pre-stimulus period -300 to 0 ms were subtracted from the ERP data. The signal was then down sampled by a factor of 4 (leading to 8 ms samples). The amplitude of the waveform (in microvolts) relative to the zero baseline is calculated for single time points in 8ms, then multiplied by a factor of 8 to achieve a measure of the area under the curve in a unit of microvolt-milliseconds. The AUC is the integral of the curve over a specified time range. Using the AUC measure, the space between the ERP waveform and baseline was divided into multiple 50ms time-windows that can be approximately mapped onto ERP component time-windows. P200 is mapped onto time unit 5 (200-250 ms), N200 on to time unit 6 (250-300 ms), P3a onto time unit 7 (300-350 ms) and P3b (350-400 ms) on time unit 8. Units are in microvolts multiplied by ms.

Sustained Attention (CPT) task

This task consists of a series of letters (B, C, D or G which are in white Arial font on a black background) presented to the participant on a computer screen for 500 milliseconds, separated by an interstimulus interval (ISI) of 1 second. The participant is requested to press two buttons with the index finger of each hand to the target stimuli. The speed and accuracy of response are equally stressed in the task instructions. There are 125 stimuli presented in total. These include 85 non-target letters (i.e., background letters); 20 pseudo-randomly presented, "1-back", target letters (that is repetitions of the previous letter), and 20 distracter stimuli consisting of checkerboard patterns (black and white 1 x 1 cm checkerboards). The checkerboards were interleaved randomly with the letter stimuli. Participants were asked to ignore the "checkerboards" which were designed to cause a distraction. A brief practice was given at the

start to ensure understanding of the task instructions. Participants were advised the test would last for 8 minutes. This task measures the processes involved in the orienting reflex, categorisation, contextual updating, sustained attention and working memory. Behavioural measures include reaction time to targets, errors of omission and errors of commission.

Results

Behavioural Data (Reaction times and error rates)

The data did not meet the criteria for parametric tests (viz, Homogeneity of Variance and Normality) for any of the performance measures reaction time, false positive (errors of commission) or false negative (errors of omission) scores. Omission errors (false negatives) consisted of target stimuli which participants did not respond to, whereas commission errors (false positives) occurred whenever participants responded to a non-target stimulus. A series of Mann-Whitney *U* tests revealed that there were no significant differences in reaction times, or omission errors between the ADHD group or controls, $p > .05$. There was a significant difference in commission errors with a higher number of errors in the ADHD group compared to control children, $U = 470.500$, $p < .05$. The behavioural data are plotted in Figure 1 below.

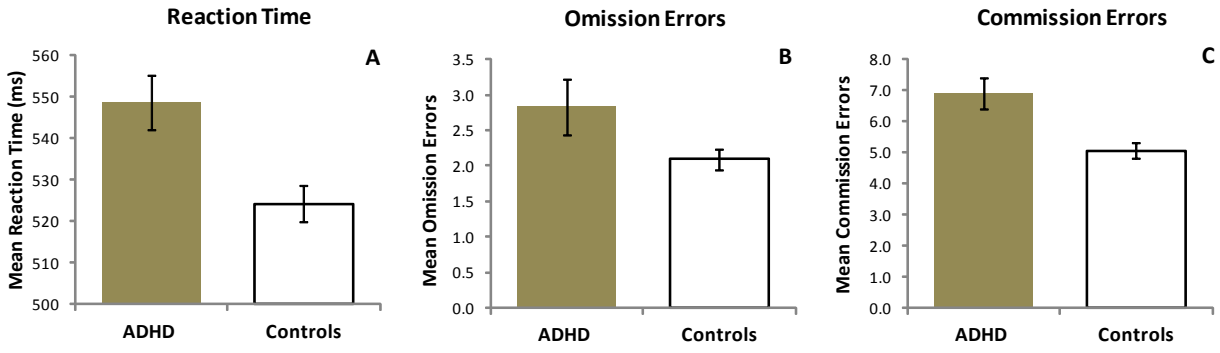


Figure 1. Mean reaction time (A), mean omission errors (B) and mean commission errors (C) for ADHD ($n = 41$) and control children/adolescents ($n = 32$), along with standard errors in the Sustained Attention Task (CPT).

Statistical Analyses for Electrophysiological data

For statistical purposes, a series of 2 x 4 x 3 mixed repeated measures analyses of variance (ANOVA) were conducted on the data. The between subjects factor was group (ADHD versus control children), while condition (Targets, Backgrounds and Distractor variables) and electrode position (Fz, FCz, Cz, and Pz) were the within subjects factors. The dependent measures were mean AUC amplitude responses (across individual time points 5 - 8). All interactions were further investigated using post-hoc tests consisting of pairwise comparisons with a Bonferroni correction for multiple testing. This is considered the most appropriate for controlling for Type 1 error in repeated measures ANOVA.

Time point 5 (P2 deflection)

The mean values (μV) and standard errors for the P2 amplitude responses for ADHD and control children are plotted in Figure 2. Mauchly's test indicated that the assumption of sphericity had been violated for the within-subject factors electrode site, χ^2 (5) = 109.93, $p < .05$, condition, χ^2 (2) = 33.19, $p < .05$ and for the interaction between condition and electrode site, χ^2 (20) = 158.68, $p < .05$. This was corrected using Greenhouse-Geisser estimates of sphericity. The corrected results are reported.

For the P2 response amplitude (time point 5), the analyses revealed a non-significant main effect of the between-subjects factor group (ADHD versus controls), $F < .01$. There were significant mains effects of the within-subjects factors electrode site (Fz, FCz, Cz, Pz), $F(1.54, 101.59) = 31.70$, $p < .001$, with the greatest positive going activation at Pz ($M = 189.41$, $SE = 32.76$) and condition, $F(1.43, 94.30) = 9.92$, $p < .01$ with Targets ($M = 107.07$, $SE = 30.53$) producing the highest positive going mean activation. There was a significant interaction between electrode site and condition, $F(3.35, 221.22) = 33.62$, $p < .001$. Post-hoc tests revealed that there was significantly greater activation at Fz between condition 1: Distractors and condition 2: Targets, with a larger mean difference in activity during the Distractor condition ($M = -185.45$, $SE = 51.68$) compared to Targets ($M = 72.18$, $SE = 36.84$), $p < .001$ and (2) Distractors and Backgrounds with increased activity in the Distractor condition ($M = -185.45$, $SE = 51.68$) compared to condition 3: Backgrounds ($M = 11.56$, $SE = 33.38$), $p < .001$. At FCz, there was a significant difference between both Distractors and Targets, with increased negative going activation in the Distractors condition ($M = -168.34$, $SE = 52.52$) compared to Targets ($M =$

76.49, *SE* = 35.81), $p < .001$. There was also a significant difference at FCz between the Distractor and Background conditions with a larger mean difference in activity in the Distractor condition (*M* = -168.34, *SE* = 52.52) relative to Backgrounds (*M* = 57.13, *SE* = 33.73), $p < .001$. At Cz, there was a significant difference between Distractors (*M* = -70.34, *SE* = 53.03) and Targets (*M* = 114.02, *SE* = 32.87) with increased positivity in the Target condition relative to Distractors, $p < .002$. There was a significant mean difference in activation also at Cz between Distractors (*M* = -70.34, *SE* = 53.03) and Backgrounds (*M* = 92.97, *SE* = 34.22), $p < .001$.

There was also a significant 3 way interaction between condition, electrode site and group was also significant, $F(6, 396) = 2.82$, $p < .02$. Post-hoc tests revealed that this was due to differences in activation during all three conditions and across electrode sites between ADHD and controls. For example, there was significantly greater activation at Fz in the ADHD group between condition 1 (Distractors, *M* = -236.85, *SE* = 66.33) and condition 2 (Targets, *M* = 70.51, *SE* = 47.28) with increased negative going activation during the Distractor condition relative to Targets, $p < .001$. The same pattern was observed at Fz between condition 1 (Distractors, *M* = -236.85, *SE* = 66.33) and condition 3 (Backgrounds, *M* = 36.93, *SE* = 42.84), with increased negativity during the Background condition, $p < .001$. At FCz, Distractors (*M* = -204.79, *SE* = 67.40) also produced a significantly enhanced negative response compared to both Targets (*M* = 99.97, *SE* = 45.96) and Backgrounds (*M* = 90.26, *SE* = 43.29), $p < .001$. At Cz, there was a significant difference in activation with increased positivity at Targets (*M* = 148.16, *SE* = 42.19) compared to Distractors which was more negative (*M* = -115.63, *SE* = 68.06). Finally at Cz, there was a significant difference in activation between Distractors (*M* = -115.63, *SE* = 68.06) and Backgrounds (*M* = 128.83, *SE* = 43.92), $p < .001$. In this case, Backgrounds eliciting increased positivity compared to Distractors which was negatively activated.

In the control group, a trend finding was observed between Distractors (*M* = -134.05, *SE* = 79.28) and Targets (*M* = 73.84, *SE* = 56.51) at Fz, with increased negativity during the Distractor condition relative whereas Targets elicited positive responses, $p = .052$. At electrode site, FCz, a trend finding was also observed between Distractors (*M* = -131.88, *SE* = 80.56) and Targets (*M* = 53.00, *SE* = 54.93), $p < .03$ and also between Distractors (*M* = -131.88, *SE* = 80.56) and Backgrounds (*M* = 24.00, *SE* = 51.74). No other interactions reached significance.

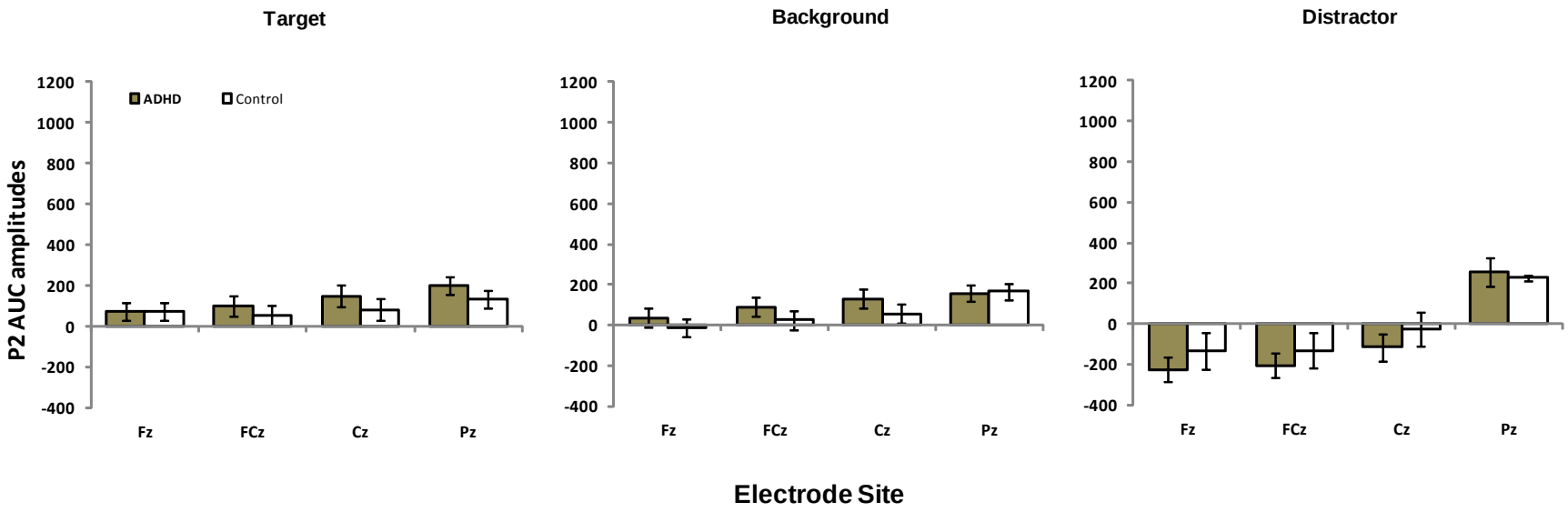


Figure 2. Mean P2 AUC amplitude responses for ADHD and Control participants in the CPT for Target (left panel), Background (middle panel) and Distractor (right panel) conditions across frontal (Fz), frontal-central (FCz), central (Cz) and parietal (Pz) electrode sites.

Time point 6 (N2 deflection)

The mean values (μV) and standard errors for the N2 amplitude responses for ADHD and control children are plotted in Figure 3. Mauchly's test indicated that the assumption of sphericity had been violated for the within-subject factors: electrode site, χ^2 (5) = 83.61, $p < .05$ and condition, χ^2 (2) = 34.11, $p < .05$ and for interaction between condition and electrode site, χ^2 (20) = 148.66, $p < .05$. This was corrected using Greenhouse-Geisser estimates of sphericity. The corrected results are reported.

For the N2 response amplitude (time point 6), the analyses revealed a non-significant main effect of the between-subjects factor group (ADHD versus controls), $F < .01$. There were significant main effects of the within-subjects factors electrode site (Fz, FCz, Cz, Pz), F(1.76, 116.40) = 121.14, $p < .001$, with greater positive going activation at Pz (M = 454.97, SE = 43.44) and condition, F(1.42, 93.73) = 4.80, $p < .02$, with greater positive going activation during the Target condition (M = 181.24, SE = 46.77).

There was a significant interaction between electrode site and condition, F(3.14, 207.02) = 3.26, $p < .03$. Post-hoc tests showed that this was due to a significant difference between Targets and Backgrounds across all electrodes (Fz, FCz, Cz and Pz). At Fz, Backgrounds produced increased negativity (M = -220.56, SE = 39.43) compared to Targets (M =-30.68, SE = 55.30). At FCz, Backgrounds (M = -103.77, SE = 39.21) revealed enhanced negativity compared to Targets which elicited positive activity (M = 29.86, SE = 53.84). At Cz, Targets elicited enhanced positivity (M = 176.48, SE = 50.92) compared to Backgrounds (M = 44.86, SE = 43.47). At Pz, a similar pattern was observed with enhanced positivity in the Targets condition (M = 549.32, SE = 48.88) compared to Backgrounds (M = 357.57, SE = 38.77).

The 3-way interaction for the within subjects effects: electrode position, condition and group was also significant, F(6, 396) = 3.57, $p < .003$. Post-hoc tests showed this was due to a significant difference at Fz between Targets (M = 21.81, SE = 70.97) and Backgrounds (M = -180.59, SE = 50.60), $p < .001$, with Backgrounds eliciting negative activity and a shift towards the positive during the Target condition. At FCz, there was also a significant difference between Targets (M = 100.56, SE = 69.10) and Backgrounds (M = -58.41, SE = 50.32), $p < .02$ with again Targets producing more positivity compared to Distractors which was negative.

At Cz, there was a significant difference again between Targets (M = 247.88, SE = 65.34) and Backgrounds (M = 81.12, SE = 55.79), $p < .01$, with increased positivity again for Targets compared to Backgrounds. At Pz, a significant difference was found between Distractors (M = 561.99, SE = 80.07) and Backgrounds (M = 367.74, SE = 49.76) and also between Targets (M = 643.94, SE = 62.74) and Backgrounds (M = 367.74, SE = 49.76) producing a larger positive response at Targets compared to both Distractor and Background conditions.

In the HC group at Fz there was a significant difference between Target (M = -83.17, SE = 84.83) and Background (M = -260.53, SE = 60.48) conditions only, $p < .002$, with enhanced negativity during the Background condition. No other interactions reached significance.

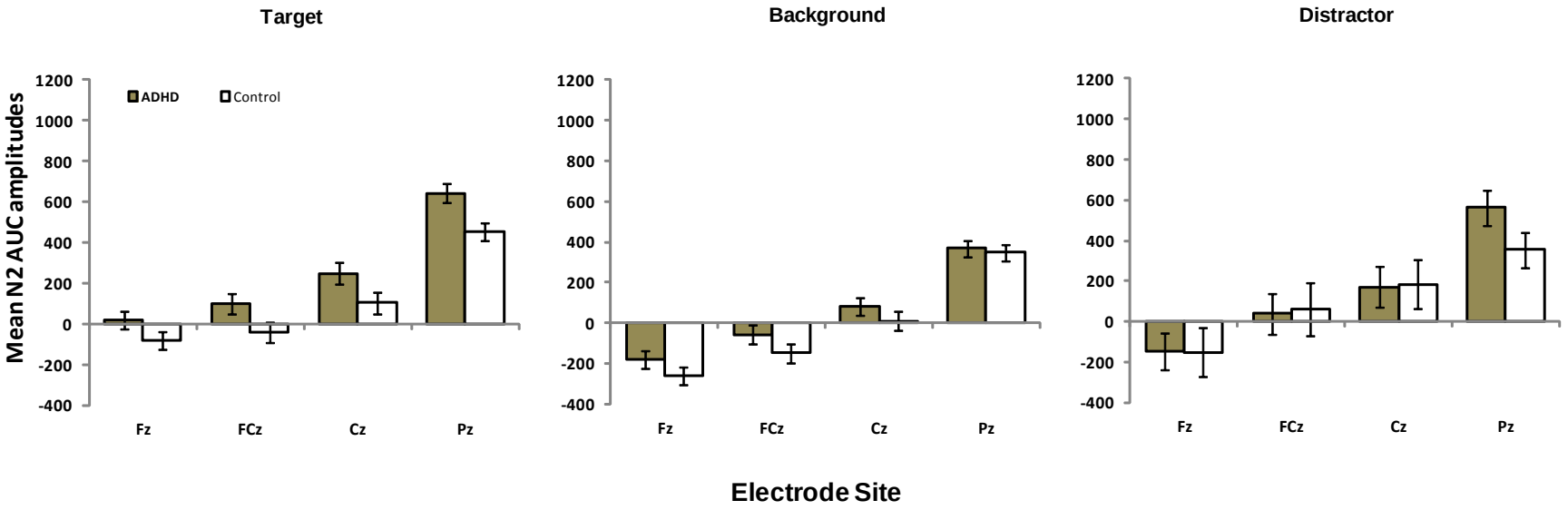


Figure 3. Mean N2 amplitude responses for ADHD and control participants in the CPT for Target (left panel), Background (middle panel) and Distractor (right panel) conditions across frontal (Fz), frontal-central (FCz), central (Cz) and parietal (Pz) electrode sites.

Time point 7 (P3a deflection)

The mean values (μV) and standard errors for the P3a amplitude responses for ADHD and control children are plotted in Figure 4.

Mauchly's test indicated that the assumption of sphericity had been violated for the within-subject factors: electrode site, χ^2 (5) = 59.11, $p < .05$, condition, χ^2 (2) = 13.80, $p < .05$ and for interaction between condition and electrode site, χ^2 (20) = 164.35, $p < .05$. This was corrected using Greenhouse-Geisser estimates of sphericity. The corrected results are reported.

For the P3a response amplitude (time point 7), the analyses revealed a non-significant main effect of the between-subjects factor group (ADHD versus controls), $F < .01$. There were significant main effects for the within-subjects factor electrode site (Fz, FCz, Cz, Pz), $F(1.93, 125.17) = 169.09$, $p < .001$, driven by significantly greater positive going activation at Pz (M = 547.94, SE = 38.07) and condition, $F(1.67, 108.87) = 60.64$, $p < .001$, which was driven by greater activation during the Distractor condition, (M = 451.43, SE = 59.93).

There was a significant interaction between condition and electrode site, $F(3.10, 201.23) = 20.29$, $p < .001$. Post-hoc tests showed that this was due to significant differences between all conditions (Backgrounds, Targets and Distractors) at the Fz electrode site. Specifically, the Distractor (M = 208.98, SE = 68.28) condition elicited increased positivity compared to the Target (M = -64.52, SE = 58.65) condition, $p < .001$. The Distractor condition (M = 208.98, SE = 68.28) elicited greater positive going activation compared to Backgrounds (M = -267.59, SE = 37.21) which was more negative, $p < .001$. Finally, the Background (M = -267.59, SE = 37.21) condition obtained increased negative going activation compared to Targets (M = -64.52, SE = 58.65), $p < .001$.

At FCz there were also significant differences across all conditions (Backgrounds, Targets and Distractors), $p < .001$. The Distractor (M = 426.70, SE = 72.26) condition elicited larger positive activity compared to the Target (M = 42.65, SE = 55.42) condition, $p < .001$. The Distractor condition (M = 426.70, SE = 72.26) elicited a more positive response compared to Backgrounds (M = -146.13, SE = 41.69) which was negative going, $p < .001$. Finally, Backgrounds (M = -146.13, SE = 41.69) were significantly more negative going activity compared to Targets (M = 42.65, SE = 55.42) which yielded greater positive going activity, $p < .001$.

At Cz there were also significant differences across all conditions (Backgrounds, Targets and Distractors), $p < .001$. The Distractor ($M = 512.12$, $SE = 65.21$) condition elicited larger positivity compared to the Targets ($M = 208.79$, $SE = 51.77$) condition, $p < .001$. The Distractor condition ($M = 512.12$, $SE = 65.21$) also elicited a more positive response compared to Backgrounds ($M = -14.51$, $SE = 44.03$) which was negative, $p < .001$. Finally, the Background ($M = -14.51$, $SE = 44.03$) condition yielded negative responses in comparison to Targets which was positive ($M = 208.79$, $SE = 51.76$), $p < .001$.

At Pz, there were significant differences between Distractors ($M = 657.93$, $SE = 50.65$) and Backgrounds ($M = 337.82$, $SE = 34.82$). Both obtained positive activity which was enhanced in the Distractor condition, $p < .001$. There was also a significant difference between Targets ($M = 648.05$, $SE = 44.96$) and Backgrounds ($M = 337.82$, $SE = 34.82$), with increased positive activity during the Target condition, $p < .001$. No other interactions reached significance, $p > .05$.

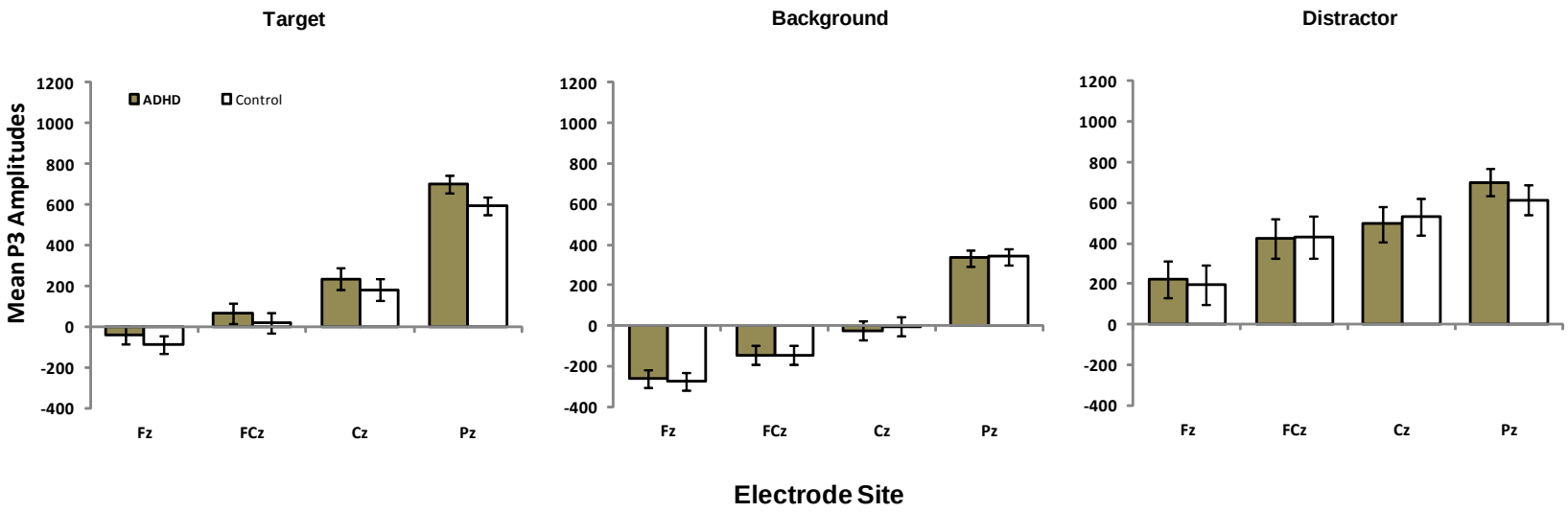


Figure 4. Mean P3a amplitude responses for ADHD and control participants in the CPT for Target (left panel), Background (middle panel) and Distractor (right panel) conditions across frontal (Fz), frontal-central (FCz), central (Cz) and parietal (Pz) electrode sites.

Time point 8 (P3b deflection)

The mean values (μV) and standard errors for mean AUC P3b amplitude responses for ADHD and control children are plotted in Figure 5. Mauchly's test indicated that the assumption of sphericity had been violated for the within-subject factors: electrode site, χ^2 (5) = 60.56, p < .05, condition, χ^2 (2) = 21.08, p < .05 and for interaction between condition and electrode site, χ^2 (20) = 149.47, p < .05. This was corrected using Greenhouse-Geisser estimates of sphericity. The corrected results are reported.

For the P3b response amplitude (time point 8), as per before the between-subjects factor group (ADHD versus controls) was not significant, p >.05. There were significant main effects for the within-subjects factors electrode site (Fz, FCz, Cz, Pz), F(1.85, 116.65) = 118.31, p < .001, driven by greater activation at Pz (M = 537.98, SE = 34.08) and condition, F(1.55, 97.80) = 40.39, p < .001, driven by greater activation during the Distractor condition. There was a significant interaction between electrode site and condition, F(2.89, 182.17) = 6.26, p < .002. Post-hoc tests showed there were significant differences between all conditions (Backgrounds, Targets and Distractors) and all electrode sites. These are outlined as follows. At Fz electrode site, the Distractor (M = 227.99, SE = 65.16) condition elicited larger positive activity compared to the Targets (M = 47.31, SE = 59.49) condition, p < .05. The Distractor condition (M = 227.99, SE = 65.16) also obtained positive activity compared to Backgrounds (M = -111.31, SE = 40.09) which was negative, p < .002. Finally, responses to Targets yielded positive responses (M = 47.31, SE = 59.49) compared to Backgrounds which were negative (M = -111.31, SE = 40.09).

At FCz, there was significantly more positive activity during the Distractor (M = 406.89, SE = 67.06) condition compared to Targets (M = 144.23, SE = 58.12), p < .003. The same pattern was observed between the Distractor condition (M = 406.89, SE = 67.06) and Backgrounds (M = -17.07, SE = 42.53), with Distractors eliciting positive activity compared to Backgrounds, p < .001. Finally, there was a significant difference in activity between Targets (M = 144.23, SE = 58.12) and Backgrounds (M = -17.07, SE = 42.53), p < .003, with Targets revealing positive responses relative to Backgrounds.

At Cz, there was significantly more positive activity during the Distractor (M = 522.79, SE = 63.15) condition compared to Targets (M = 262.01, SE = 52.64), p < .003. The same pattern was observed between the Distractor condition (M = 522.79, SE = 63.15) and Backgrounds (M =

49.12, *SE* = 42.00), with Distractors eliciting more positive activity compared to Backgrounds, $p < .001$. Finally, there was a significant difference in activity between Targets (*M* = 262.01, *SE* = 52.64) and Backgrounds (*M* = 49.12, *SE* = 42.00), $p < .003$, with Targets eliciting increased positivity relative to Backgrounds.

At Pz, there was a significant difference between the Distractor (*M* = 773.07, *SE* = 51.04) condition compared to Targets (*M* = 597.62, *SE* = 44.02), $p < .001$. The same pattern was observed between the Distractor condition (*M* = 773.07, *SE* = 51.04) and Backgrounds (*M* = 243.26, *SE* = 32.13), with Distractors eliciting more positive activity compared to Backgrounds, $p < .001$. Finally, there was a significant difference in activity between Targets (*M* = 597.62, *SE* = 44.02) and Backgrounds (*M* = 243.26, *SE* = 32.13), $p < .001$, with Targets showing increased positivity relative to Backgrounds. No other interactions reached significance, $F < .01$.

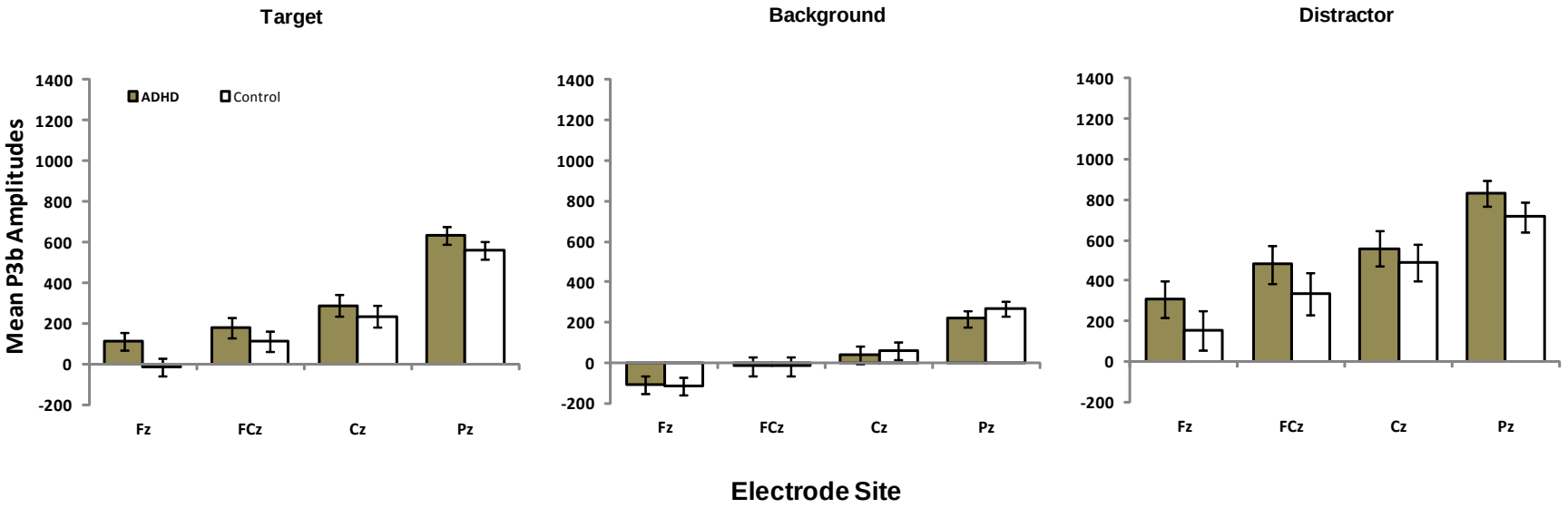


Figure 5. Mean P3b amplitude response for ADHD and control participants in the CPT for Target (left panel), Background (middle panel) and Distractor (right panel) conditions across frontal (Fz), frontal-central (FCz), central (Cz) and parietal (Pz) electrode sites.

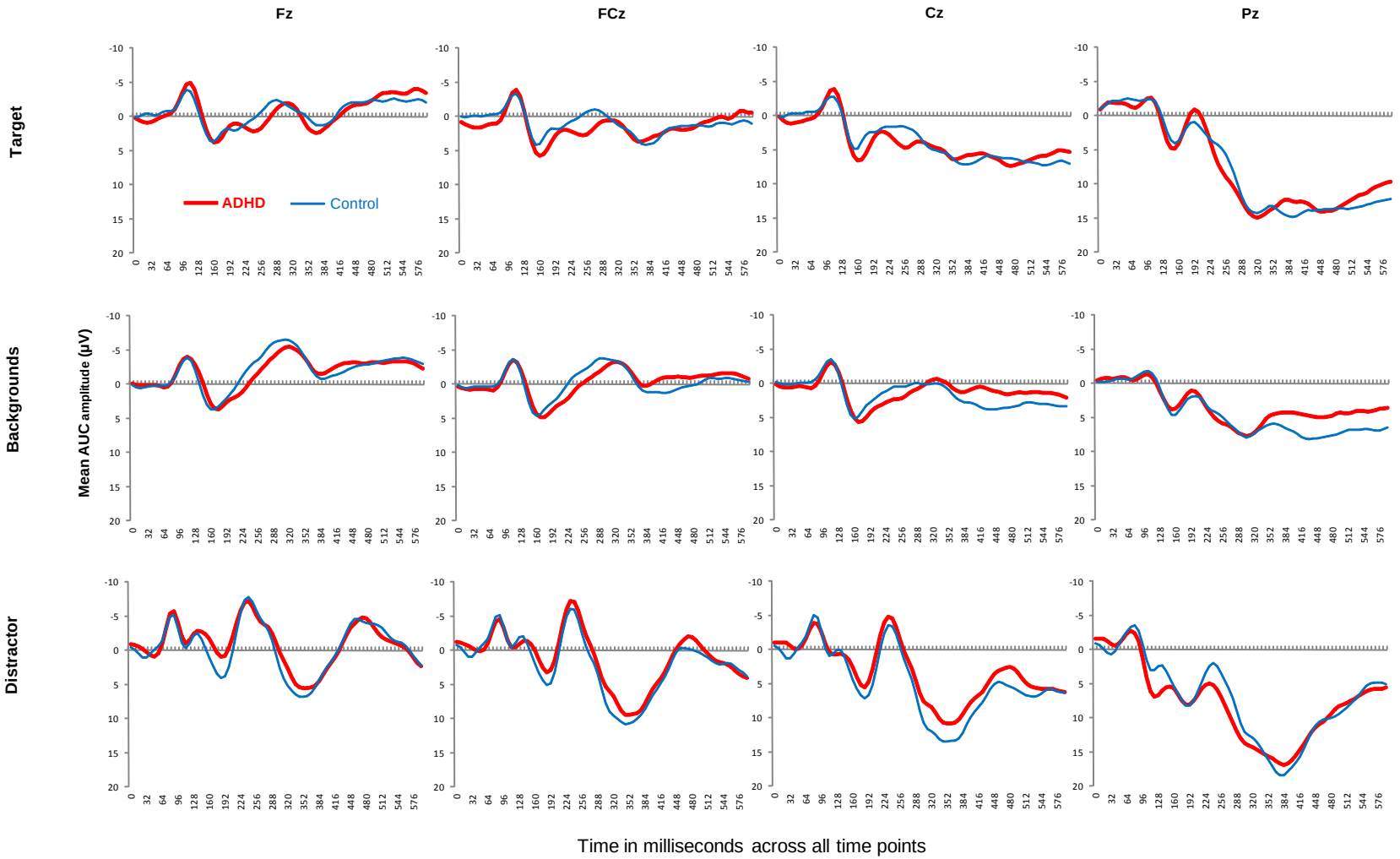


Figure 6. Grand average curves of the AUC ERPs of children/adolescents with ADHD (bold line) and healthy controls (thin line) across all AUC timepoints (P2, N2, P3a and P3b) for Targets (top panel), Backgrounds (middle panel) and Distractors (bottom panel) at electrode positions: Fz, FCz, Cz and Pz

PUFA fractions in Children/Adolescents with ADHD and Healthy Control Children

The means and standard deviations are presented in Table 1. For all plasma PC analyses, a series of independent-samples *t* tests were conducted to compare the plasma fatty acid levels between ADHD and control children in the CPT. The key fatty acid indices chosen were from the omega-3 and omega-6 series, namely (1) c18:3n-3 (ALA), (2) c20:5n-3 (EPA), (3) c22:5n-3 (DPA), (4) c226n-3 (DHA) and (5) Total n-3 and (6) c18:2n6 (LA), (7) c18:3n6 (GLA), (8) c20: 4n6 (AA) and (9) total n-6 respectively. Given the large number of tests, the false discovery rate correction (FDR) for multiple testing was employed for all analyses (Benjamini & Hochberg, 1995; this correction procedure is more conservative with the lower *p* values, but not as conservative as a Bonferroni correction). The relationships that survived correction only are reported below.

CPT

There were significant differences between the ADHD and control groups for ten out of the thirteen fatty acids indices any of the omega-3/6 fatty acid levels. From the omega-6 series there were significant differences for c20:2n6, $t(65) = -2.76$, $p = .01$, c20:3n6, $t(69) = -3.41$, $p = .001$, c20:4n6 (AA), $t(69) = -7.83$, $p = .001$, c22:4n6 (a metabolite of AA), $t(69) = -5.40$, $p = .001$, and total n-6, $t(69) = -4.60$, $p = .001$. From the omega-3 series, there were significant differences between ADHD and control children for c18:3n3 (ALA), $t(69) = -4.60$, $p = .003$, c20:5n3 (EPA), $t(69) = -5.72$, $p = .003$, c22:5n3 (DPA), $t(69) = -6.62$, $p = .004$, c22:6n3 (DHA) $t(69) = -11.08$, $p = .006$ and total n-3 $t(69) = -12.09$, $p = .01$. In all instances, LC-PUFA fractions in both omega-3 and 6 series as reported above were significantly higher in controls compared to ADHD.

Table 1. Mean LC-PUFA fractions in plasma choline phosphoglycerides (PPC) in ADHD and HC groups for the CPT

CPT task (ADHD: *n* = 33, HC: *n* = 38)					
	ADHD		**Healthy Control**		
	M	*SD*	*M*	*SD*	
Omega 6					
c18: 2n-6 (LA)	25.00	3.60	24.76	3.44	
c18: 3n6	0.11	0.07	0.10	0.06	
c20: 2n6	0.26	0.06	0.34	0.08	**
c20: 3n6	2.98	0.68	3.66	0.96	**
c20: 4n6 (AA)	8.00	1.50	11.63	2.25	**
c22: 4n6	0.29	0.06	0.39	0.08	**
c22: 5n6	0.31	0.22	0.25	0.08	
Total n6	36.94	4.23	41.11	3.39	**
Omega 3					
c18: 3n3 (ALA)	0.22	0.09	0.34	0.13	**
c20: 3n3	-	-	0.13	0.04	
c20: 5n3 (EPA)	0.53	0.20	0.99	0.45	**
c22: 5n3	0.62	0.24	1.06	0.31	**
c22: 6n3 (DHA)	1.86	0.40	3.71	0.92	**
Total n3	3.23	0.53	6.23	1.42	**

Note: * $p < .05$, ** $p < .01$

PUFA fractions and ERP measures

The Kolmogorov-Smirnov test revealed that scores were normally distributed for all ERP measures and therefore met the criteria for parametric data. Pearson coefficients were calculated to assess relationships between Targets, Backgrounds and Distractor stimuli and 7 key fatty acid indices. The strength of the associations between the fatty acid indices (omega-6: ALA, c18:3n6, AA, c20:4n6 and Total n-6 and omega-3: ALA: c18:3n3, EPA: c20:5n3, DHA: c22:6n3 and Total n-3) and earlier and later P3 (P3a and P3b) amplitudes measures at four electrode sites (Fz, FCz, Cz, Pz) were assessed using Pearson correlation coefficients. The performance data was not normally distributed and therefore Spearman Rho correlations for non-parametric data were applied. Again, given the large number of correlations (84 in the ADHD and 84 in the HC group), the FDR for multiple testing was performed.

Performance Data

There was a trend finding between DHA and errors of omission (i.e., the failure to respond to target trials) in the ADHD group, $r(40) = -.302$, $p = .058$ which did not survive correction (adjusted value $p > 0.05$. In the healthy control group, there were 2 significant relationships, the first a significant negative relationship between ALA (c18:3n3) and omission errors, $r(32) = -.494$, $p = .004$ (adjusted $p = .042$). The second was a positive relationship between AA (c20:4n6) and omission errors, $r(32) = .517$, $p = .002$ (adjusted $p = .042$).

P3a and PUFA fractions

None of the relationships between any of the fatty acid indices or P3a ERP measures in either the ADHD or control group survived the FDR correction, $p > .005$. The largest non-significant relationship in the ADHD group was between AA (c20:4n6) and P3a amplitude responses to Targets at Pz, $r(39) = -.400$, $p = .012$. The largest non-significant correlation in the control group was observed in the Target condition between EPA and Cz, $r(28) = -.419$, $p = .026$.

P3b and PUFA fractions

There were no significant relationships between any of the fatty acid indices and P3b ERP measures in either the ADHD or control groups, $p > .005$. In the ADHD group, there was a trend finding between Total omega-3 and P3b amplitude response to Targets at Pz, $r(39) = -.296$, $p = .067$. The second largest non-significant correlation was observed also at Pz (in the Target condition) between AA (c20:4n6), $r(39) = -.288$, $p = .076$. In the healthy control group, the largest non-significant correlation was observed between EPA (c20:5n3) and P3b amplitude responses to Distractors at FCz, $r(33) = .318$, $p = .072$. The second largest non-significant correlation was between Total omega-6 and P3b amplitude responses to Distractors at Pz, $r(33) = .321$, $p = .073$

Brief Discussion & Conclusion

The primary objective of this study was to ascertain whether earlier and later P3 components implicated in sustained and selective attention processes differed between children and adolescents with and without ADHD. However, as per before, additional time points which incorporate the P2 and N2 were also measured across fronto-central and parietal scalp regions due to their involvement in selection and WM processes. Secondary analyses explored the relationship between fatty acid indices and the P3 family, it was predicted that omega-3 fatty acids would be positively associated with P3 responses to Targets. There were no aprori hypotheses concerning the relationship between omega-3/6 and the Backgrounds and Distractors conditions. The results of the performance data revealed that there were no significant differences in RT or omission errors between cases and controls. However, the ADHD group made a significantly greater number of commission errors that is, responding to non-target stimuli, compared to control children, which is thought to be an indicator of impulsiveness (Halperin, et al., 1991; Hooks, Milich, & Pugzles Lorch, 1994). This finding is in line with the existing literature of commission deficits during this task in ADHD (Losier, et al., 1996a; Rubia, Russell, et al., 2001; Willcutt, et al., 2005).

The electrophysiological findings were unexpected as there was no main effect of group (ADHD versus control children) for any of the mean AUC amplitude ERP responses across time points 5 – 8 (P2, N2, P3a and P3b), suggesting that sustained / selective attention was not impaired in ADHD during this CPT task. Therefore, the findings of this study do not support previous ERP literature which suggest children with ADHD are impaired in selective and/or sustained attention processes as indexed by smaller P3 ERP amplitudes during both target and non-target conditions of the CPT (Banaschewski, et al., 2004; Barry, Johnstone, et al., 2003b; Brandeis, Banaschewski, et al., 2002; Jonkman, Kemner, Verbaten, Koelega, Camfferman, v.d. Gaag, et al., 1997; Kratz, et al., 2011; Liotti, et al., 2007; Seifert, et al., 2003). There were also no group differences in the earlier deflections (i.e., P2, N2) which have also been reported to be reduced in ADHD compared to controls during tasks of visual attention (Perchet, et al., 2001). Of note, is that the sustained / selective attention deficit theory in child ADHD can be quite controversial with some studies reporting no group differences in P3 waves (Lazzaro et al., 1997; Tucha et al., 2009; Zamorano et al., 2008). A dissociation between sustained and selective

attention capabilities have also been proposed based on findings that children with ADHD are able to perform as well as control children on selective attention tasks while their performance during sustained attention tasks demonstrate impairment (DeShazo Barry, Klinger, Lyman, Bush, & Hawkins, 2001). Arguably, the duration of this task was not long enough to fully capture sustained attention which will be discussed further in the limitations section. Another consideration for the absence of group differences is that the children in this study were in the main representative of a community sample. Previous research has suggested that children with ADHD in the community present with less severe symptoms than those recruited from the clinic (Sprafkin, et al., 2007).

Generic Task Effects

There were some generic task relevant effects including persistently greater negative going activation during the Distractor condition at early time points at frontal-central scalp regions. The exception was at parietal regions in which responses to Backgrounds shifted towards the positive. AUC amplitude responses for all conditions were maximal at Pz which supports the literature demonstrating the role of the posterior parietal cortex in visual-spatial selective attention (Behrmann, Geng, & Shomstein, 2004). In addition, it supports the role of later time points captured by the P3b wave which is enhanced in response to target detection, often requiring a motor or cognitive response (Friedman, et al., 2001a).

Of note, is that at both time point 7 (P3a) and 8 (P3b), in all instances, the Distractor condition elicited greater positive going activity at frontal-centro and parietal scalp regions (Fz, FCz, Cz and Pz) maximal at Pz compared to both Targets and Backgrounds. The findings suggest that despite the instruction to ignore the Distractors (checkerboards) both groups demonstrated increased distractibility towards irrelevant stimuli as by captured by enhanced positivity in both P3a and P3b amplitude responses. However, it seems that the healthy control group were able to refocus their attention following the intrusions as demonstrated by the lower mean number of both commission errors compared to the ADHD group. In addition, this effect was also confirmed by the statistically higher number of commission errors demonstrated by the ADHD group. A final point in relation to the greater activation during the Distractor condition is that arguably the checkerboards created an oddball effect, that is, greater activation to rare / novel stimuli during a succession of regular stimuli which is often captured by the P3a wave

(Friedman, et al., 2001a). The resulting ERPs are sequentially made up of the MMN and the novelty P3 (Friedman, et al., 2001a).

Blood measures of LC-PUFA and associations with performance data

The LC-PUFA levels in plasma choline phosphoglycerides measures were contrasted between the children with ADHD recruited during the MAAFA trial and the healthy control group. There were significant differences as predicted in key omega-3 and omega-6 fatty acids indices. Overall there was a pattern of persistently higher levels of both omega-3/6 in the healthy control group compared to ADHD. These findings support previous research suggesting abnormal levels of fatty acids in ADHD (Antalis, et al., 2006; Stevens, et al., 1996; Stevens, et al., 1995; Young, et al., 2004). These findings warrant further investigation to explore whether the differences reflect dietary intakes of fatty acids or alterations in fatty acid metabolism.

In relation to the correlational analysis for the performance data and PUFA fractions, there were no significant relationships between omega-3/6 and behavioural scores in the ADHD group. A significant negative relationship was observed in the healthy control group between the omega-3, ALA and errors of omission, suggesting that as omega-3 increased - omission errors decreased. Also in the healthy control group, there was a trend finding between lower levels of DHA and higher omission errors.

Furthermore, in the healthy control group, there was a significant positive relationship between the omega-6, AA and errors of omission implying as omega-6 levels increased, omission errors also increased. The AA/DHA ratio is especially important for membrane fluidity and glia cells (Gustafsson et al., 2010; Hulbert, 2003; Joardar, Sen, & Das, 2006). The ratio of these two fatty acids are also relevant for incorporation into cell membranes as they compete for the same desaturase and elongation enzymes (Gustafsson, et al., 2010). Therefore, a higher or excessive intake of the omega-6, AA, would automatically suppress the metabolism of the omega-3, DHA resulting in diminished levels of available DHA in the brain (Gustafsson, et al., 2010). AA is present throughout the brain but specifically concentrated in 26 varying regions of the brain with highest levels found in the hippocampus, cingulate, caudate, putamen, post-centralis, occipital, temporal, frontal and amygdala brain regions (Brenna & Diau, 2007b). Most of these brain areas have been found to be abnormal in structure and function in ADHD patients (Rubia, Halari, Taylor, et al., 2011; Valera, et al., 2007). DHA is likely to positively impact

cognition via its role in advancing neurotransmission (Wurtman, 2008). For example, DHA is able to increase the amount of dendritic spines, and elevate specific synapses of hippocampal neurons, in particular on excitatory glutamatergic synapses; a role which AA is not able to play (Wurtman, 2008). In contrast, AA plays a key role in the synthesis of eicosanoids and is the main substrate for the synthesis of the 2-series prostaglandins, the 4-series leukotrienes and thromboxanes A2 (Le, Meisel, de Meijer, Gura, & Puder, 2009). Higher levels of EPA down-regulate AA and simultaneously reduce pro-inflammatory eicosanoids and cytokines (Calder, 2008).

Associations between LC-PUFA and ERPs

The results of the present study did not support the hypothesis that omega-3 would be positively related to ERP responses to Targets. The largest non-significant relationship was observed between AA (c20_4n6) and P3a amplitude responses at parietal scalp regions in the ADHD group, suggesting that as AA increased, responses to target stimuli decreased. This relationship although non-significant was unexpected as it suggests a negative effect of AA in the electrical activation of P3a responses to the target stimuli in ADHD. The importance of AA in the brain for both structure and function cannot be refuted. Nevertheless upon release, AA can be metabolised to a countless number of bioactive derivatives called eicosanoids, which are associated with a range of chronic diseases (Rett & Whelan, 2011b). However, eicosanoids also play a key role in tissue homeostasis and the resolving of inflammation (Calder, 2006; Rett & Whelan, 2011a). It is thought that only when the membrane is damaged and free AA is released that it can be potentially harmful although not acting directly but as a consequence of its metabolites (Horrobin, 1990).

A further observation of note, is that the standard progression of the development of the visual P3 during the course of adolescence is a decrease in both amplitude and latency (Berman, et al., 1990; Berman, et al., 2006; Hill & Shen, 2002; Iacono, et al., 2002). This is thought to reflect patterns of cortical changes occurring in the adolescent brain involving neuronal and synaptic alterations including the finalisation of frontal myelination (Berman, et al., 2006; Luna, et al., 2004; Luna & Sweeney, 2004; Sowell, Thompson, & Toga, 2004). Therefore, the negative association between AA and P3 waves may also reflect the general decrease observed during adolescence. There is limited research in human studies in which to base these findings on.

However, one study found an association between higher levels of AA with lower symptoms of emotional problems suggesting that AA, which is rich in amygdala brain regions, may be involved more with emotional processes than measures of cognition (Kohlboeck et al., 2011).

One final observation, it may be that the FDR was too stringent in correction and an alpha criterion of for example, .005 (5 chances in a thousand) although arguably arbitrary would be less conservative and still minimise Type 1 error to 5 chances in a thousand.

Limitations

In relation to limitations of the study, task difficulty is worth mentioning. During sustained attention tasks the participant must demonstrate an element of vigilance, in other words the ability to sustain attention over long periods of time. The task employed in this study lasted for a total of 8 minutes which may not have been long enough to fully capture group differences. Furthermore, other investigations have provided confirmation of a differential alteration in performance over time as demonstrated by a larger decrement of performance and/or alterations in the intra-individual performance variability over time in children with ADHD relative to control children (Hooks, et al., 1994; Tucha, et al., 2009). During CPT's that have been modified into difficult and easy versions, participants without ADHD have demonstrated an increase in P3 amplitudes to unexpected stimuli from the easy to difficult version which was not observed in the ADHD group. This deviation was not evident however in responses to novel targets, and during this condition both groups displayed a reduction in P3 amplitudes suggesting that ADHD and control children share a similar reduction for easy task versions but not for difficult task versions. This finding has also been confirmed in other sustained attention tasks and has led to the notion that although ADHD and healthy control children may share the same attentional capacity, children with ADHD seem unable to allocate their attention when the demands of the task increased (Jonkman et al., 2000; Lopez et al., 2006; Steger, Imhof, Steinhausen, & Brandeis, 2000; van der Stelt, van der Molen, Boudewijn Gunning, & Kok, 2001). Therefore, arguably a more difficult version that the CPT task employed in this study, may elicit similar differences in selective attention processes in ADHD compared to control children as those observed previously. Future studies may consider that a Distractor condition may be an unnecessary interruption during a CPT task. Finally, there is the notion that clinically referred cases with

ADHD may present more severe symptoms than a community sample (Angold, et al., 1999; Sprafkin, et al., 2007) thus allowing greater capacity to capture group differences.

Conclusion

In conclusion, this study does not support previous literature which suggests that children with ADHD have abnormal P3 responses during both the target and non-target conditions of the CPT. The findings of this study demonstrate that children with ADHD are however impaired in the inhibitory measure of the performance data as reflected by higher commission errors compared to control children which supports previous literature finding similar deficits in ADHD. Despite differences in performance data, there were no group differences in brain function as measured by ERP responses which was unexpected. However, it should be considered that a minority of previous research has also confirmed no differences in attention capacity between ADHD and control children during other CPT task. The absence of group as discussed is likely to be due to task design which may not have been too short to fully capture sustained attention deficits. Furthermore, although fatty acids were significantly associated with performance, none of the relationships between ERP measures of brain function and fatty acids indices survived correction. Further research with larger sample sizes and a more demanding task design would be recommended to explore the role of fatty acids in sustained attention further.

Chapter Ten: Study 1C.

Emotion Processing in ADHD and Healthy Matched Controls

Introduction

Children with ADHD do not only suffer from hyperactive-impulsive and/or attention deficit symptoms but frequently have added difficulty with affect processing such as emotional dysregulation, extreme emotional reactivity and emotional instability (Schlochtermeier, et al., 2011). There is some evidence also that emotion dysfunction exists in children with ADHD on a behavioural level as evidenced by an inability to correctly identify the facial expressions of others especially emotions of fear, anger and sadness (Pelc, et al., 2006; Singh, et al., 1998; Yuill & Lyon, 2007). Facial expressions provide significant non-verbal social cues to affective states and are immediate indicators of emotional dispositions in other people (Eimer & Holmes, 2002). In ADHD, impaired interpersonal relationships have been reported and in children this is manifested within peer, sibling, teacher and parental relationships (de Boo & Prins, 2007; Greene, et al., 2001; Pelc, et al., 2006). Both lesion and neuroimaging research has illustrated that the amygdala and orbitofrontal cortex play an important role in the processing of affective facial expressions (Berlin, et al., 2004; Rolls, 2000). Other prefrontal regions such as the right anterior cingulate, right inferior parietal cortex, inferotemporal cortex, hippocampus and ventromedial occipitotemporal cortex are also implicated in the evaluation and examination of faces and facial expressions (Adolphs, et al., 1996; Blair, et al., 1999; Eimer & Holmes, 2002).

Symptoms of ADHD often overlap with symptoms of related disorders such as mood disorders (e.g., pediatric bi-polar emotional problems, anxiety disorders and bi-polar disorders (for a review see NICE, 2008) (Ryan-Krause, 2010a, 2010b). It is thought that approximately 50% to 60% of children with ADHD also meet the criteria for an additional psychiatric disorder (Reiff & Tippins, 2004; Ryan-Krause, 2010a). Despite, the knowledge that emotional problems frequently co-exist in ADHD, much less is known about the reasons for this (Taylor, Dopfner, et al., 2004). For these reasons, the investigation of affect processing in ADHD is especially relevant.

LC-PUFAs have an important role in the neurodevelopment of cognitive and emotional function throughout childhood (Ryan, et al., 2010). Supplementation of fatty acids (in particular

EPA) to clinical populations has been found to elevate mood and alleviate depression (Fontani, Corradeschi, Felici, Alfatti, Bugarini, et al., 2005; Freeman, et al., 2006; Peet & Horrobin, 2002a). Despite, the documented relationship between emotional lability and omega-3 fatty acids, there is only one study to date which has explored the relationship between LC-PUFA and affect processing in child/adolescents groups (Gow et al., 2009) and therefore more research in this area is needed.

As mentioned, in the introduction, several mechanisms of action have been proposed to account for the potential benefits of essential fatty acids, predominately related to their structural role in the brain and neurotransmission (Sinn & Howe, 2008). In relation to the brain, both DHA and AA are found in elevated quantities in the grey matter of the cerebral cortex, in particular in the membranes of neuronal synapses (Gow, Matsudaira, Taylor, Rubia, Crawford, Ghebremeskel, Ibrahimovic, Vallee-Tourangeau, et al., 2009; McNamara, 2006; McNamara, et al., 2009; McNamara, Jandacek, et al., 2010). Both EPA and DHA are linked with many important functions related to neural activity such as neurotransmission, ion channel, myelination membrane fluidity, enzyme regulation and gene expression (Lauritzen, et al., 2001; Sinn & Howe, 2008). Inadequate supplies of DHA *in-utero* are associated with impaired learning and attention in addition to emotional irregularities such as increased depression, anxiety and aggression in animal models (Fedorova & Salem, 2006). These irregularities have been found to be in part related to alterations in neurotransmission function (Mathieu, et al., 2008). For example, dietary induced deficiencies in DHA result in a deregulation of the meso-cortico-limbic dopaminergic pathway which in turn is implicated in emotion and reward processes (Zimmer, et al., 2002). Rodents receiving a chronically deficient omega-3 diet lead to a reduction in the release of acetylcholine and serotonin in the hippocampus (Mathieu, et al., 2008). Furthermore, the amount of omega-3 PUFA in the diet influenced the serotonergic receptor and the muscarinic receptor binding (Mathieu, et al., 2008).

In human studies, cumulative evidence suggests that lower PUFA levels may be linked with behavioural problems commonly associated with ADHD (Edwards, et al., 1998; Gesch, et al., 2002; Nemets, et al., 2006; Osher, et al., 2006; Peet, et al., 1998). Several research trials have found supplementation with omega-3 fatty acids to be advantageous in reducing symptoms of child depression (Nemets, et al., 2006), anti-social, violent and psychopathic behaviours

(Corrigan, et al., 1994; Gesch, et al., 2002; Zaalberg, et al., 2010), and aggression (which is linked to both depression and anxiety) (Itomura, et al., 2005).

Event Related Potentials and Face Processing

Event related potentials (ERPs) are an imaging technique with a high temporal resolution which is advantageous for the study of emotion processing and allows the investigation of automaticity during different, temporally separate, stages of emotion processing (Rellecke, Sommer, & Schacht). Seminal research by Halgren and Marinkovic (1994) proposed an ERP model for the appraisal and response to emotional stimuli. In this model, there are 2 stages, "orienting" and "event integration" in distinguishing conscious and non-conscious emotion perception. *Orienting* can be described as the automatic interruption of continuing processing so as to direct attention towards a new and considerably hostile event in order to mobilise cognitive and behavioural resources for flight or fight action (Halgren, Baudena, Heit, Clarke, Marinkovic, Chauvel, et al., 1994; Liddell, et al., 2004). The orienting response is considered to be free from conscious consideration and is captured by the N2/P3a/slow wave complex peaking around 200, 280 and 350 ms post-stimulus onset (Halgren, Baudena, Heit, Clarke, Marinkovic, Chauvel, et al., 1994; Kenemans, et al., 1992; Liddell, et al., 2004). Evidence suggests that N2 is altered by emotional expressions and in particular face stimuli (Bentin, et al., 1996; Halgren, Baudena, Heit, Clarke, Marinkovic, Chauvel, et al., 1994; Halgren, Baudena, Heit, Clarke, Marinkovic, & Clarke, 1994; Liddell, et al., 2004; Sokolov & Boucsein, 2000). The P3a has been linked with the automatic features of the orienting response which are implicated in the perception of threatening and/or novel stimuli (Friedman, et al., 2001a; Johnston, et al., 1986; Lagopoulos, et al., 1998). The *event integration* aspect of the model is characterised by the N4/P3b and this stage of the time sequence (circa 430-600 ms post-stimulus) is involved in the cognitive integration to generate conscious emotional experience (Liddell, et al., 2004). The N4 deflection is considered to be a marker of semantic processing (Kiefer & Spitzer, 2000; Liddell, et al., 2004). The P3b is generated in the response to the careful registration of the stimulus, created by the early orienting response and followed by the succeeding updating of the stimulus framework (Halgren, Baudena, Heit, Clarke, Marinkovic, Chauvel, et al., 1994; Liddell, et al., 2004). In contrast to the fairly sizeable adult ERP literature - and much smaller developmental literature in healthy

populations - in face processing, the neurophysiological correlates of emotional dysfunction in children/adolescents with ADHD is virtually unexplored.

Williams and colleagues (2006) used an ERP face recognition task to demonstrate that signals of potential threat as depicted in facial expression of fear are given precedence over neutral and positive (e.g., happy faces) signals (Williams, et al., 2006). Fearful faces were persistently notable by increased positivity, linked with an active shift from temporal, frontal regions (first 120 ms) to more dispersed cortical sources (120 -220 ms) and again back to the medial fronto-centro region (220 – 450 ms) (Williams, et al., 2006). Faces depicting happiness, by contrast, elicited a separate increased wave of negativity, observed at a later time point of approximately 230-350 ms, localised to the fusiform area of the temporal cortex (Williams, et al., 2006). The results indicate that fear signals seem to be given priority in neuronal processing schemes, over positive signals which may be held back until alertness for possible danger is resolved (Williams, et al., 2006). Furthermore, while fear may be processed by the use of parallel pathways instigated ahead of structural encoding, neural systems involved in the processing of positively valenced stimuli (e.g., happy faces) may be more localised and depend on structural encoding (Williams, et al., 2006). Another study by Liddell and colleagues (2004) found that the P3b wave, which is associated in the merging of emotional features, was enhanced to supraliminal perception of fear (Liddell, et al., 2004). The findings therefore also support the notion that systems for evaluating threat related signals may be instigated involuntarily and without the necessity for conscious recognition of these signals (Liddell, et al., 2004).

Another study by Williams et al. (2004) employed ERPs to investigate the time course of neural responses of perception of fear relative to neutral between conscious (overt) and non-conscious (covert) face processing in 20 healthy volunteers. Non-conscious fear perception (both discrimination and detection) elicited greater N2 responses at fronto-central sites (Fz, Cz), earlier P1 response (e.g., within 100 ms post-stimulus onset relative to neutral) and by contrast a more prominent N4 in relation to conscious perception (Williams, et al., 2004). N2 may provide a temporal correlate of the early sensory processing of prominent facial configurations whereas the N4 may index the conscious amalgamation of emotion stimuli in WM, subserved by larger cortical activity (Liddell, et al., 2004; Williams, et al., 2004).

Differences in emotion related ERP responses have been reported between adolescents with ADHD (n = 51) and typically developing controls (n = 51) (Williams, Hermens, et al.,

2008). These differences were characterised by higher self-rated scores of both depression and anxiety as rated by the Depression, Anxiety and Stress scales (DASS), difficulties correctly identifying threat related faces (e.g., anger and fear) alongside differences in ERPs marked by a clear reduction in early stages of processing over occipital activity (circa 120 ms), proceeded by an amplification of activity associated with structural encoding (120-220 ms), followed by a decrease and general slowing of activity over temporal areas known to sub-serving context processing (300-400 ms) (Williams, Hermens, et al., 2008). The ADHD group also displayed reductions in the P120 waves and increased N170 deflections to facial expressions (Williams, Hermens, et al., 2008). Hermann and colleagues (2010) have also investigated ERP responses to positive (i.e., happy faces), negative (i.e., fear, anger and sad faces) and neutral pictures in adults with and without ADHD (Herrmann, et al., 2010). The results demonstrated less reactivity to happy faces in ADHD relative to controls supporting the dysfunctional motivational-reward system theory in ADHD (Herrmann, et al., 2009; Sonuga-Barke, 2003, 2005).

Gow and colleagues (2009) reported for the first time the relationship between a cognitive bias ERP measurement and PUFA fractions in the blood samples of adolescent boys with ADHD (Gow, Matsudaira, Taylor, Rubia, Crawford, Ghebremeskel, Ibrahimovic, Vallée-Tourangeau, et al., 2009). The findings demonstrated a positive association between EPA and a cognitive bias in orientation to overt expressions of happiness relative to both sad and fearful faces as indexed by midline frontal P300 amplitude. Furthermore, exploratory analyses reported a significant positive correlation between DHA and the right temporal N170 response to fear. The right temporal N170 response was also negatively correlated AA/DHA ratio to concealed expressions of fear. The results suggest that both EPA and DHA may be implicated in different features of affect processing in ADHD (Gow, Matsudaira, Taylor, Rubia, Crawford, Ghebremeskel, Ibrahimovic, Vallée-Tourangeau, et al., 2009) and provide insights into the current, inconsistent literature on PUFA intervention in ADHD and depression.

The present study aimed to assess differences in ERP responses to facial stimuli depicting five emotions (fear, sad, happy and anger) contrasted with a neutral face (i.e., with no expression) between ADHD and controls. It was hypothesised that these differences would be characterised by less reactivity to emotional expressions of happy faces as indexed by P3 and N2 AUC amplitudes in the ADHD group and enhanced reactivity to fear in the healthy control group across frontal-central and parietal scalp regions. Furthermore, it was hypothesised that the

healthy control group would demonstrate enhanced responses for the late N4/P3b *inhibitory* component, implicated in the integration of emotional processes (Liddell et al, 2004) given the evidence suggesting ADHD may be impaired in correctly identifying the emotional status of others (Singh, et al., 1998). In relation, to the relationship between PUFA and ERP responses to facial expressions of emotion, it was predicted that (1) omega-3 levels would be negatively correlated with brain function as indexed by the N4/P3b complex and N4 wave to both positive and negative stimuli (i.e., facial expressions of fear, sad, happy and angry faces); (2) omega-3 PUFA, (in particular EPA) would be positively correlated with P3 responses to facial expressions of happiness (i.e., the higher the omega-3 - the greater the activation to happy faces); and finally (3) omega-6 would be positively associated with N4 responses to negative stimuli (i.e., facial expressions of fear, anger and sad faces) in both cases and controls.

Method

Participants

The EEG/ERP data for the ADHD children had previously been collected during the MAAFA trial. During the MAAFA trial, a total of 76 male children/adolescents were drawn from various special educational settings (e.g., boarding schools, mainstream schools with provision for children with emotional and behavioural difficulties) in and around the London area and were screened to ensure they met the criteria for ADHD according to the DSM-IV. As per before, this included a short semi-structured interview, (Children's Interview for Psychiatric Syndromes, *ChIPS*) based on DSM-IV criteria (ChIPS see Rooney, Fristad, Weller & Weller, 1999) and that both Parent and Teacher Connor Rating Scales (CPRS/CTRS) were equal to or above 65 (> 95th percentile). The EEG/ERP data was age matched to the healthy control group. Intelligence quotient (IQ) for all participants had to be higher than 70 on the prorated IQ as assessed using the Kaufman Brief Intelligence test (K-BIT).

In relation to the recruitment of the healthy age and gender matched controls, participants were screened in the same way as the ADHD group to ensure that their IQ was higher than 70 using the K-BIT and that they did not meet criteria for ADHD according to the DSM-IV. This included the ChIPS interview and ensuring scores of the Conner's Parent and Teacher Rating

Scale ADHD index *t* score were collectively lower than 65. Their teachers were advised of the recruitment criteria and pre-screened children in terms of suitability for each group e.g., ensured that the healthy controls were not known to have behavioural difficulties or special educational needs. As well as completing the battery of clinical questionnaires, and providing a 16 ml blood sample, the healthy controls were tested on all EEG/ERP tasks so as to provide control data for the pre-existing EEG/ERP data collected during the MAAFA trial. Exclusion criteria were as per previously reported in Chapters 8 and 9.

Data were available from the task for a total of 63 male adolescents. This was made up of baseline data from 31 children/adolescents that had been recorded during the Maudsley Adolescence ADHD Fatty Acid (MAAFA) trial and met criteria for ADHD and 32 healthy age and gender matched control children.

Age, IQ, Handedness and Medication

The mean age for the ADHD group was 14.00 years (M = 14.00, SD = 1.10) versus 14.46 years for the HC group (M = 14.46, SD = 1.12). There were no statistically significant differences in age between the ADHD group (M = 14.00) and the control group (M = 14.46), t (65) = -1.72, p = .09.

In the ADHD group, 28 males were right handed, one was left handed and two were ambidextrous. In the control group, 28 were right handed, and four were left-handed. A Chi-square test for independence indicated no significant group differences in the handedness distribution, χ^2 (2, 73) = 3.71, p = .15.

Twenty two of the ADHD participants were medication naïve and the remaining nine underwent a 48 hour wash out period for stimulant medication, in line with current EEG practice.

There were statistically significant differences between measures of composite (overall) IQ between the control group (M = 118.73, SD = 13.58) and the ADHD group (M = 96.32, SD = 13.96), t (67) = -6.73, p > .001, with ADHD scoring lower in IQ than controls. It should be considered that the selection of groups recruited in this study was not random (as in most case-control studies) and therefore covarying for IQ would violate the ANCOVA assumptions altering the group effect in potentially problematical ways and resulting in spurious findings (Bridgett &

Walker, 2006; Dennis, et al., 2009; Miller & Chapman, 2001). However, in order to address any potential associations between IQ and measures that differed between groups, correlations were investigated between those measures and IQ.

Procedure & Materials

Children and adolescents below the age of 16 years old were accompanied by an appropriate adult (parent, teacher or guardian) to the Institute of Psychiatry at The Maudsley Hospital where approximately 16 ml of blood was taken by a qualified phlebotomist. Blood assessments required eight hours fasting beforehand, although water was permitted, and this was explained fully to the parent and child prior to the appointment. The blood was taken by a qualified phlebotomist, transported by motor-cycle courier to the Science Centre at the London Metropolitan University where it was spun before storing at -80 degrees Celsius. Following the blood sample all participants were given a complimentary breakfast at the Maudsley restaurant. They were then taken to a quiet, well lit testing room to complete all the self-reported questionnaires. Instructions were given for each questionnaire and assistance during completion where necessary. All participants in the healthy control group were given also an EEG/ERP assessment. On occasion, data collection took place over 2 visits, with the child assessment and screening sometimes taking place at school. Participants were given £20.00 for their participation and all associated travel expenses were refunded to the parent/guardian. During the MAAFA trial, any children taking stimulant medication for ADHD were required a wash-out for 48 hours prior to the EEG recording.

Blood Analysis

Total lipids were extracted from 1 ml of plasma according to the Folch method. The red cells were homogenized in chloroform and methanol (2:1 v/v) containing 0.01% butylated hydroxytoluene as an antioxidant, under nitrogen. Fatty acid methyl esters (FAMEs) were prepared by heating the extracted total lipid in 4ml of 15% acetyl chloride in methanol for 3h at 70°C, under nitrogen in a sealed vial. FAMEs were separated by a gas-lipid chromatograph (HRGC MEGA 2 series, Fisons Instruments, Italy) fitted with a capillary column (60 m x 0.25 mm x 0.25 μm, BPX70). Hydrogen was used as a carrier gas, and the injector, oven and detector temperatures were 235°C, 200°C and 250°C, respectively. FAMEs were identified by

comparison with relative retention times of authentic standards and calculation of equivalent chain length values. Peak areas were quantified by a computer chromatography data system (EZChrom Chromatography Data System, Scientific Software Inc., San Ramon, CA, USA). This study reports data from plasma phopshatidylcholine (PPC) only.

ERP recording

EEG data were collected from 26 electrodes using an adapted 10-20 system following an internationally standardized protocol LabNeuroTM (Brain Resource, 2010). Participants sat in a light and sound attenuated room with an ambient temperature of 24˚C. A NeuroScan Quik cap and NuAmps amplifier (sampling rate = 500 Hz) were employed to collect EEG data from electrode sites. Data was recorded relative to a virtual ground, but referenced offline to linked mastoids. Horizontal eye movements were recorded with electrodes placed 1.5cm lateral to the outer canthus of each eye. Vertical eye movements were recorded with electrodes placed 3mm above the middle of the left eyebrow and 1.5cm below the middle of the left bottom eye-lid. Skin resistance was < 5 kOhms. A continuous acquisition system was employed and data was EOG corrected offline (Gratton, Coles, & Donchin, 1983). A low pass filter with attenuation of 40dB per decade above 100 Hz was employed prior to digitization.

ERP area under the curve

Average ERPs were calculated for event types corresponding to a stimulus type in each paradigm. For each channel, the individual single-trial epochs were filtered with a low-pass Tukey filter function that attenuates frequencies above 25 Hz. A cosine ramp from 1 down to 0.5 between 25Hz and 35Hz is used as an envelope on the FFT data in the Tukey filter. The single trials were then averaged to form conventional ERPs. The averages of the pre-stimulus period -300 to 0 ms were subtracted from the ERP data. The signal was then down sampled by a factor of 4 (leading to 8 ms samples). The amplitude of the waveform (in microvolts) relative to the zero baseline is calculated for single time points in 8ms, then multiplied by a factor of 8 to achieve a measure of the area under the curve in a unit of microvolt-milliseconds. The AUC is the integral of the curve over a specified time range. Using the AUC measure, the space between the ERP waveform and baseline was divided into multiple 50ms time-windows that can be approximately mapped onto ERP component time-windows.

P200 is mapped onto time unit 5 (200-250 ms), N200 on to time unit 6 (250-300 ms), P3a onto time unit 7 (300-350 ms) and P3b (350-400 ms) on time unit 8. Units are in microvolts multiplied by ms.

Emotion Processing Task

EEG data were recorded during an emotion perception task containing 48 grey scale stimuli of facial expressions. The stimuli represented 3-D facial expressions of happiness, sadness, anger, fear and disgust relative to neutral faces and were chosen from a standandised set of stimuli (Gur, Sara, Hagendoorn, Marom, Hughett et al., 2002). The stimuli were made up of eight different individuals representing each expression. The images were tailored for orientation (i.e., so that the eyes of each image were at the central horizontal in all cases) and equivalent luminance. A maximum of 192 stimuli (8 different individuals representing each expression recurred four times) were shown pseudo-randomly under both covert (to measure non-conscious, automatic processing) and overt (to measure controlled processing) conditions.

In the covert condition, the facial expressions were shown for 10 ms, followed by a neutral mask for 150 ms. An ISI of 1,100 ms between target and mask was used to make sure that the total length of stimulus plus ISI was the same across conditions (1, 267 ms). In the overt condition, the duration of each stimulus was 500 ms, with inter-stimulus interval (ISI) of 767 ms. The mask was spatially offset somewhat (randomly, 1 degree in the course of the four diagonals) to control for the possible perceptual priming effects (in other words, the perceptual difference due to the arrangement of fear-neutral opposed to neutral-neutral target-mask pairs). Participants were advised to focus on each face in preparation for post-test questions to ensure that attention was paid. This particular task has been employed in a number of published research papers to date (see Williams et al., 2006; Williams et al., 2008). This PhD reports data from the overt condition only.

Results

Statistical Analyses for Electrophysiological data

The data were analysed using a series of mixed model 4 x 5 analyses of variance (ANOVA). The between subjects factor was group (ADHD versus control children), while facial expressions (Fear, Neutral, Sad, Happy and Anger) and electrode position (Fz, FCz, Cz, and Pz) were the within subjects factors. The dependent measures were the AUC amplitude responses (at different time points). As per previous tasks, timepoints 5 – 8 were included in the analysis. However, previous research suggests that facial recognition especially those of fear elicit larger and faster responses within the 150 ms post-stimulus range (Williams, et al., 2004) and for that reason an earlier time point (4) was included in the analysis. The time points of interest were: time point 4 (P2: 150-200 ms), 5 (N2: 200-250 ms), 6 (P3a: 250-300 ms), 7 (N4/P3b: 300-350 ms) and 8 (N400: 350-400 ms). Significant main effects of electrode site or condition were not further followed up with post-hoc tests. However, all group or interaction findings were followed up with post-hoc tests and pairwise comparisons. Bonferoni corrected for multiple testing.

Time point 4 (P2 deflection)

The mean values (μV) and standard errors for the AUC P2 amplitude responses for ADHD and control children are plotted in Figure 1. Mauchly's test indicated that the assumption of sphericity had been violated for the within-subject factors: electrode site, χ^2 (5) = .117, $p < .05$, and for the interaction between electrode site and faces, χ^2 (77) = .005. This was corrected using Greenhouse-Geisser estimates of sphericity. The corrected results are reported.

For the P2 AUC responses (time point 4), the analyses revealed a trend finding for the main effect of the between-subjects factor group (ADHD versus controls), $F(1, 61) = 3.24$, $p = .07$, with greater negative activation in the healthy controls ($M = -162.47$, $SE = 30.68$) compared to ADHD ($M = -83.74$, $SE = 31.17$). There were significant main effects for the within-subjects factor electrode site (Fz, FCz, Cz, Pz), $F(1.42, 86.49) = 21.36$, $p < .001$, with greater activation at Fz ($M = -155.73$, $SE = 22.73$) and also for faces, $F(4, 244) = 8.65$, $p = .001$, with happy faces producing greater positive going activation ($M = 160.93$, $SE = 26.29$).

There was a trend finding for the interaction between electrode site and group, $F(3, 183) = 2.32$, $p = .07$. Post-hoc tests revealed that this was due to significantly greater difference in

activation at FCz electrode site between ADHD and controls, $p < .05$ with enhanced negativity for controls compared to ADHD. The same pattern was observed at Cz with greater negativity in controls compared to ADHD, $p < .05$.

There was a significant interaction between the within-subjects factors faces and group, $F(4, 244) = 2.84$, $p < .03$. Post-hoc tests revealed that this was due to significant differences between ADHD and controls in P2 responses to fear, neutral and angry faces. The control group displayed greater negative going activation to facial expressions of sad compared to ADHD, $p < .05$. There was also a significant difference in responses to neutral faces between ADHD and controls, $p < .05$. Finally, there was a significant difference between facial expressions of anger between ADHD and controls, $p < .05$. As per before the control group displayed greater negative going activation relative to the ADHD group.

There was a significant interaction between electrode site and faces, $F(6.47, 394.46) = 2.77$, $p < .02$. Post-hoc tests revealed this was due to significant differences between all facial expressions and electrode sites, with happy faces generating the greatest negative activation at Fz, FCz, and Cz, this shifted to sad faces generating the highest negative activation at Pz electrode site, $p < .05$.

Finally, a trend finding was observed for the 3 way interaction between electrode site, faces and group, $F(12, 732) = 1.56$, $p = .09$. Post-hoc analyses showed that this was due to significant differences at Fz for responses to fear and anger between ADHD and controls. There were also significant differences between FCz for responses to neutral, sad and angry faces and Cz for responses to neutral and angry faces. In all cases activation was attenuated to these facial expressions in ADHD relative to controls, $p < .05$.

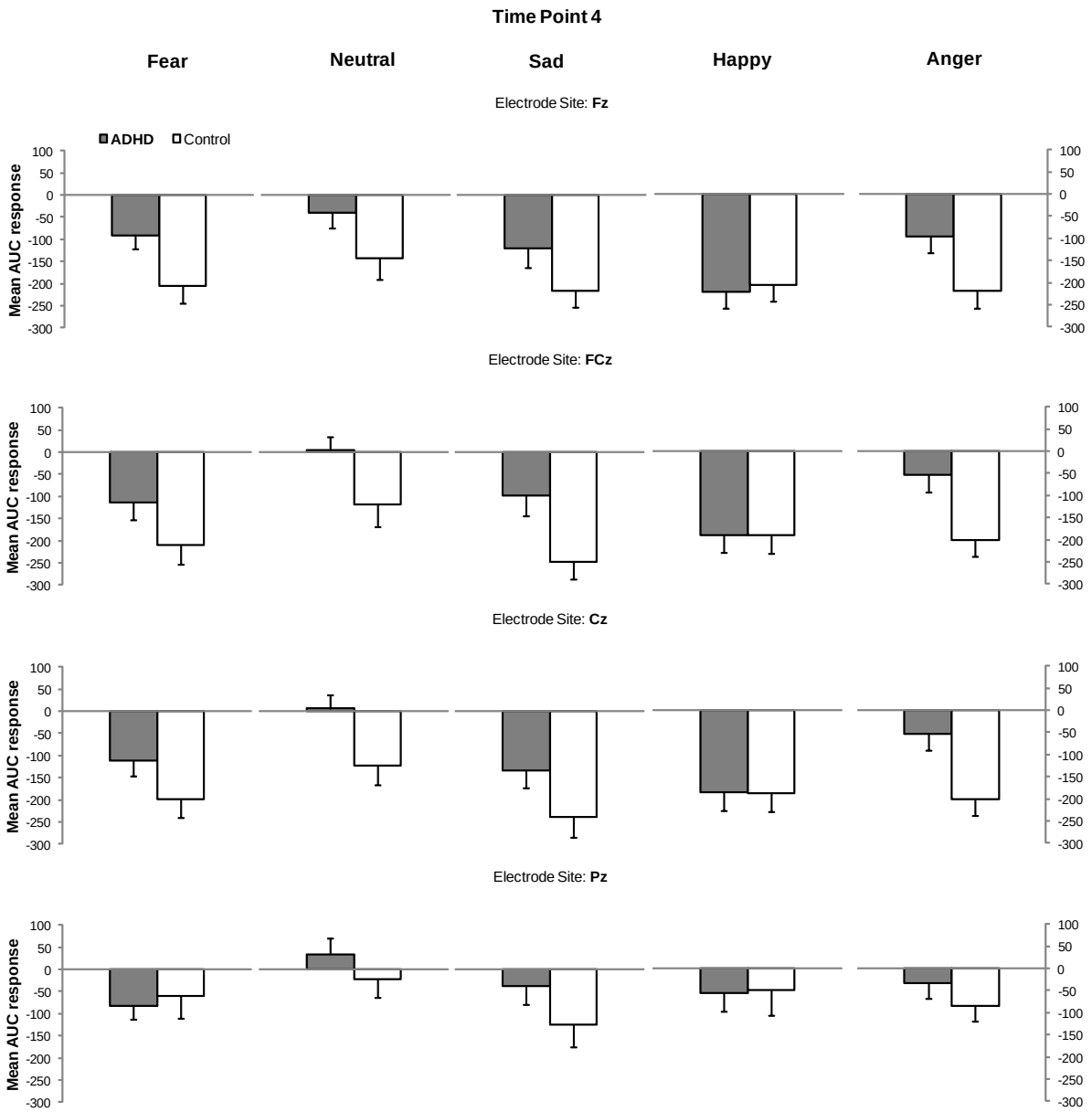


Figure 1. Mean AUC amplitude responses for electrode sites Fz, FCz, Cz and Pz for facial expressions of fear, neutral, sad, happy and anger at time point 4.

Time point 5 (N2 deflection)

Mauchly's test indicated that the assumption of sphericity had been violated for the within-subject factors: electrode site, χ^2 (5) = .102, $p < .05$, and for the interaction between electrode site and faces, χ^2 (77) = .009. This was corrected using Greenhouse-Geisser estimates of sphericity. The corrected results are reported.

For the N2 AUC responses (time point 5), the analyses revealed a significant main effect of the between-subjects factor group (ADHD versus controls), $F(1, 61) = 7.12$, $p > .02$ with greater activation to all facial expressions for the control group (M = -130.25, SE = 28.52) relative to ADHD (M = -21.73, SE = 28.97) . There were significant main effects for the within-subjects factor electrode site (Fz, FCz, Cz, Pz), $F(1.34, 83.37) = 38.26$, $p < .001$ with greater activation at Fz (M = -118.17, SE= 21.04). A trend finding was observed for the within-subjects factor faces, $F(4, 244) = 2.07$, $p = .086$ with greater negative activation to angry faces, (M = -97.55, SE = 24.30).

There was also a non-significant trend for the interaction between electrode site and faces, $F(7.12, 434.23) = 1.91$, $p < .066$. There were significant differences between facial expressions of fear (M = -54.56, SE = 25.34) and neutral (M = -143.24, SE = 31.21, fear (M = -54.56, SE = 25.34) and happy (M = -142.29, SE = 27.03) and fear (M = -54.56, SE = 25.34) and anger (M = -138.08, SE = 26.47), $p < .05$. Facial expressions of neutral elicited the greatest negative response, while responses for fear produced the smallest negative response. The mean values (μV) and standard errors for the AUC N2 amplitude responses for ADHD and control children are plotted in Figure 2.

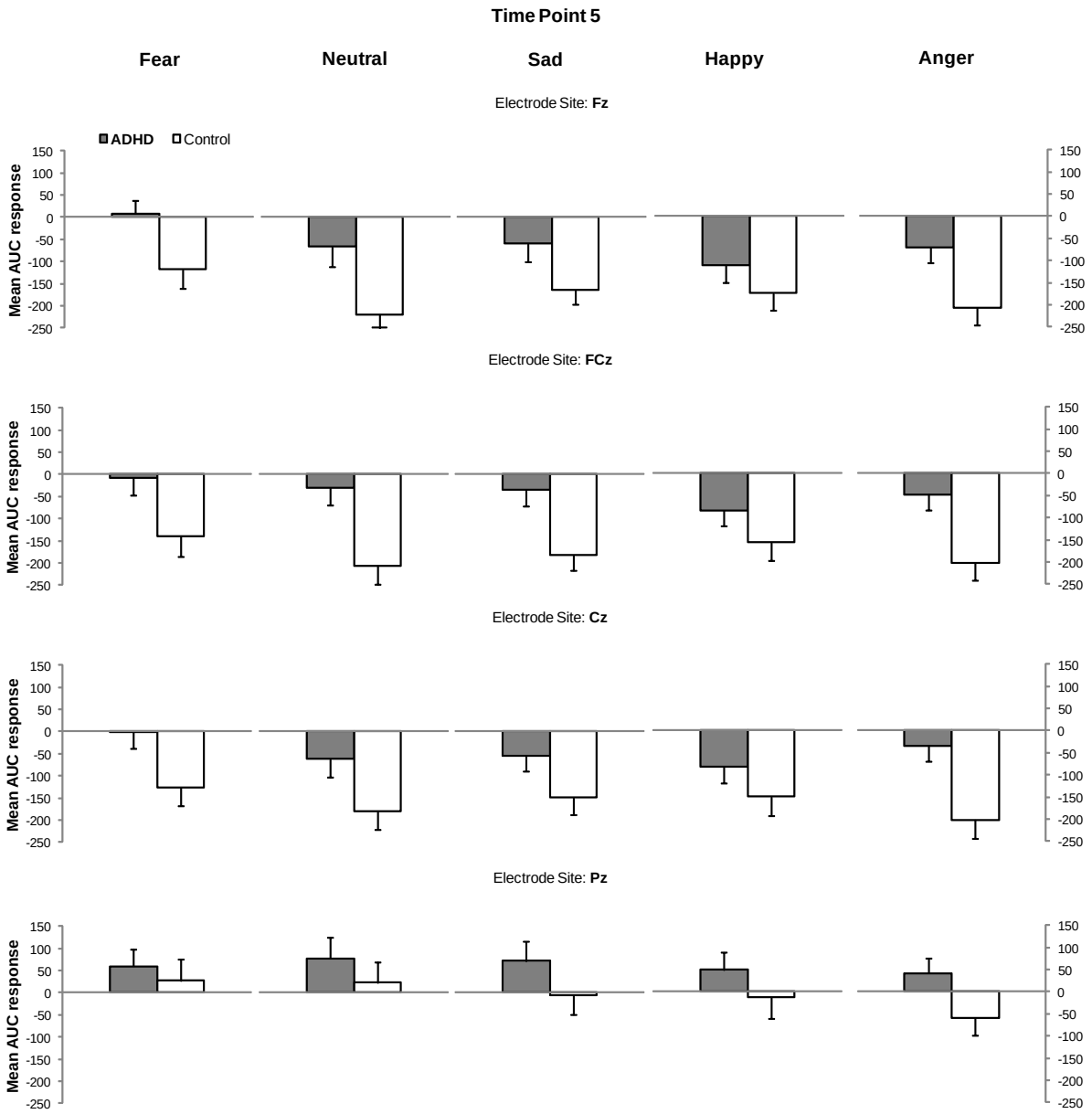


Figure 2. Mean AUC amplitude responses for electrode sites Fz, FCz, Cz and Pz for facial expressions of fear, neutral, sad, happy and anger at time point 5.

Time point 6 (P3a deflection)

The mean values (μV) and standard errors for the P3a AUC amplitude responses for ADHD and control children are plotted in Figure 3.

Mauchly's test indicated that the assumption of sphericity had been violated for the within-subject factors: electrode site, χ^2 (5) = .129, $p < .05$, faces, χ^2 (9) = .672, $p < .05$ and for the interaction between electrode site and faces, χ^2 (77) = .013. This was corrected using Greenhouse-Geisser estimates of sphericity. The corrected results are reported.

For the P3a AUC responses (time point 6), the analyses revealed a no significant main effect of the between-subjects factor group (ADHD versus controls), $p > .05$. There was a significant main effect for the within-subjects factor electrode site (Fz, FCz, Cz, Pz), $F(1.40, 85.48) = 74.83$, $p < .001$, again this was driven by greater negative activation at Fz, (M = -171.66, SE = 23.75). The within-subjects factor faces was also significant, $F(3.29, 200.90) = 3.84$, $p < .01$ with greater negative activation to neutral faces, (M = -162.84, SE = 32.24).

There was a trend finding the interaction between electrode site and faces, $F(7.25, 442.06) = 1.99$, $p = .053$. Post-hoc tests showed that this was due to a significant difference between facial expressions of fear (M = -98.98, SE = 30.38) and neutral (M = -238.84, SE = 34.71) at Fz electrode site, $p < .05$. There was also a significant difference between facial expressions of fear (M = -95.76, SE = 35.53) and neutral (M = -232.63, SE = 35.88) at Cz, $p < .05$. On both occasions, neutral faces produced an increase in negative activity across frontal-central scalp regions.

No other interactions reached significance.

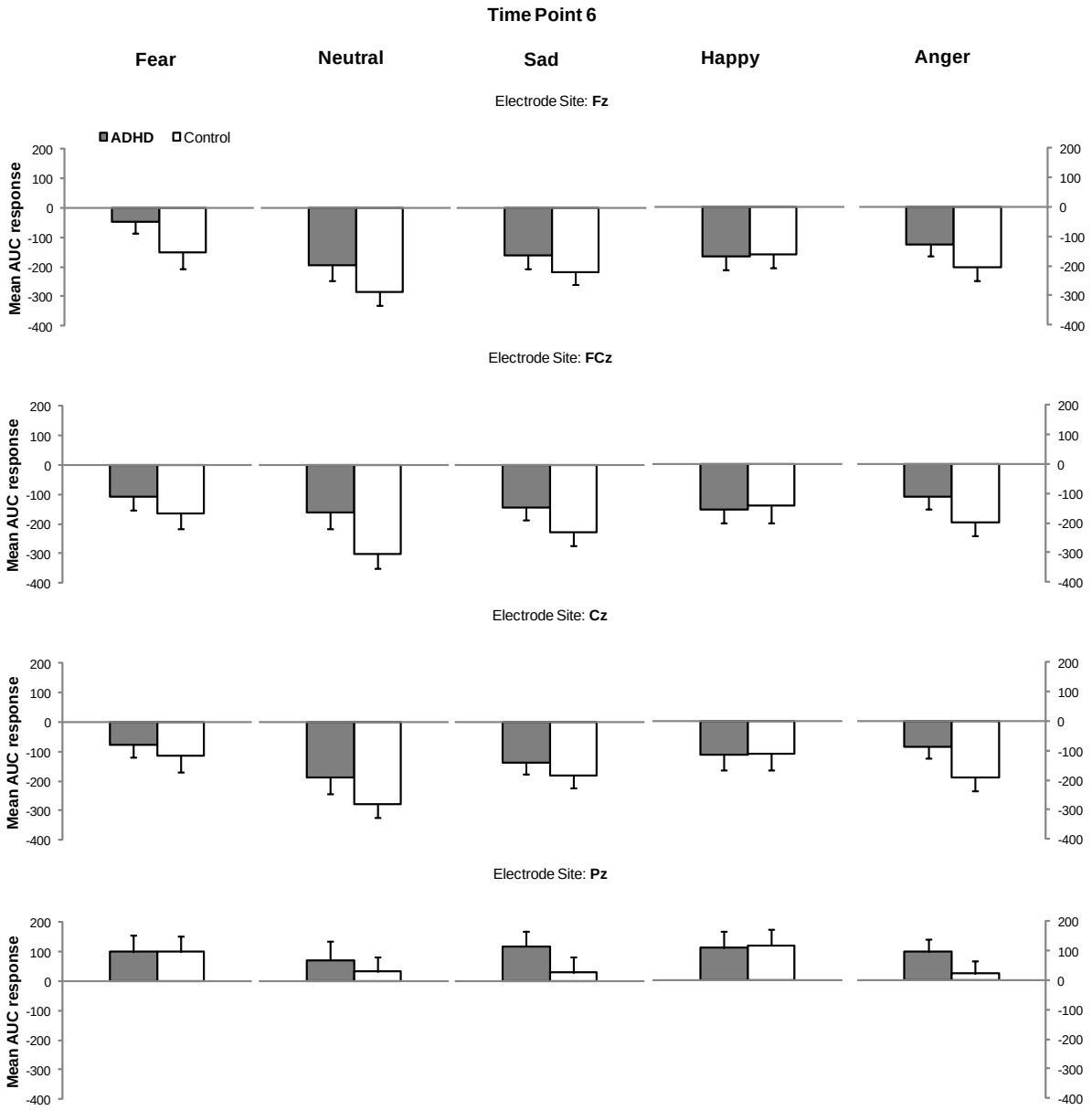


Figure 3. Mean AUC amplitude responses for electrode sites Fz, FCz, Cz and Pz for facial expressions of fear, neutral, sad, happy and anger at time point 6.

Time point 7 (N4/P3b deflection)

The mean values (μV) and standard errors for the AUC N4/P3b amplitude responses for ADHD and control children are plotted in Figure 4.

Mauchly's test indicated that the assumption of sphericity had been violated for the within-subject factors: electrode site, χ^2 (5) = .176, $p < .05$, faces, χ^2 (9) = .681, $p < .05$ and for the interaction between electrode site and faces, χ^2 (77) = .006. This was corrected using Greenhouse-Geisser estimates of sphericity. The corrected results are reported.

For the N4/P3b AUC responses (time point 7), the between-subjects factor group (ADHD versus controls) was not significant, $p > .05$. There was a significant main effect of the within-subjects factor electrode site (Fz, FCz, Cz, Pz), $F(1.54, 93.75) = 96.43$, $p < .001$ which was due to greater activation at FCz, (M = -209.64, SE = 25.93).

A trend finding was observed for the within-subjects factor faces, $F(3.27, 199.59) = 2.18$, $p = .086$ with greater mean activation to sad faces, (M = -172.51, SE = 29.00). None of the interactions reached significance, $p > .05$.

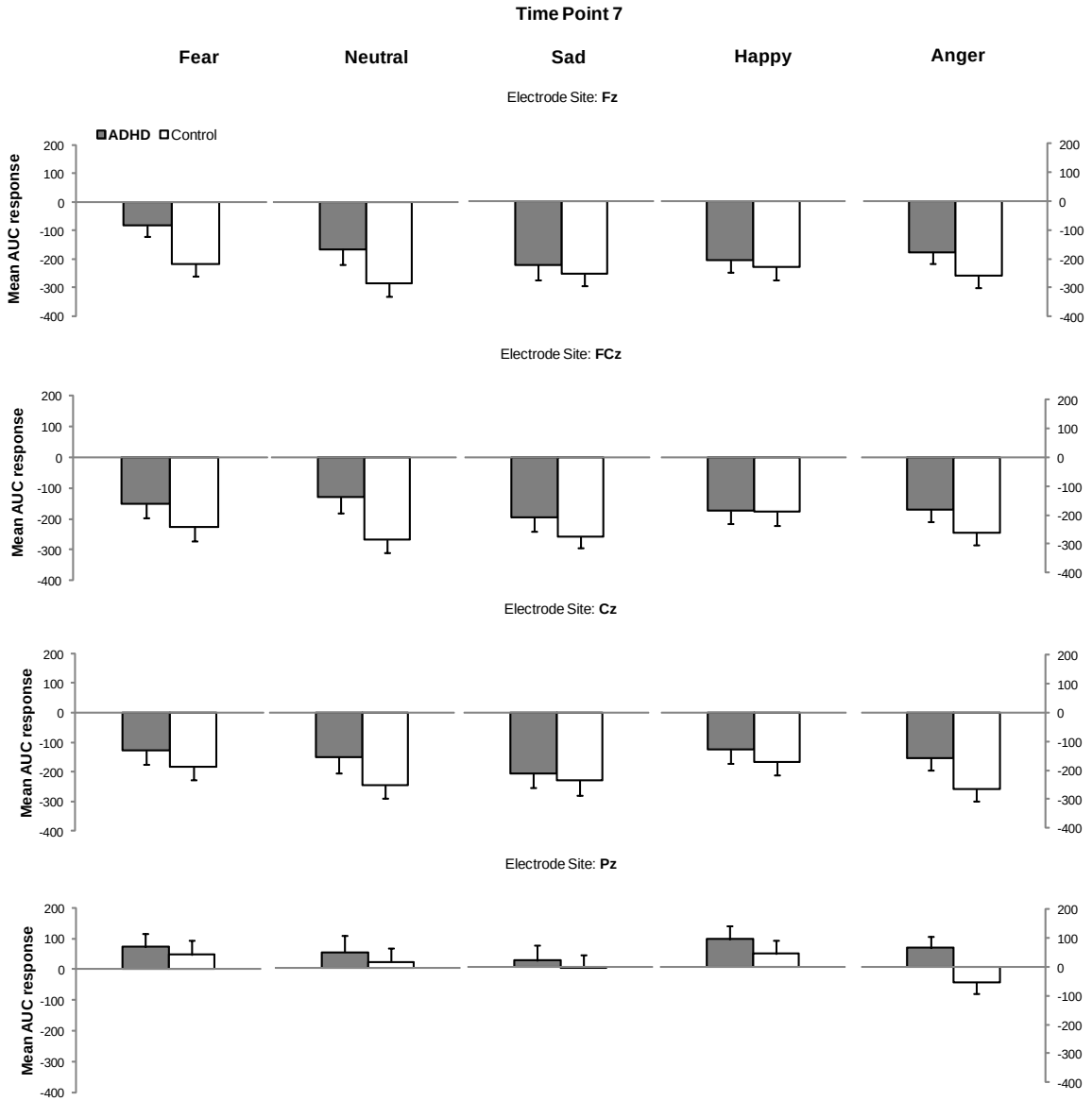


Figure 4. Mean AUC amplitude responses for electrode sites Fz, FCz, Cz and Pz for facial expressions of fear, neutral, sad, happy and anger at time point 7.

Time point 8 (N4 deflection)

The mean values (μV) and standard errors for the AUC N4 amplitude responses for ADHD and control children are plotted in Figure 5.

Mauchly's test indicated that the assumption of sphericity had been violated for the within-subject factors: electrode site, χ^2 (5) = .183, $p < .05$, faces, χ^2 (9) = .719, $p < .05$ and for the interaction between electrode site and faces, χ^2 (77) = .022. This was corrected using Greenhouse-Geisser estimates of sphericity. The corrected results are reported.

For the N4 AUC responses (time point 8), the analyses revealed a significant main effect of the between-subjects factor group (ADHD versus controls), $F(1, 61) = 5.42$, $p < .03$ with significantly lower activation in N4 responses in ADHD (M = -146.32, SE = 31.93) relative to controls, (M = -250.67, SE = 31.43).

There were significant main effects for the within-subjects factor electrode site (Fz, FCz, Cz, Pz), $F(1.58, 96.13) = 65.72$, $p < .001$, this was driven by higher negative activation at Fz (M = -250.33, SE = 23.70). A trend finding was observed for the within-subjects factor faces, $F(3.45, 210.52) = 2.26$, $p = .073$, this was driven by greater mean activation to angry faces, (M = -233.44, SE =26.10). None of the interactions reached significance, $p > .05$.

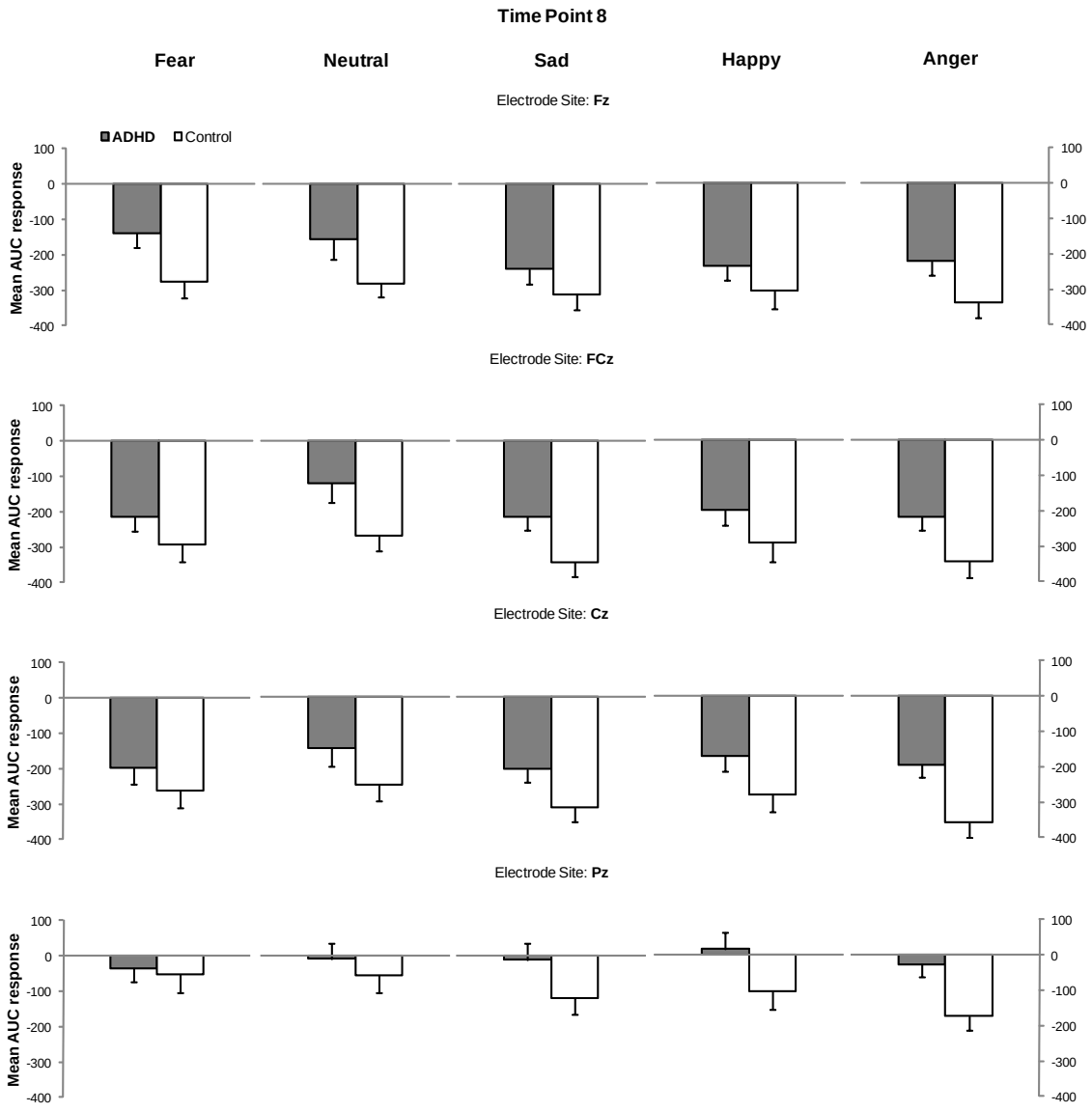


Figure 5. Mean AUC amplitude responses for electrode sites Fz, FCz, Cz and Pz for facial expressions of fear, neutral, sad, happy and anger at time point 8.

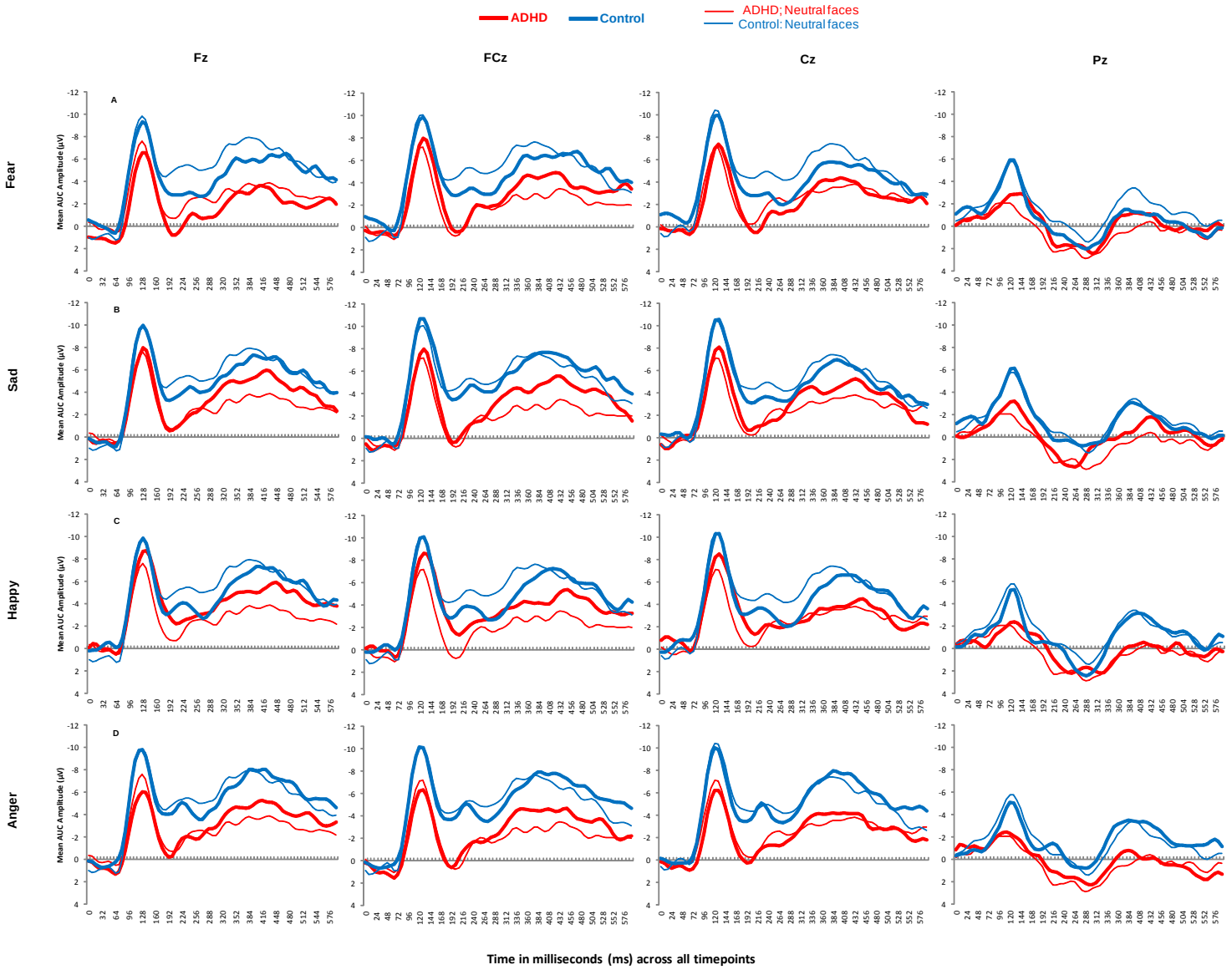


Figure 6. Grand average curves of the AUC ERPs of children/adolescents with ADHD (bold line) and healthy controls (thin line) across all AUC timepoints for the facial expressions of Fear (A), Sad (B), Happy (C) and Anger (D) at electrode positions: Fz, FCz, Cz and Pz

LC-PUFA Fractions between Children/Adolescents with ADHD and Healthy Control Children

The means and standard deviations are presented in Table 1. For all plasma PC analyses, a series of independent-samples *t* tests were conducted to compare the plasma fatty acid levels between ADHD and control children for the emotion processing task. The key fatty acid indices chosen were from the omega-3 and omega-6 series, namely (1) c18:3n-3 (ALA), (2) c20:5n-3 (EPA), (3) c22:5n-3 (DPA), (4) c226n-3 (DHA) and (5) Total n-3 and (6) c18:2n6 (LA), (7) c18:3n6 (GLA), (8) c20: 4n6 (AA) and (9) total n-6 respectively. Given the large number of tests, the false discovery rate (FDR) correction for multiple testing was employed for all analyses (Benjamini & Hochberg, 1995; this correction procedure is more conservative with the lower *p* values, but not as conservative as a Bonferroni correction). The relationships that survived correction only are reported below.

Emotion processing task

There were significant differences between the ADHD and control groups for ten out of the thirteen fatty acids indices. From the omega-6 series there were significant differences for c20:2n6, $t(65) = -3.48$, $p = .01$, c20:3n6, $t(66) = -4.28$, $p = .001$, c20:4n6 (AA), $t(66) = -9.23$, $p = .001$, c22:4n6 (a metabolite of AA), $t(66) = -5.65$, $p = .001$, and total n-6, $t(66) = -5.61$, $p = .002$. From the omega-3 series, there were significant differences between ADHD and control children for c18:3n3 (ALA), $t(66) = -3.51$, $p = .002$, c20:5n3 (EPA), $t(66) = -5.88$, $p = .003$, c22:5n3 (DPA), $t(66) = -7.53$, $p = .004$, c22:6n3 (DHA) $t(69) = -11.08$, $p = .006$, c22:6n3, $t(66) = -11.32$, $p = .006$ and total n-3 $t(66) = -11.32$, $p = .01$. In all instances, LC-PUFA fractions in both omega-3 and 6 series as reported above were significantly higher in controls compared to ADHD.

Table 1. Mean LC-PUFA fractions in plasma choline phosphoglycerides (PPC) in ADHD and HC groups for the emotion processing task.

Emotion Processing Task (ADHD: *n* = 29, HC: *n* = 38)				
	ADHD		**Healthy Control**	
	M	*SD*	*M*	*SD*
Omega 6				
c18: 2n-6 (LA)	24.45	3.94	24.76	3.44
c18: 3n6	0.12	0.10	0.09	0.06
c20: 2n6	0.25	0.05	0.31	0.07 **
c20: 3n6	2.80	0.62	3.66	0.96 **
c20: 4n6 (AA)	7.65	1.25	11.63	2.25 **
c22: 4n6	0.29	0.05	0.39	0.08 **
c22: 5n6	0.27	0.15	0.25	0.08
Total n6	35.83	4.37	41.11	3.39 **
Omega 3				
c18: 3n3 (ALA)	0.24	0.10	0.34	0.13 **
c20: 3n3	-	-	0.13	0.04
c20: 5n3 (EPA)	0.53	0.16	0.99	0.45 **
c22: 5n3	0.61	0.18	1.06	0.31 **
c22: 6n3 (DHA)	1.91	0.50	3.71	0.92 **
Total n3	3.29	0.66	6.23	1.42 **

Note: * $p < .05$, ** $p < .01$

IQ and correlations for significant results

To test for potential confounds of IQ, all significant between-group findings were correlated with IQ. The Kolmogorov-Smirnov test revealed that scores were normally distributed for the ERP responses for both N2 and N4 responses at Fz to angry faces in both the ADHD and healthy control (HC) group and therefore met the criteria for parametric data. There were no significant relationships between N2 or N4 responses at Fz to angry faces and composite IQ in the ADHD or HC group, $p > .05$.

PUFA fractions and ERP measures for the Emotion Processing Task

The Kolmogorov-Smirnov test revealed that scores were normally distributed for all ERP measures and therefore met the criteria for parametric data. Pearson coefficients were calculated to assess relationships between AUC amplitude responses to facial expressions of Fear, Sad, Happy and Anger and 7 key fatty acid indices. The strength of the associations between the fatty acid indices (omega-6: LA, c18:3n6, AA, c20:4n6 and Total n-6 and omega-3: ALA: c18:3n3, EPA: c20:5n3, DHA: c22:6n3 and Total n-3) and early and late components, namely, P3a, the combined N4/P3b complex and N4 AUC amplitudes measures at four electrode sites (Fz, FCz, Cz, Pz) were assessed using Pearson correlation coefficients. All analyses were subjected to correction for multiple testing employing the FDR.

P3a (time point 6) and PUFA fractions

None of the relationships at this time point survived the FDR correction for multiple testing in either the ADHD or healthy control group.

Of note, are some relationships which were significant at an alpha criterion of .005 and .01 prior to correction. In the ADHD group, the largest non-significant correlation was observed between c18:2n6 (LA) and AUC amplitude responses to Fear at Fz electrode site, $r(30) = .539$, $p = .002$. Furthermore, at Fz electrode site, c22:6n3 (DHA) was negatively correlated with AUC amplitude responses to Fear, $r(30) = -.542$, $p = .002$. A non-significant negative correlation was also observed at Fz between Total n-3 and responses to Fear, $r(30) = -.464$, $p = .01$. Also at Fz, there was a positive relationship between c20:5n3 (EPA) and AUC amplitude responses to Sad faces, $r(30) = .513$, $p = .004$. Finally, at Pz, c18:3n3 (ALA) was negatively correlated with facial expressions of Fear, $r(30) = - .478$, $p = .008$.

In the healthy control group, a significant negative correlation was observed at FCz between c18:3n6 (LA) and AUC amplitude responses to Happy faces, $r(32) = -.403$, $p = .005$. At FCz, there was a negative correlation between c18:3n6 (LA) and AUC amplitude responses to Happy faces, $r(32) = -.419$, $p = .02$ which did not reach significance. At Cz, a significant negative correlation was observed between c18:3n6 (LA) and AUC amplitude responses to Happy faces, $r(32) = -.532$, $p = .002$.

N4/P3b (time point 7) and PUFA fractions

None of the relationships at this time point survived the FDR correction for multiple testing in either the ADHD or healthy control group.

Of note, are some relationships which were significant at an alpha criterion of .005 and .01 prior to the FDR correction. In the ADHD group, there was a significant positive relationship between c18:3n6 (LA) and AUC amplitude responses to Fearful faces at Fz, $r(30) = .503$, $p = .005$. Also at Fz was a non-significant correlation between Total n-6 and AUC amplitude responses to Fear, $r(30) = .451$, $p = .012$. LA was also positively correlated with AUC amplitude responses to Sad faces at Cz, $r(30) = .440$, $p = .01$. Finally, there was a significant negative relationship between c18:3n3 (ALA) and responses to Happy faces, $r(30) = -.514$, $p = .004$.

In the healthy control group none the largest non-significant correlation was observed at Fz between Total n-6 and AUC amplitude responses to Fear, $r(30) = -.450$, $p = .01$.

N4 (time point 8) and PUFA fractions

At time point 8 (N4) only 2 relationships remained significant following the FDR correction for multiple testing, and 3 relationships were significant at trend level only, $p = .056$ in the ADHD group only. The first significant relationship was between Total n-3 and AUC amplitude responses to Happy faces, $r(30) = -.551$, $p = .002$ (adjusted $p = .04$). The second was a significantly negative relationship between ALA (c18:3n3) and N4 amplitude responses to Happy faces at Cz, $r(30) = -.577$, $p = .001$ (adjusted $p = .03$). The first trend finding was a negative correlation at FCz, c18:3n3 (ALA) was significantly negatively correlated with AUC amplitude responses to Happy faces, $r(30) = -.585$, $p = .001$ (adjusted $p = .056$). The second trend finding, was at Pz, c18:3n3 (ALA) was significantly correlated with AUC amplitude responses to Happy faces, $r(30) = -.518$, $p = .003$ (adjusted $p = .056$). Finally, at Cz, P3b

amplitude responses to Happy faces was significantly correlated with Total omega-3, $r(30) = -.543$, $p = .002$ (adjusted $p = .056$).

Of note, are some relationships, again in the ADHD group, which were significant at an alpha criterion of .01 and 0.05 prior to the FDR correction. The first a positive correlation between c20:5n3 (EPA) and AUC amplitude responses to Sad faces at Fz, $r(30) = .393$, $p = .03$. The second at Fz, between c18:3n6 (LA) and AUC amplitude responses to Fear, $r(30) = .433$, $p = .02$. The third was observed between c18:3n6 (LA) and AUC amplitude responses to Anger, $r(30) = .380$, $p = .04$. Finally, a positive correlation was observed at Fz between Total n-6 and AUC amplitude responses to Anger, $r(30) = .443$, $p = .01$.

Brief Discussion and Conclusion

In the present study, the emotion processing of children and adolescents with ADHD was examined. The main findings demonstrated significant group differences in N2 and N4 responses with a trend finding for group at the early P2 waves. In all instances, the ADHD group had significantly less negative activation to facial expressions of emotional as well as neutral faces compared to healthy control children. There was a significant main effect for electrode site with Fz in all cases (with the exception of time point 7: P3b/N4 which was greater at FCz) generating significantly greater activation. ERP responses to facial expressions varied according to time point with greater activation to angry faces at both N2 and N4 deflections. However, earlier P2 waves generated increased activation to happy faces; at P3a this shifted to greater activation to neutral faces and finally at N4/P3b there was a trend for increased activation to sad faces supporting the notion that emotional responses alter in their temporal processing. There were also significant negative associations between N4 and omega-3 fatty acid indices in the ADHD group which supported the hypothesis that as omega-3 increased N4 responses became more negative, i.e., more similar to that observed in the healthy controls. There were some trend findings between ERP measures and omega-3/6 which were in line with the study's hypothesis and are discussed in later sections.

N2 and N4 deflections

The results suggest that children and adolescents with ADHD relative to controls differed in their ERP responses to facial stimuli as captured by N2 deflections reflecting the orienting response and the later time point, N4, which is considered to reflect the event integration aspect of face processing (peaking at circa 430 ms post-stimulus). Both the N2 and N4 wave are modulated specifically by facial expressions of emotion and the results of this study confirm their involvement during affect processing (Bentin, et al., 1996; Halgren, Baudena, Heit, Clarke, Marinkovic, Chauvel, et al., 1994; Sokolov & Boucsein, 2000). The N2 is known to be automatically enhanced in relation to biological or novel stimuli (Balconi, 2005; Balconi & Lucchiari, 2005). Both the N4, which is associated with semantic processing , and the late P3b, which is implicated in event integration, have been found to be abnormal in clinical populations (including maltreated children and those with psychopathy) during face processing studies (Herrmann, et al., 2009; Ibanez, et al., 2011; Pollak, Klorman, Thatcher, & Cicchetti, 2001). The present study confirms for the first time that both N2 and later N4 are also abnormal in our group of children with ADHD relative to typically developing control children. Of note, the findings were unspecific to either negative or positive facial expressions as responses were observed to all emotions as well as during neutral faces over the different time points. This may in part be interpreted as evidence for generic deficits in early information processing (Banaschewski et al., 2006) rather than a specific emotion recognition deficit. Although, less is understood about the N4/P3b in relation to face processing in children/adolescents with ADHD, it is known to reflect the conscious emotional experience and subsequent stimulus updating processes (Halgren, Baudena, Heit, Clarke, Marinkovic, Chauvel, et al., 1994). These findings are also in line with the emotion processing model proposed by Halgren and associates (1994) which described two stages of emotion perception, namely: orienting and event integration. The first stage of the model, orienting, is captured by the N2 wave as also demonstrated in our study while the latter stage, event integration, is captured by the later N4 (circa 430-600 ms post stimulus) and reflects the cognitive integration of the emotional experience (Halgren, Baudena, Heit, Clarke, Marinkovic, Chauvel, et al., 1994).

Affect processing

The present study also confirms that there were significant differences in the processing of emotional stimuli at P2 and P3a only. Trend findings were observed at N2, N4/P3b and N4 which will be discussed in individual sections.

P2 responses

There was a significant finding for condition with P2 responses to happy faces (followed by sad, fear, angry and neutral faces respectively) displaying greater activation at midline frontal scalp regions, possibly reflecting the role of the anterior cingulate cortex (Etkin, Egner, & Kalisch, 2011). The P2 wave is considered a marker of attention reflecting early visual selection in emotion processing (Frenkel & Bar-Haim, 2011). In clinical populations, that is in anxious versus non-anxious patients the P2 wave has been found to be attenuated to threat related stimuli (Bar-Haim, Lamy, & Glickman, 2005). Early components such as the P2 are considered to be related to the basic structural encoding/configural recognition of faces, modulated to emotion as early as 120-160 milliseconds and our results further lend support to this notion (Ashley, Vuilleumier, & Swick, 2004; Eimer & Holmes, 2002; Pizzagalli et al., 2002). In other words, ADHD patients are less able to encode and/or successfully recognise facial expressions of emotion at this early time point. There was also a significant interaction between faces and group at P2 with the healthy control group in all cases showing greater negative going activation to sad, neutral and happy faces at frontal and central scalp regions (FCz and Cz) relative to the ADHD group. These findings suggest that ADHD are impaired in early emotion processing as well as neutral face processing as captured by attenuated P2 responses to positive, neutral and negative stimuli.

P3a responses

At P3a time point, across all participants, responses to neutral faces were given precedence and elicited the highest mean activation, maximal at Fz. The significant difference at this time point was driven by the mean difference in activation between responses to neutral and fearful faces. The greater activation found for neutral faces is likely to be due to the detection and contextual updating process. The P3a is associated with the detection of novel and threatening stimuli, it is also linked to the automatic features of the orienting response (Friedman, et al.,

2001b; Lagopoulos, et al., 1998). It seems plausible that at this time point, both groups were attempting to decipher the emotional expression of the neutral face.

N2, N4/P3b and N4 trend findings

Trend findings were also observed for the different facial expressions at time points 5 (N2), 7 (N4/P3b) and 8 (N4). At N2, there was a trend finding for faces with anger at Fz eliciting the greatest negative activity in the control group compared to ADHD. At N4/P3b and N4, which were both maximal at frontal scalp regions (FCz and Fz respectively), a similar response to negative stimuli was observed with greater activation to anger and sad respectively. These findings suggest that ADHD children show attenuation in activation towards negative emotional stimuli at these time points. The P3b is considered to be implicated in the merging of emotional features (Liddell, et al., 2004). In contrast, the N2 deflection may provide a temporal correlate of the early sensory processing of prominent facial configurations whereas the N4 wave may index the conscious amalgamation of emotion stimuli in WM, sub-served by greater cortico-amygdala pathways (Liddell, et al., 2004; Williams, et al., 2004). These findings lend some support to the ERP literature proposing emotion dysfunction in ADHD (Williams, Hermens, et al., 2008) in particular to negative emotions.

Blood measures of LC-PUFA and associations with ERPs

The LC-PUFA levels in plasma choline phosphoglycerides measures were contrasted between the children with ADHD recruited during the MAAFA trial and the healthy control group. There were significant differences as predicted in key omega-3 and omega-6 fatty acids indices. Overall there was a pattern of persistently higher levels of both omega-3/6 in the healthy control group compared to ADHD. These findings support previous research suggesting abnormal levels of fatty acids in ADHD (Antalis, et al., 2006; Stevens, et al., 1996; Stevens, et al., 1995; Young, et al., 2004). These findings warrant further investigation to explore whether the differences reflect dietary intakes of fatty acids or alterations in fatty acid metabolism.

In relation to the correlational analyses between plasma levels of LC-PUFA and ERP measures, only 2 relationships in the ADHD group survived correction for multiple testing. Both were in line with the study's prediction, that omega-3 would be negatively related to N4 AUC amplitudes. The first relationship was a negative relationship between N4 amplitude responses to

happy faces and the total omega-3 ratio. Therefore, the higher the omega-3 the more negative the response to happy faces, i.e., the more similar the activation to that of the controls. The second was also a negative association between N4 responses to happy faces and the parent omega-3 compound, ALA. Again, the higher the omega-3 the more negative the N4 and more similar to the N4 of controls. N4 is involved in the integration of the existing attended to event and related cognitive context in order to facilitate neural coding and in this study was found be abnormal in ADHD compared to controls, the relationships suggest that as omega-3 fatty acids increase the N4 responses decrease. N4 amplitudes were less negative in ADHD in all conditions (i.e., facial expressions) relative to control, and the relationship suggests that as omega-3 increases N4 responses also decrease, in other words become more negative, which is in line with the healthy control responses. It may be speculated that omega-3 has a modulating effect on the observed neuronal responses which is possibly restricted in part due to potential alterations in neurotransmitter functions, which are known to be impaired in ADHD and may explain the less negative N4 AUC responses compared to control children (Banaschewski, et al., 2005; Castellanos & Tannock, 2002a). This theory also supports the finding of reduced omega-3 levels in the ADHD group compared to controls. Animal studies have shown that diets deficient in omega-3 perinatally result in impairments in (1) visual function, which has also been found to be impaired in ADHD (Tannock, Banaschewski, & Gold, 2006), (2) neurotransmission processes and (3) in the capacity to learn (Aid et al., 2003; Alessandri, et al., 2004; Chalon, 2006; Innis & Friesen, 2008). In addition, the emotional irregularities observed in n-3 deficient rodents are thought to be in part explained by a dysregulation of both dopaminergic and glutamatergic neurotransmitter systems (Lavialle, Denis, Guesnet, & Vancassel, 2010). The balance of omega-3/6 is especially important as higher n-6 intakes will create an imbalance consequently reducing DHA accessibility for neural metabolism (Lavialle, et al., 2010). Although, guidelines have been set for adolescents to consume circa 250 mg per day, it is thought that approximately 30 to 80% of Western populations are not meeting these recommendations (Sioen, Huybrechts, Verbeke, Camp, & De Henauw, 2007; Sioen, Matthys, De Backer, Van Camp, & Henauw, 2007). Furthermore, one other study also found negative associations between N4 responses during a semantic memory test and total PUFAs but in a schizophrenic population (Condray, et al., 2008). Although, discussion of this study's preliminary findings are purely speculative and further research ideally with larger sample sizes and a longitudinal design (so that the diet of the

pregnant mother and later developmental outcomes of the child can be mapped) are needed to confirm these relationships.

Of note, is that some of the relationships prior to the correction also in the ADHD group were also in line with the study's hypotheses, that is, that omega-6 would be positively associated with N4/P3b responses to negative stimuli (i.e., responses to fear, angry and sad faces). Both measures of LA and total omega-6 ratios were positively associated with fear, anger and sad faces implying that as omega-6 increased, N4/P3b responses to negative stimuli also increased, that is became more positive in activation. This finding is in line with previous research demonstrating that increased levels of omega-6 (and simultaneous low levels of omega-3) are positively associated with homicide rates, neuroticism, suicidal and depressive behaviour (Conklin, Manuck, et al., 2007; Hibbeln, 2001, 2007).

Study Limitations

The main limitation of the study is arguably that plasma measures are not as robust as red blood cell measures. Although, plasma choline phosphoglycerides (PPC) are the main fraction containing AA, EPA and DHA, red blood cells are a more stable measure and representative of fatty acids fractions over time. Future studies therefore should also examine the red blood cells alongside plasma PC measures to ensure reliability.

Conclusion

This study in particular implicates impairments in both N2 and N4 AUC amplitude responses in ADHD compared to control children. Specifically, children with ADHD in this study display decreased activation in the early orienting phase and again in the later deflections associated with stimulus updating and event integration supporting previous research suggesting emotional dysfunction in ADHD. This study also confirmed that in plasma measures of both omega-3 and 6 were lower in ADHD relative to controls. The generic absence of positive associations between LC-PUFA and brain function were unexpected and not in line with the study's hypothesis. The N4 wave which was found to be dysfunctional in this study in ADHD was also negatively related to omega-3 potentially suggesting that in ADHD, as omega-3 increases, emotional responses decrease, that is become more negative which is in line with the healthy control responses. Therefore, it can be suggested that omega-3 is implicated in the ability to modulate emotional responses in assessments of brain function. However, these preliminary

relationships warrant further investigation to fully extrapolate the role of omega-3 in emotion processing.

Chapter 11: MAAFA ADHD Subgroup Intervention Analyses

(ERP Studies 2A, 2B & 2C)

Introduction

Several randomised, placebo controlled, double blind trials have explored the plausibility of omega-3 supplementation to ADHD symptoms in various clinical and community populations but with inconsistent designs and consequently results. On a behavioural and/or literacy level, some improvements in behaviour and concentration have been reported; (1) following supplementation with LC-PUFA in underachieving mainstream schoolchildren (who also had symptoms of ADHD and Developmental Coordination Disorder (Richardson & Montgomery, 2005), (2) a community sample of children that scored 2 *SD* higher than the mean (i.e., over the 90th percentile) on Conners abbreviated ADHD Index which assesses the severity of attentional/cognitive, hyperactive and impulsive problems (Sinn & Bryan, 2007) and (3) in a clinical sample of children previously assessed and diagnosed with ADHD (Johnson, et al., 2009). For a review of this research, please refer to Chapter 2 of the thesis.

On a neuropsychological level, improvements have also been reported in the capability to switch and control attention (as measured by the Creature Counting Task) in children with ADHD receiving omega-3/6 polyunsaturated fatty acids at 15 weeks compared to placebo (Sinn, Bryan, & Wilson, 2007). The LC-PUFA and neurophysiological/imaging research is extremely limited, in that, there are only 4 EEG/ERP studies and 1 fMRI study published in the literature to date and only two of them in ADHD (Boucher, et al., 2011; Fontani, Corradeschi, Felici, Alfatti, Migliorini, et al., 2005; Gow, Matsudaira, Taylor, Rubia, Crawford, Ghebremeskel, Ibrahimovic, Vallée-Tourangeau, et al., 2009; McNamara, Able, et al., 2010; Sumich, et al., 2009). Two of these studies from our team have been conducted in ADHD and have reported significant relationships between blood levels of LC-PUFA and (i) ERP responses during an emotion processing task (Gow et al., 2009) and (ii) with resting state EEG and memory in children with symptoms of ADHD (Sumich, et al., 2009). Gow and colleagues (2009) reported evidence for a cognitive bias between EPA and P3 amplitude responses to happy faces relative to facial expressions of both fear and sadness. Sumich and colleagues (2009) reported significant

associations in resting state EEG measures during both eyes open and eyes closed conditions and DHA and EPA respectively. In relation to the two other EEG studies, the first was a supplementation trial in healthy adults which reported a reduction in RT, i.e., faster performance in the omega-3 group compared to placebo during both Go/NoGo and sustained attention tasks. At the study end point, an increase in P3 amplitude was reported during both Go and NoGo trials along with a shift towards lower theta and alpha at resting state in the active group (Fontani, Corradeschi, Felici, Alfatti, Migliorini, et al., 2005). The second involved a prospective, longitudinal study in Greenland Inuit, fish eating, school children. During a continuous visual recognition task children with higher levels of DHA displayed a shorter N4 ERP latency deflection and larger late positive component, which in turn, are related to both recognition memory processes. Elevated DHA measures were also related to enhanced N4 amplitude and positive associations were also observed between DHA and behavioural performance of memory (Boucher, et al., 2011). The final study employed fMRI in the context of a RCT in healthy adolescent boys. At both baseline and endpoint, the blood measures of DHA were positively associated with activation in the DLPFC and negatively associated with RT (McNamara, Able, et al., 2010). Increased activation in the prefrontal cortex activation in relation to DHA, which is known to be impaired in ADHD, suggests that dietary intakes of omega-3, DHA play a role in the modulation of functional fronto-cortical activity (McNamara, Able, et al., 2010).

In addition, there is some evidence for abnormal fatty acids concentrations in the erythrocytes of children and adults with ADHD (Antalis, et al., 2006; Chen, et al., 2004; Colter, et al., 2008; Germano, et al., 2007; Mitchell, et al., 1987; Mitchell, et al., 1983). For a review, please refer to Chapter 2.

However, in spite of the positive findings reporting some efficacy of omega-3 supplementation in children with symptoms of ADHD, other clinical trials investigating the relationship between LC-PUFA in ADHD and/or hyperactivity have reported little or no effect (Arnold, et al., 1989; Belanger, et al., 2009; Hirayama, et al., 2004; Raz, et al., 2009; Voigt, et al., 2001). It should be noted that there are many variables that could be accountable for the wide variation in findings and hence replicability of these trials. Primarily, there are very few trials which employ the same design, supplement, dose and duration of supplementation. The three clinical trials finding improvement in symptoms of ADHD mentioned earlier in the introduction which arguably demonstrate the most promise for PUFA efficacy have employed a double-blind,

one-way crossover design for a six month period and all have used the same formula with is a combination of omega-3 and 6 (Johnson, et al., 2009; Richardson & Montgomery, 2005; Sinn & Bryan, 2007). A critique of the least successful trials has been given previously in Chapter 2 but essentially there are few trials which employ the same design, supplement, dose and duration of supplementation which in turn have implications for replication and results. The least successful trials collectively have a number of notable misguidings in their design. For example, they have either (1) employed a fortified (fish oil) food product (e.g., fermented soybean milk, steamed bread and bread rolls, see Hirayama et al., 2004) as opposed to a direct source of PUFA and arguably lowered the bioavailability of the product, (2) lacked statistical power due to small sample size (Arnold, et al., 1989; Belanger, et al., 2009), (3) have used DHA only supplementation (Voigt, et al., 2001) without first checking the micronutrient and fatty acids status of the individual at baseline to assess for high/low status or variability within the omega-3 index (von Schacky & Harris, 2007) (4) have employed supplementation with short-chain plant derived EFA (dietary sources of ALA do not provide substantial quantities of the key omega-3's EPA and DHA due to very poor hepatic conversion to the LC-PUFAs required for incorporation in the brain. Furthermore, the metabolic pathway from ALA to DHA is notoriously complex and low in efficiency[9]) (Raz, et al., 2009), (5) have used omega-6 only (e.g., LA or GLA) when there is adequate evidence to demonstrate that Western diets are already overloaded with dietary LA as it is present in almost all commonly consumed commercial foods and typical intakes are 12-17 grams per day for women and men (Rett & Whelan, 2011b) or (6) supplementation has been for a very short duration (e.g., 1 month) (Arnold, et al., 1989) which does not tally with the duration necessary (i.e., approx 6 weeks) to physiologically alter the RBC fatty acid status.

A small number of open-label studies have reported some improvements in behavioural ratings of ADHD however the sample sizes in all the studies were small, i.e., < 30 (9, 16, 20 and 30 respectively) and therefore need replicating to ensure validity (Germano, et al., 2007; Harding, et al., 2003; Joshi, et al., 2006; Sorgi, et al., 2007).

In light of the collective evidence documenting the potential benefit of omega-3/6 supplementation in ADHD, this study sought to test whether 12 weeks of omega-3/6

[9] Written correspondence with Dr Robert Peers, General, Preventative and Nutritional Medical Practitioner, Victoria, Austrlia, 19th February 2012.

supplementation would significantly enhance the performance (Studies 2A and 2B only) and neuronal activity of children as measured by ERP's with ADHD during the same ERP tasks reported in Studies 1A, 1B and 1C, measuring sustained attention, inhibition and emotion processing. The data reported in these 3 studies (2A, 2B and 2C) are from a subgroup of children with ADHD who took part in a larger randomised, placebo-controlled, double-blind, clinical trial (MAAFA) led by our research group. In all studies (2A, 2B, 2C), activation was assessed at baseline (0 months prior to supplementation) and then again at follow up (after 12 weeks of active or placebo supplementation).

1. The first prediction in relation to Study 2A was that ERP assessments of brain function would significantly differ between active and placebo groups following 12 weeks of supplementation in children/adolescents with ADHD. The hypothesis was based on the notion that omega-3 has a modulating role in impulse control in ADHD (Freeman, et al., 2006), along with the reported increase in P3 amplitudes in the Fontani study (2005) following supplementation in healthy individuals. It was therefore predicted differences would be indexed by increases in activation in both P3 and N2 responses during the Go/NoGo task. It was further predicted that the performance data (commission and omission errors) would be significantly negatively correlated with omega-3 in the active group compared to placebo at the follow up assessments. In other words, as omega-3 increased over 12 weeks, both commission and omission errors will decrease.

2. The second objective in relation to Study 2B was to ascertain whether a marker of attention, the P3, would significantly differ in the omega-3/6 supplementation group relative to placebo during the sustained attention task. The hypothesis was based on animal and human studies suggesting the omega-3 fatty acids have a modulating effect in cognition (Drover, et al., 2009; Hashimoto, et al., 2011) and in particular sustained attention processes (McNamara, Able, et al., 2010). More specifically, it was predicted that an increase in P3 (which is an index of attention) would be present in the active group compared to placebo following 12 weeks of supplementation. As per the Go/NoGo task, it was further predicted that the performance data (commission and omission errors) would be significantly negatively correlated with omega-3 in the active group compared to placebo at the follow up assessments. In other words, as omega-3 increased over 12 weeks in the active supplementation group, both commission and omission errors would decrease.

3. The final aim sought to test whether there would be significant differences in ERP amplitude responses to positive (i.e., happy faces) and negative (i.e., sad and fearful faces only) facial stimuli. This was based on previous research by our team which found an association between P3 amplitude responses and EPA to happy faces (Gow et al., 2009) alongside collective research showing that omega-3 may have a modulating effect in emotion processes including mood (Freeman, et al., 2006). It was predicted that these differences would be characterised by greater activation in N2 and N4 responses in the active group compared to the placebo group in children/adolescents with ADHD during an emotion processing task.

Procedure & Materials

This was a randomised, placebo-controlled, double blind trial over duration of 12 weeks. The sample size was calculated using an effect size of 0.7 due to its clinical relevance to clinicians with expertise in ADHD. This standardized effect size was considered realistic at the time as both stimulant medication and Atomoxetine have shown effect sizes of 0.9 to 1.2. This resulted in a total of 38 patients (included an additional 10% drop out rate) in each intervention arm to reach 80% power. The MAAFA trial was carried out according to the guidelines and principles of (1) International Conference on Harmonisation Good Clinical Practice guidelines (CPMP/ICH/135/95), (2) The European Union Clinical Trials Directive (2001/20/EC), (3) The Associated UK Medicines for Human Use (Clinical Trials) Regulations (2004), and (4) the Data Protection Act (1998). The trial was a clinical trial approved by the Medicines and Healthcare Products Regulatory Agency, the Clinical Trial Authorisation, the Research and Development Office at the Institute of Psychiatry, and the St Thomas Hospital Research Ethics Committee (application reference number: 06/Q0702/19). It was registered in the European Clinical Trials Database (EudraCT: 2005-005330-12) and had an International Standard Randomised Controlled Trial Number (ISRCTN: 27741572).

Treatment Compliance

Participants were asked to return their pill bottles at the final assessments so that a pill count could be carried out to check compliance. At six weeks parents and teachers were also contacted by telephone or email to monitor adverse effects and compliance. At the end of the study, parents and participants were verbally asked again about compliance and the change in LC-PUFA in the blood samples of participants recorded. The details of which are reported in the PhD thesis of Dr Matsudaira.

Participants

Information sheets about the study were circulated with permission by the Head teacher to the parents and asked to contact the study team should they be interested in their son taking part. Following parental permission both parent and teacher were asked to complete and return the Conners' Parent/Teacher Rating Scale. Informed consent/assent was obtained from participants and their parents, according to the National Health and Medical Research Council (UK) guideline and all participants (as well as parents and teachers) were fully briefed on the ethical considerations of the study. They were also advised they could withdraw at any point with no obligation. The eligibility and screening criteria for the MAAFA trial were as per previously reported in Chapters 8, 9 and 10. Once the child was deemed eligible i.e., by fulfilling criteria for symptoms of ADHD, formal screening was scheduled which was normally held at the child's school. Once criteria for entry to the study were met, a baseline appointment was scheduled at the Maudsley for an EEG/ERP assessment and blood test.

Randomisation into groups was carried out by the Mental Health & Neuroscience Clinical Trials Unit based at the Institute of Psychiatry, KCL. Allocation was stratified by whether the child attended a day or boarding school and their age group (12 – 14 years and 15 – 17 years) using minimisation randomization. Randomisation took place 24 hours prior to their appointment during which the trial medication was dispensed. Instructions were given not to take the first dose of the study drug until the Research Worker has confirmed with the school that the blood results are normal. The investigative medicinal product or active treatment was Equazen eye q (dose of active: x 6 daily; each containing 400 mg fish oil and 100 mg evening primrose oil with active ingredients EPA: 93 mg, DHA: 29 mg, Gamma-Linolenic acid; GLA: 10 mg, and Vitamin E: 1.8 mg) or identical placebo (medium chain triglycerides: MCT) for a three month

period. These were provided in four identical bottles labeled with an identifying code and in compliance with Good Manufacturing Process. Equazen eye q is a food supplement and not a licensed medication in the U.K. but approval was sought from the Medicines and Healthcare Products Regulatory Agency to give permission for its use in this way for the purpose of the study only.

As per previously reported in studies 1A, 1B and 1C, children/adolescents were accompanied by an appropriate adult (parent, teacher or guardian) to the Institute of Psychiatry at The Maudsley Hospital where approximately 16 ml of blood was taken by a qualified phlebotomist. Blood assessments required 8 hours fasting beforehand, although water was permitted, and this was explained fully to the parent and child prior to the appointment. The blood was taken by a qualified phlebotomist and analysed by The Institute of Brain Chemistry and Human Nutrition, London Metropolitan University. Following the blood sample all participants were given a complimentary breakfast at the Maudsley restaurant. They were then taken to a quiet, well lit testing room to complete an EEG/ERP assessment.

Another appointment was scheduled for the second (follow up) assessment (i.e., a repeat of the same EEG/ERP assessment) after 12 weeks of supplementation with either active (fish oil) or placebo (medium chain triglycerides). Participants were given £20.00 for their participation and all associated travel expenses were refunded to the parent/guardian/school. During the MAAFA trial, any children taking stimulant medication for ADHD were required a wash-out for 48 hours prior to the EEG recording.

EEG Technique

EEG and ERP were collected using The Brain Resource Company internationally standardised techniques. Those on stimulant medication underwent a wash-out period of 48 hours prior to the EEG. Participants sat in a light and sound attenuated room with an ambient temperature of 24 ˚C. A NeuroScan Quik cap and NuAmps amplifier (sampling rate = 500 Hz) were employed to collect EEG data from electrode sites. In the ERP peak scoring data were filtered with a 25Hz low-pass filter, and base lined to average amplitude of zero.
EEG data analysis. The data are measured in terms of "Area under the curve (AUC)". That is the space between the ERP waveform and baseline. It is divided into multiple 50ms time-windows that can be mapped onto ERP component time-windows. For example, N2 is mapped onto time

unit 5 (200-250 ms), N250 on to time unit 6 (250-300 ms), P3a onto time unit 7 (300-350 ms) and P3b (350-400 ms) on time unit 8. Units are in microvolts multiplied by ms.
Task descriptions for the Go/NoGo, CPT and Emotion processing are as per Chapter 8, 9 and 10 respectively and will not be repeated here.

Results for Study 2A: Conflict Response Inhibition (Go/NoGo) Task

Behavioural Data (Reaction times and error rates)

Baseline and follow up data from the MAAFA trial for the Go/NoGo task were available for 28 participants with ADHD only; of which 16 children/adolescents were in the active (fish oil supplementation) group and 12 in the placebo group. A repeated measures analysis of variance (ANOVA) was performed on the behavioural data measures of errors of commission (NoGo stimuli), and omission (Go stimuli) and average reaction times.

The within-subjects factors were time points: (1) baseline at 0 months and (2) follow up at 3 months and behavioural measure (e.g., commission, omission or reaction time). The between-subjects factor was intervention (placebo or active supplementation). There were no significant main effects of any of the within-subjects factors for behavioural measure (reaction time, commission or omission errors) between baseline or follow-up, $F < .01$. The between subjects factor intervention was also non-significant, $p > .05$. There was a significant interaction between omission errors and intervention, F (1, 26) = 6.61, $p < .02$, this was driven by a significant difference between omission errors at baseline between placebo and active groups with a greater number of omission errors in the placebo group (M = 4.33, SE = 1.18) compared to the active group (M = 1.81, SE = .42) at baseline. There were no further significant interactions, $p > .05$.

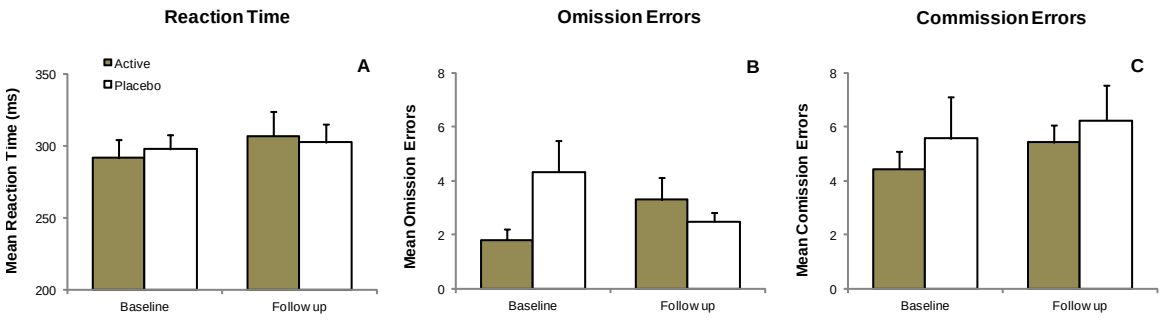


Figure 1. Mean reaction time (A), mean omission errors (B) and mean commission errors (C) for children/adolescents with ADHD in active (n = 15) and placebo (n = 11) groups at baseline (0 months) and follow up (at 12 weeks of supplementation) assessments for the Go/NoGo task

IQ, Medication and Handedness

In relation to IQ for verbal, non-verbal and composite scores, there were no significant differences in the ADHD group between baseline and follow-up intervention (e.g., active versus placebo) groups, $p > .05$. The means for the composite IQ scores were $M = 95.23$, $SD = 15.23$ for the placebo group versus $M = 95.19$, $SD = 11.20$ for the active group.

Twenty one of these children were medication naive; the remaining seven underwent a washout of stimulant medication 48 hours prior to testing in line with standard EEG practise.

Twenty six were right handed, 1 was left handed and 1 was ambidextrous. An independent t test confirmed that there were no significant differences in handedness between active and placebo groups, $p > .05$.

Statistical Analyses for the electrophysiological data

For statistical purposes, series of 2 x 2 x 4 mixed repeated measures analyses of variance (ANOVA) were carried out on the electrophysiological data at time points 5 (P2), 6 (N2), 7 (P3a) and 8 (P3b). Participants were tested at both time points: baseline and follow up. The between subjects factor was intervention (active/LC-PUFA supplementation versus placebo), while testing time (baseline at 0 months versus 3 months of supplementation (Go versus NoGo), condition (Go versus NoGo) and electrode position (Fz, FCz, Cz, and Pz) were the within subjects factors. The dependent measures were the AUC amplitude range (time point 5- 7). All interactions were further investigated using post hoc tests consisting of pairwise Comparisons

with a Bonferroni correction for multiple testing. This is considered the most appropriate for controlling for Type 1 error in repeated measures analysis of variance.

Time point 5 (P2 response amplitude)

Mauchly's test indicated that the assumption of sphericity had been violated, χ^2 (5) = 68.62, $p < .05$ for the within-subject factors electrode site and the interactions between condition and electrode site, χ^2 (5) = 29.28, $p < .05$, time and electrode site, χ^2 (5) = 23.78, $p < .05$, and the 3-way interaction between time, electrode site and condition, χ^2 (5) = 16.61, $p < .05$. This was corrected for using Greenhouse-Geisser estimates of sphericity. The corrected results are reported as follows. The main effect of the between-subjects factor intervention (active/LC-PUFA supplementation versus placebo) was not significant, $p = .16$. The within-subjects factor testing time (0 and 3 months) was significant, $F(1, 26) = 7.95$, $p < .01$, with baseline testing time eliciting increased activation compared to follow up testing. The main effect of the within-subjects factor, electrode position (Fz, FCz, Cz, Pz) was also significant, $F(1.25, 32.57) = 10.38$, $p < .003$ with increased activation at Pz and the smallest activation at Fz. There was no significant main effect of the within-subject factor, condition, $p > .05$. There was a significant interaction between electrode site and condition, $F(1.86, 48.46) = 10.78$, $p < .001$. Post hoc tests revealed that there was significantly increased activation in the Go condition ($M = 436.41$, $SE = 59.12$) relative to NoGo ($M = 273.42$, $SE = 44.42$) at the Pz electrode site.

The 3-way interaction between testing time, condition and electrode site was significant, $F(2.15, 55.87) = 6.15$, $p < .004$. Post-hoc tests showed that this was due to a significant greater activation at baseline (0 months) at Pz in the Go ($M = 497.89$, $SE = 56.85$) compared to NoGo ($M = 286.96$, $SE = 48.49$), $p < .001$. At follow-up (3 months), both Fz and Pz showed significantly increased activation in the Go condition ($M = 170.73$, $SE = 42.61$) compared to the NoGo condition ($M = 107.71$, $SE = 33.43$), $p < .03$ as did Pz (Go: $M = 374.93$, $SE = 66.71$ versus NoGo: $M = 259.88$, $SE = 55.18$), $p < .01$.

No other interactions reached significance, $F < 1$.

Time point 6 (N2 response amplitude)

Mauchly's test indicated that the assumption of sphericity had been violated, χ^2 (5) = 39.91, $p < .05$ for the within-subject factors electrode site and the interactions between condition and electrode site, χ^2 (5) = 25.46, $p < .05$, time and electrode site, χ^2 (5) = 36.73, $p < .05$, and the 3-way interaction between time, electrode site and condition, χ^2 (5) = 23.81, $p < .05$. This was corrected for using Greenhouse-Geisser estimates of sphericity. The corrected results are reported as follows. The main effect of the between-subjects factor intervention (active/LC-PUFA supplementation versus placebo), at time point 6, was not significant, $F < 1$. The main effects of the within-subjects factors testing time (0 and 3 months), F (1, 26) = 4.29, $p < .05$ were significant, again with increased activation observed at baseline compared to follow-up. There were significant main effects for electrode position (Fz, FCz, Cz, Pz), F(1.61, 41.96) = 77.76, $p < .001$ with greater activation again at Pz and the smallest at Fz, and condition, F(1.00, 26.00) = 13.68, $p < .002$ with increased activation for the Go (Targets) condition compared to NoGo.

There was a significant interaction between electrode site and condition, F (2.00, 52.02) = 64.81, $p < .001$. Post-hoc tests revealed that this was due to greater activity at Pz in the Go (M = 656.19, SE = 42.28) condition compared to NoGo (M = 656.19, SE = 42.28).

The 3-way interaction testing time, condition and electrode site was significant, F(1.97, 51.44) = 6.10, $p < .05$. Post-hoc tests revealed that this was again due to higher activation at baseline (testing time 1) at Pz in the Go (M = 733.16, SE = 56.05) condition relative to NoGo (M = 296.81, SE = 60.58), p =.006. However, at the follow up (testing time 2: 12 weeks) there was significantly greater activation in the NoGo condition (M = -54.32, SE = 52.15) at FCz compared to Go (M = -16.38, SE = 55.80), p = .036. Also, at follow up (testing time 2), there was significantly greater activation in the Go condition (M = 116.91, SE = 58.85) relative to NoGo (M = 16.46, SE = 52.21) at Cz. No other interactions reached significance, $p > .05$

Time point 7 (P3a response amplitude)

Mauchly's test indicated that the assumption of sphericity had been violated, χ^2 (5) = 47.60, $p < .05$ for the within-subject factors electrode site and the interactions between condition and electrode site, χ^2 (5) = 36.07, $p < .05$, testing time and electrode site, χ^2 (5) = 30.02, $p < .05$, and the 3-way interaction between time, electrode site and condition, χ^2 (5) = 13.74, $p < .05$. This was corrected for using Greenhouse-Geisser estimates of sphericity. The corrected results are reported as follows. At time point 7, the main effect of the between-subjects factor intervention (active/LC-PUFA supplementation versus placebo) was not significant, $p > .05$. The main effect of the within-subjects factors testing time (0 and 3 months) was significant, $F(1, 26) = 7.95$, $p < .01$, as per before, activation was higher at baseline compared to follow testing. There was also a significant main effect of electrode site (Fz, FCz, Cz, Pz), $F(1.56, 40.77) = 54.85$, $p < .001$, driven by the difference between Pz ($M = 409.18$) which produced the greatest activation relative to Fz ($M = -5.05$) which was smallest.

There was a significant interaction between electrode site and condition, F (2.05, 53.29) = 17.80, $p < .001$. Post-hoc tests showed that this was due to significantly greater activity at Pz during Go ($M = 521.25$, $SE = 38.87$) trials compared to NoGo ($M = 297.10$, $SE = 41.42$). There was a trend finding for the 3 way interaction time, condition and electrode site, $p = .064$. No other interactions reached significance, $F < 1$.

Time point 8 (P3b response amplitude)

Mauchly's test indicated that the assumption of sphericity had been violated, χ^2 (5) = 44.15, $p < .05$ for the within-subject factors electrode site and the interactions between condition and electrode site, χ^2 (5) = 34.59, $p < .05$, and testing time and electrode site, χ^2 (5) = 29.24, $p < .05$. For the P3b response amplitude (time point 8), the main effect of the between-subjects factor intervention (active/LC-PUFA supplementation versus placebo) was not significant, $F < 1$. The main effects of the within-subjects factor testing time (0 and 3 months) were significant $F(1, 26) = 4.37$, $p < .05$, again driven by higher activation at baseline relative to follow up.

The main effect of the within-subjects factor, electrode position (Fz, FCz, Cz, Pz), $F(1.61, 41.94) = 15.29$, $p < .001$ was also significant, again with greater activation at Pz compared to Fz.

There was a significant interaction between electrode site and condition, $F(1.83, 47.60) = 6.67$, $p < .001$. Post-hoc tests revealed that this was due to greater activation at FCz during the NoGo ($M = 101.58$, $SE = 47.25$) trials compared to Go ($M = -16.92$, $SE = 59.83$), $p < .02$.

The 3-way interaction between testing time, condition and electrode site was also significant, $F(2.57, 66.82) = 3.74$, $p < .03$. Post-hoc tests showed that this was due to significantly increased activation at Pz between baseline ($M = 295.35$, $SE = 54.63$) and follow up($M = 181.25$, $SE = 48.63$) in Go trials. There was also a significant difference at the FCz electrode site with greater activation at baseline ($M = 177.23$, $SE = 57.53$) in the NoGo condition ($M = 25.93$, $SE = 55.55$). No other interactions reached significance, $p > .05$.

Results for Study 2B: Sustained Attention Task (Continuous Performance Task, CPT)

Group and Stimulant Medication

Baseline and follow up data from the MAAFA trial for the sustained attention task were available for 26 participants with ADHD only; of which 15 children/adolescents were in the active (fish oil supplementation) group and 11 in the placebo group.

Behavioural Data (reaction times and error rates)

A series of 2 x 2 repeated measures analyses of variance (ANOVA) were carried out on the performance data with testing time (baseline and follow up) were the within subject factors and intervention as the between subject factor . Reaction time, commission and omission errors were the dependent variables. Mauchly's test indicated that the assumption of sphericity had been not been violated for any of the within-subject factors.

The main effect of the between-subjects factor intervention (active/LC-PUFA supplementation versus placebo) was not significant; $F < 1$ for any of the dependent variables: reaction time, errors or commission and omission. The within-subjects factor testing time (0 and 3 months) was also not significant for any of the performance measures, $p > .05$. There were no significant interactions between any of the performance measures and testing time, $p > .05$. Although, for commission errors a trend finding was observed for testing time, $F(1, 23) = 3.35$, $p = .08$, with a higher number of commission errors at follow up ($M = 8.79$, $SE = 1.43$) compared to baseline ($M = 6.59$, $SE = 1.00$).

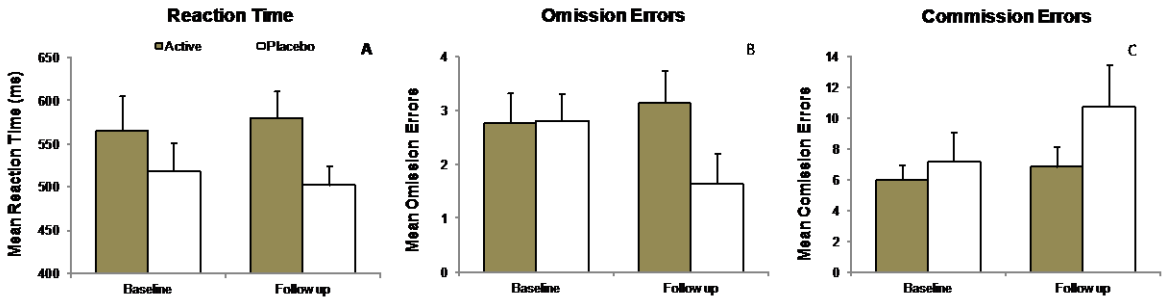


Figure 1. Mean reaction time (A), mean omission errors (B) and mean commission errors (C) along with standard errors for children/adolescents with ADHD in active (n = 15) and placebo (n = 11) groups at baseline (0 months) and follow up (at 12 weeks of supplementation) assessments during the CPT

IQ, Handedness and Medication

In relation to the 3 measures of IQ, the Kolmogorov-Smirnov tests of normality confirmed that verbal and non-verbal scores met the criteria for parametric tests. Composite scores of IQ were not normally distributed, $p < .05$. A series of independent samples t tests revealed there were no significant differences between measures of verbal or non-verbal IQ between placebo or active groups at baseline, $p > .05$. Mann-Whitney U test confirmed that measures of composite IQ were not significant between active and placebo groups, $p < .05$.

Twenty two of these children were medication naive; the remaining 4 underwent a washout of stimulant medication 48 hours prior to testing in line with standard EEG practise.

Twenty-five children were right handed and one was left handed. An independent t test confirmed there were no significant differences between active and placebo group in handedness, $p < .05$.

Statistical Analyses and Electrophysiological data

For statistical purposes, a series of 2 x 3 x 4 mixed repeated measures analyses of variance (ANOVA) were carried out on the electrophysiological data (P2, N2, P3a and P3b) collected during the Maudsley Adolescent ADHD Fatty Acid trial. The between subjects factor was intervention (active/LC-PUFA supplementation versus placebo), while testing time (baseline at 0 months versus 3 months of either active or placebo supplementation), condition (Targets, Distractors and Backgrounds) and electrode position (Fz, FCz, Cz, and Pz) were the within subjects factors.

Time point 5 (P2 deflection)

Mauchly's test indicated that the assumption of sphericity had been violated for the within-subject factors: electrode site, χ^2 (5) = .262, $p < .05$ and condition, χ^2 (2) = .726, $p < .05$, the interaction between testing time (baseline and follow up) and electrode site, χ^2 (5) = .598, $p < .05$, condition and electrode site, χ^2 (20) = .032, $p < .05$ and the 3-way interaction between testing time, condition and electrode site, χ^2 (20) = .127, $p < .05$. This was corrected using Greenhouse-Geisser estimates of sphericity. The corrected results are reported.

For the P2 response amplitude (time point 5), the main effect of the between-subjects factor intervention (active/LC-PUFA supplementation versus placebo) was not significant, $F < 1$. The within-subjects factor testing time (0 and 3 months) was not significant, although a trend finding was observed, $p = .093$, with higher mean activation at baseline compared to follow up (12 weeks). The main effects of the within-subjects factors factor electrode position (Fz, FCz, Cz, Pz), $F(1.71, 41.07) = 25.31$, $p < .001$ were significant, with higher activation at Pz and the lowest activation at Cz. The main effects of condition were also significant, $F(1.29, 30.87) = 5.12$, $p < .03$, with Distractors eliciting the greatest negative activity and Targets eliciting greater positive activity.

There was a significant interaction between electrode site and condition, $F(3.04, 72.88) = 188.82$, $p < .001$. Post-hoc tests showed that there were significant difference between all conditions (Backgrounds, Targets and Distractors) and all electrode sites with the exception of a trend finding at Fz for Targets and Distractors, $p = .057$. These are outlined below as follows:

At Fz electrode site, the Distractor (M = -234.66, SE = 60.70) condition elicited larger negative activity compared to the Targets (M = -36.61, SE = 55.50) condition, $p < .001$. The Distractor condition (M = -234.66, SE = 60.70) also obtained increased negative activity compared to Backgrounds (M = -8.00, SE = 51.94) which was negative, $p < .001$.

At FCz, the Distractor (M = -203.21, SE = 61.95) condition elicited larger negative activity compared to the Targets (M = 7.15, SE = 57.76) condition, $p < .004$. Finally, there was a significant difference between the Distractor condition (M = -203.21, SE = 61.95) and Backgrounds (M = 22.92, SE = 55.96), Distractors were more negative compared to Backgrounds, $p < .001$.

At Cz, the Distractor (M = -97.90, SE = 63.87) condition elicited larger negative activity compared to the Targets (M = 83.49, SE = 63.87) condition which was positive, $p < .04$. Finally, there was a significant difference between the Distractor condition (M = -97.90, SE = 63.87) and Backgrounds (M = 51.42, SE = 58.60), Distractors elicited a negative compared to Backgrounds which was positive, $p < .02$.

At Pz, there was a significant difference between the Distractor condition (M = 250.70, SE = 56.49) and Backgrounds (M = 100.78, SE = 50.95), Distractors elicited an enhanced positive response compared to Backgrounds, $p < .05$.

No further interactions reached significance, $p > .05$.

Time point 6 (N2 deflection)

Mauchly's test indicated that the assumption of sphericity had been violated for the within-subject factors: electrode site, χ^2 (5) = .135, $p < .05$ and condition, χ^2 (2) = .528, $p < .05$, the interaction between testing time (baseline and follow up) and electrode site, χ^2 (5) = .501, $p < .05$, condition and electrode site, χ^2 (20) = .030, $p < .05$ and the 3-way interaction between testing time, condition and electrode site, χ^2 (20) = .115, $p < .05$. This was corrected using Greenhouse-Geisser estimates of sphericity. The corrected results are reported.

For the N2 response amplitude (time point 6), as per before the main effect of the between-subjects factor intervention (active/LC-PUFA supplementation versus placebo) was not significant, $F < 1$. The main effects of the within-subjects factor testing time (baseline: 0 months and follow up at 3 months) was significant, $F(1, 24) = 6.88$, $p > .02$, again the same pattern was repeated as per before, with higher activation at baseline compared to follow up testing. The main effect of the within-subjects factor, electrode position (Fz, FCz, Cz, Pz) was also significant, $F(1.40, 33.56) = 90.83$, $p < .001$, again driven by greater positive activity at Pz (M = 458.14) compared to the smallest positive activity which was at Cz (M = 53.99). There was a trend finding for condition, $p = .053$ which was greater for the Target condition. Post-hoc tests revealed that this was due to differences between Targets and Background conditions at FCz, Cz and Pz electrode sites. At FCz, Targets (M = -39.29) produced significantly lower negative activity compared to Backgrounds (M = -141.62) condition. At Cz, (M = 152.25) Targets produced greater positive activity compared to Backgrounds which was negative (M = -1.81). Finally, at Pz, Targets (M = 596.17) also produced greater positive activity compared to Backgrounds (M = 318.06).

There was a trend finding for the interaction between electrode site and condition, $p = .06$. No other interactions reached significance, $p > .05$.

Time point 7 (P3a deflection)

Mauchly's test indicated that the assumption of sphericity had been violated for the within-subject factors: electrode site, χ^2 (5) = .157, $p < .05$, condition and electrode site, χ^2 (20) = .030, $p < .05$ and the 3-way interaction between testing time, condition and electrode site, χ^2 (20) = .121, $p < .05$. This was corrected using Greenhouse-Geisser estimates of sphericity. The corrected results are reported.

For the P3a response amplitude (time point 7), the main effect of the between-subjects factor intervention (active/LC-PUFA supplementation versus placebo) was not significant, although a trend finding was observed, $F(1, 24) = 3.30$, $p = .082$ with greater activation in the placebo group. The main effect of the within-subjects factor, electrode position (Fz, FCz, Cz, Pz) was significant, $F(1.47, 35.24) = 94.64$, $p < .001$ again driven by greater activation at Pz. The main effect of the within-subjects factor condition, $F(2, 48) = 44.62$, $p > .001$ with Distractors ($M = 396.14$, $SE = 83.92$) showing the greatest activation and Backgrounds ($M = -33.13$, $SE = 52.61$) the smallest.

There were three interactions of note. The first, a significant interaction between electrode site and condition, $F(3.06, 73.41) = 9.91$, $p < .001$. Post-hoc tests showed that this was due to significant differences across all electrode sites between the different conditions as outlined below.

At Fz electrode site, the Distractor ($M = 128.78$, $SE = 100.12$) condition elicited larger positive activity compared to the Targets ($M = -140.13$, $SE = 79.50$) condition, $p < .001$. The Distractor condition ($M = 128.78$, $SE = 100.12$) elicited positive responses compared to Backgrounds ($M = -260.89$, $SE = 55.66$) which was negative, $p < .001$.

At FCz electrode site, the Distractor ($M = 341.67$, $SE = 102.11$) condition elicited larger positivity compared to the Targets ($M = -26.8$, $SE = 75.72$) condition, $p < .001$. The Distractor condition ($M = 341.67$, $SE = 102.11$) elicited positive responses compared to Backgrounds ($M = -152.26$, $SE = 58.71$), $p < .001$.

At Cz electrode site, the Distractor ($M = 432.93$, $SE = 88.46$) condition elicited larger positivity compared to the Targets ($M = 189.17$, $SE = 76.73$) condition, $p < .01$. The Distractor condition ($M = 432.93$, $SE = 88.46$) elicited positive responses compared to Backgrounds ($M =$-36.13, $SE = -36.13$), $p < .001$. Finally, there was a significant difference between Targets ($M =$

189.17, *SE* = 76.73), which obtained positive responses compared to Backgrounds (*M* =-36.13, *SE* = -36.13) which were negative, $p < .001$.

At Pz electrode site, the Distractor (*M* = 681.20, *SE* = 73.12) condition elicited larger positivity compared to the Background (*M* = 316.77, *SE* = 54.78) condition, $p < .001$. Distractors elicited an enhanced positive response compared to Backgrounds. Finally, there was a significant difference between Targets (*M* = 698.58, *SE* = 56.14), which obtained increased positivity compared to Backgrounds (*M* = 316.77, *SE* = 54.78), $p < .001$.

There was also a significant interaction between electrode site and intervention, $F(3, 72) = 2.87$, $p < .05$. Post-hoc tests confirmed that this was due to a difference at trend level, $p = .054$ at FCz in the placebo group (*M* = 203.04, *SE* = 111.25) which elicited greater positive activity compared to responses in the active group which were reduced (*M* = -94.15, *SE* = 95.27).

A trend finding was observed between testing time (0 and 3 months) and condition, $F(2, 48) = 3.09$, $p = .054$. No other interactions reached significance.

Time point 8 (P3b deflection)

Mauchly's test indicated that the assumption of sphericity had been violated for the within-subject factors: electrode site, χ^2 (5) = .155, $p < .05$, condition, χ^2 (2) = .597, $p < .05$, and for the interactions: condition and electrode site, χ^2 (20) = .036, $p < .05$, testing time (baseline and 3 months) and electrode site, χ^2 (5) = .601, $p < .05$ and finally the 3-way interaction between testing time, condition and electrode site, χ^2 (20) = .087, $p < .05$. This was corrected using Greenhouse-Geisser estimates of sphericity. The corrected results are reported.

For the P3b response amplitude (time point 8), the main effect of the between-subjects factor intervention (active/LC-PUFA supplementation versus placebo) was not significant, $p > .05$. The main effect of the within-subjects factor, electrode position (Fz, FCz, Cz, Pz) was significant, $F(1.52, 36.64) = 50.80$, $p < .001$, again driven by the difference between Pz, which produced the greatest positive activity ($M = 544.99$) and Fz which produced the smallest ($M = 66.16$). There was also a significant main effect of the within-subject factor, condition, $F(1.42, 34.20) = 27.83$, $p > .001$ with Distractors producing significantly greater activity ($M = 486.64$) and Backgrounds the smallest ($M = 49.82$).

There was a significant interaction between electrode site and condition, $F(2.69, 64.57) = 7.66$, $p < .001$. Post-hoc tests showed that this was due to significant differences as outlined below. At Fz electrode site, the Distractor ($M = 228.56$, $SE = 77.90$) condition elicited larger positive activity compared to Backgrounds ($M = -72.91$, $SE = 56.65$), $p < .001$.

At FCz, there was a significant difference between the Distractor condition ($M = 415.28$, $SE = 78.89$) and Targets ($M = 115.72$, $SE = 74.79$), $p < .001$. In this instance, Distractors elicited enhanced positive responses compared to Targets. There was also a significant difference between Distractors ($M = 415.28$, $SE = 78.89$) and Backgrounds ($M = 7.42$, $SE = 56.09$), $p < .001$.

At Cz, there was a significant difference between the Distractor condition ($M = 513.44$, $SE = 79.93$) and Backgrounds ($M = 50.50$, $SE = 54.96$), $p < .001$. In this instance, Distractors elicited enhanced positive responses compared to Backgrounds. There was also a significant difference between Targets ($M = 271.70$, $SE = 81.90$) and Backgrounds ($M = 50.50$, $SE = 54.96$), $p < .001$.

At Pz, there was a significant difference between the Distractor condition (M = 789.28, SE = 70.50) and Backgrounds (M = 214.27, SE = 47.73), $p < .001$. In this instance, Distractors elicited enhanced positive responses compared to Backgrounds. There was also a significant difference between Targets (M = 631.42, SE = 54.47) and Backgrounds (M = 214.27, SE = 47.73), $p < .001$.

No other interactions reached significance, $p > .05$.

Results for Study 2C: Emotion Processing (Faces) Task

Group and Stimulant Medication

Baseline and follow up data from the MAAFA trial for the emotion processing task were available for 20 participants only with ADHD; of which 11 children/adolescents were in the active (LC-PUFA supplementation) group and 9 in the placebo group. Fourteen of these children were medication naive; the remaining 6 underwent a washout of stimulant medication 48 hours prior to testing in line with standard EEG practise.

Statistical Analyses and Electrophysiological data

For statistical purposes, a series of 2 x 3 x 4 mixed repeated measures analyses of variance (ANOVA) were carried out on the electrophysiological data at time points 4 (P2), 5 (N2) and 8 (N4). The between-subjects factors were intervention (e.g., active versus placebo) supplementation. The within-subjects factors were testing time: e.g., (i) baseline at 0 months and (ii) follow up at 3 months. The within-subjects factors electrode position (Fz, FCz and Pz) and faces (Fear, Sad and Happy). These factors were selectively chosen on the basis of the results of the previous analysis coupled with previous literature in this area (Williams, et al., 2004). The midline frontal and parietal electrode sites were identified as regions of interest. The timepoints of interest in this study were the early deflections e.g., time point 4 (P2) and time point 5 (N2) and the later time point 8 (N4) responses, again driven by previous research which has employed the same task and demonstrated the significance of these time points in emotion processing. Furthermore, these time points mirror differential stages in cognitive processing (Frenkel & Bar-Haim, 2011; Williams, et al., 2004) .

Time point 4 (P2 deflection)

Mauchly's test indicated that the assumption of sphericity had been violated for the within-subject factors: electrode site, χ^2 (2) = 20.47, $p < .05$, and for the interactions between electrode site and testing time, χ^2 (2) = 25.56, $p < .05$, electrode site and faces, χ^2 (35) = .019, $p < .05$, electrode site and faces, χ^2 (9) = 22.80, $p < .05$ and the 3-way interaction between testing time,

electrode site and faces, χ^2 (9) = 30.51, $p < .05$. This was corrected using Greenhouse-Geisser estimates of sphericity. The corrected results are reported.

For the time point 4 (P2) AUC responses the between-subjects factor intervention (active versus placebo) was not significant, $p > .05$. There was a significant main effects for the within-subjects factor electrode site (Fz, FCz, Pz), $F(1.18, 21.18) = 8.60$, $p < .01$ with Fz (M = -150.66, SE = 29.17) producing the greatest negative activity and Pz the smallest (M = -69.09, SE = 29.17). There was also a significant interaction between electrode site and faces, $F(2.38, 42.93) = 4.09$, $p < .02$. Post-hoc tests revealed that this was due to significantly greater negative activity between facial expressions of sadness between Fz (M = -153.40, SE = 39.12) and Pz (M = -72.19, SE = 32.82). In addition, there were significant differences between facial expressions of happiness between Fz (M = -183.48, SE = 33.06) and FCz (M = -146.87, SE = 27.67) which increased negativity at Fz. This was also the case between Fz (M = -183.48, SE = 33.06) and Pz (M = -48.53, SE = 35.78), and finally, FCz (M = -146.87, SE = 27.67) and Pz (M = -48.53, SE = 35.78) with greater negative responses at frontal and frontal central scalp regions relative to parietal sites.

There was a significant interaction between faces and intervention, $F(2, 36) = 4.75$, $p < .02$. Post hoc tests confirmed that this was driven by significantly greater activity in the active (fish oil) group between facial expressions happy faces (M = -173.79, SE = 38.49) compared to those of fear (M = -99.41, SE = 37.06). No other interactions reached significance.

Time point 5 (N2 deflection)

Mauchly's test indicated that the assumption of sphericity had been violated for the within-subject factors: electrode site, χ^2 (2) = 28.65, $p < .001$, and for the interactions between electrode site and testing time, χ^2 (2) = 12.26, $p < .01$, electrode site and faces, χ^2 (9) = .21.29, $p < .02$, and for the 3-way interaction between testing time, electrode site and faces, χ^2 (9) = 33.26, $p < .001$. This was corrected using Greenhouse-Geisser estimates of sphericity. The corrected results are reported.

For the N2 AUC responses (time point 5), the between-subjects factor intervention (active versus placebo) was not significant, $p > .05$. There was a significant main effect for the within-subjects factor electrode site (Fz, FCz, Pz), F (1.10, 19.84) = 17.67, $p < .001$, again with greater activation at Fz. None of the other main effects reached significance, $p > .05$.

There was a significant interaction between electrode site and faces, F(2.55, 45.96) = 3.34, $p < .05$. Post hoc tests confirmed that this was driven by significant differences between Fear, Sad and Happy faces across electrodes as outlined below.

There was a trend for facial expression of fear between FCz (M = -27.14, SE = 40.23) and Pz (M = 45. 12, SE = 41.30) with Pz producing greater positive activity compared to FCz which was negative. For sad faces, there were significant differences between electrode sites Fz (M = -98.24, SE = 41.35) and Pz (M = 72.34, SE = 47.39) and also between FCz (M = -65.54, SE = 34.33) and Pz (M = 72.34, SE = 47.39).In all cases, responses to sad faces produced greater positive activity at parietal scalp regions compared to frontal and frontal-central regions which were negative.

For happy faces, there were significant differences in activity between Fz (M = -82.78, SE = 34.60) and FCz (M = -41.48, SE = 35.67) electrode sites as well as Fz (M = -82.78, SE = 34.60) and Pz (M = 65.32, SE = 40.24) and finally FCz (M = -41.48, SE = 35.67) and Pz (M = 65.32, SE = 40.24). The same pattern reoccurred with greater positive activity occurring at parietal scalp regions. No other interactions reached significance, $p > .05$.

Time point 8 (N4 deflection)

Mauchly's test indicated that the assumption of sphericity had been violated for the within-subject factors: electrode site, χ^2 (2) = 23.61, $p < .001$, and for the interactions between electrode site and testing time, χ^2 (2) = 8.79, $p < .02$, electrode site and faces, χ^2 (9) = 34.16, $p < .001$ and the 3-way interaction between testing time, electrode site and faces, χ^2 (9) = .019, $p < .002$. This was corrected using Greenhouse-Geisser estimates of sphericity. The corrected results are reported.

For the N4 AUC responses (time point 8), the main effect of the between-subjects factor intervention (active versus placebo) was not significant, $p > .05$. There were significant main effects for the within-subjects factor electrode site (Fz, FCz, Pz), $F(1.14, 20.56) = 27.38$, $p < .001$ with significantly greater activation at Fz (M = -197.2, SE = 33.39), followed by FCz (M = -197.55, SE = 35.05) compared to Pz (M = -11.67, SE = 33.97). There was a trend finding for faces, $F(2, 36) = 2.61$, $p < .08$ with sad faces producing the greatest activity (M = -171.34, SE = 34.43) and fearful faces the smallest (M = -115.94, SE = 32.22). There was a significant interaction between testing time and electrode, $F(1.42, 25.64) = 4.24$, $p < .05$. Post-hoc tests showed that this was due to significant differences between Fz (M = -209.16, SE = 41.42) and Pz (M = 24.55, SE = 40.08) and also between FCz (M = -204.93, SE = 43.80) and Pz (M = 24.55, SE = 40.08) at baseline. In both cases, frontal and frontal-central scalp regions produced greater negativity activity compared to parietal regions which was positive. There were also significant differences in the follow up group (testing time 2: 12 weeks) between Fz (M = -185.88, SE = 42.50) and Pz (M = -47.90, SE = 42.29) and also between FCz (M = -190.17, SE = 45.34) and Pz (M = -47.90, SE = 42.29), FCz producing the greatest activity and Pz the smallest.

In relation to the 3-way interaction between testing time, electrode site and faces, there was a significant difference at testing time 2 (follow up at 3 months) between facial expressions of fear (M = -127.14, SE = 43.89) and anger (M = -299.99, SE = 43.19) at Fz, $p < .02$. In the follow up group, there was also a significant difference at FCz between facial expressions of fear (M = -134.79, SE = 48.85) and sadness (M = -268.56, SE = 51.14) $p < .02$, as well as between fear (M = -134.79, SE = 48.85) and anger (M = -302.40, SE = 48.12), $p < .05$.

There was a trend finding for the 3-way interaction between testing time, electrode site and intervention, $F(2, 36) = 2.63$, $p = .056$. Pot-hoc tests showed that this was due to significant

differences in the placebo group at baseline between all electrode sites (Fz, FCz and Pz), $p < .05$. In all cases Fz elicited the largest negative activation (M = -204.39, SE = 61.44) compared to FCz (M = -140.29, SE = 64.97) and Pz (M = 79.46, SE = 59.00) which was positive. At testing time 2 (follow up), also in the placebo group there was a trend finding between FCz (M = -141.43, SE = 67.25) and Pz (M = -2.22, SE = 62.72). The highest negative activity was observed at FCz compared to the Pz electrode site which elicited the smallest activity.

In the active group, there were significant differences at baseline between Fz (M = -213.92, SE = 55.57) and Pz (M = -30.36, SE = 53.77) and also between FCz (M = -269.56, SE = 58.76) and Pz (M = -30.36, SE = 53.77), $p < .05$. The largest difference in responses was at FCz and the lowest at Pz. At testing time 2 (follow up) in the active treatment group there were significant differences between Fz (M = -256.55, SE = 57.02) and Pz (M = -93.58, SE = 56.73) and FCz (M = -238.91, SE = 60.83) and Pz (M = -93.58, SE = 56.73), $p < .05$. As per before the largest negative activation was observed found at Fz and the smallest at Pz. No other interactions reached significance, $p > .05$.

Brief Discussion and Conclusion

The present study is the first study that tested whether twelve weeks of omega-3/6 supplementation would significantly alter the task performance and the neuronal activity of children with ADHD compared to placebo during 2 tasks of executive function (EF) and an emotional processing task as measured by event related potentials (ERPs). The overall findings of this study were essentially negative as no significant effects of omega-3/6 were observed in any of the ERP waveforms between baseline and follow up assessments during the 3 tasks employed in this study. There was a trend finding for a significant group effect during the sustained attention which will be discussed further in the task relevant section. However, this was not in line with study's hypotheses, which predicted greater activation in P3 in the active group following 12 weeks of supplementation, as it was observed in the placebo group. In the emotion processing task there was a significant group by condition interaction, with greater negative neuronal activation in the active group relative to the placebo group to happy faces which is in line with previous research findings by our team and will be discussed further in the task relevant section (Gow et al., 2009). Finally, there were no significant differences in behavioural performance in the two EF tasks (Go/NoGo or CPT) between the omega-3/6 supplementation and placebo groups at either baseline or follow up.

Collectively, there was little support for a significant effect of omega 3/6 on performance or assessments of brain function in ADHD. However, the limitations of the research should be considered and will be discussed individually (that is per task) and collectively in later sections in the discussion. The results reported here cannot be directly compared to other studies as there are no previous published studies exploring the effects of omega 3/6 supplementation to brain function in ADHD. There are a few studies which have demonstrated some significant effects of LC-PUFA supplementation (or fish consumption) to cognition, however these were conducted in both healthy school children and adult populations (Boucher, et al., 2011; Fontani, Corradeschi, Felici, Alfatti, Migliorini, et al., 2005; McNamara, Able, et al., 2010). There were some generic effects and interactions relative to the task demands which will be discussed individually under the relevant sub-headings. Following, the individual task discussions, the generic limitations of all three studies will also be addressed.

Go/NoGo task

There were no significant main effects of intervention on any of the ERP measures during the Go/NoGo task. In addition, there were no significant main effects of intervention on the performance data. The findings confirmed that across time points, baseline testing time produced significantly greater activation compared to follow up. This suggests that the practise effect may have reduced brain activation at the follow up assessment compared to baseline. Across all time points there was a significant effect of electrode which produced greater activity at Pz and smallest activation at Fz. The role of the posterior parietal cortex is especially relevant during tasks of visual-spatial attention, a function which is essential for Go/NoGo task performance, underlying both executive and inhibitory processes (Behrmann, et al., 2004). In all instances, Go trials produced significantly increased activation in ERP responses which arguably reflects the parietal mechanisms associated with response preparation, target detection and selective attention that have been previously reported (Banaschewski, et al., 2004).

Chapter 8 presents the results from a larger baseline sample of children with ADHD and the comparison to healthy control children during the same Go/NoGo task and no group differences were observed. This was unexpected and not in line with the studies hypothesis. However, the lack of group differences reported in Chapter 8 is also relevant for the findings obtained in this ADHD supplementation subgroup study. For example, as there are no apparent group differences in ERP assessments of brain function between ADHD and healthy controls in Chapter 8, then within this cohort the anticipated normalisation effect of LC-PUFA was unlikely. It should be considered, that the research study was originally designed around the limited existing literature and none of the ERP findings especially in terms of group differences could have been pre-empted. This study does not support the role of omega-3/6 supplementation in inhibitory or executive processes associated with the Go/NoGo task. Furthermore, it does not lend support to the work of Fontani and colleagues (2005) whose study reported a significant effect of omega-3 supplementation as demonstrated by a reduction of reaction time (i.e., faster performance) and P3 amplitude during both Go and NoGo trials. However, the Fontani study was in healthy adult males and therefore is not directly translational to this study.

Limitations of Study 2A (Go/NoGo task)

One limitation of the study is the blocked task design, where NoGo and Go trials are presented in separate blocks as opposed to intermixed. This arguably made the task too easy as there was no prepotent tendency to respond in the block design of the task. Furthermore, the NoGo trials to be inhibited were also conflict trials (inhibit to the word "PRESS"), thus measuring motor and interference inhibition at the same time. This was designed so the neurophysiologic substrates of both motor and interference inhibition functions could be assessed within one task. The rationale being that motor and interference inhibition involve similar neural networks in children and adults (Rubia, et al., 2006a). The disadvantage, however, is that the neurophysiologic underpinnings of motor response inhibition and interference inhibition cannot be assessed independently from each other.

Continuous Performance Task (CPT)

The results of this study showed no significant effects of omega-3/6 supplementation on any of the performance or ERP measures at baseline or follow up time points. There was a trend finding for group at P3a; however this was due to significantly greater activation in the placebo group relative to the active group. This finding was unexpected and not in line with the study's hypothesis which predicted twelve weeks of omega 3/6 supplementation would enhance neuronal activation relative to placebo. There was a reoccurring pattern across time points which were also observed during the Go/NoGo task in that parietal scalp regions (as measured by Pz electrode site) generated increased activation compared to frontal and frontal-central regions. This is likely to reflect the demands of the task which had a greater load on selective attention processes which are in turn are associated with parietal mechanisms (Behrmann, et al., 2004). The effect of condition was similar to the results reported in Chapter 9, in that, distractors produced significantly greater activation in amplitude responses implying that the group demonstrated a greater level of attention / distractibility towards irrelevant stimuli in the early orienting ERP responses captured by P2 deflections and early and later P3 associated with working memory updating, novelty, and selective and sustained attention respectively. At time point 5, N2 deflections were enhanced to targets maximal at posterior scalp regions (i.e., Pz) potentially reflecting the mismatch negativity component (MMN). The MMN occurs in response to deviant or rare events, occurring within a succession of same type stimuli, in this CPT task, the targets were the rare stimuli in a succession of regular alphabetical letters.

Limitations of Study 2B (CPT)

The main limitations of this study were arguably the duration and difficulty of the task as previously discussed in Chapter 9. Briefly, as the task measured sustained attention it should require the participant to maintain a level of vigilance over long periods of time. The task employed in this study lasted for a total of 8 minutes and was shorter than similar attentional tasks such as The Test of Variables of Attention (T.O.V.A.) and the Conners' Continuous Performance Test (CPT II) which range from 14 to 21 minutes depending on age. There is also the question of task difficulty. Previous research in other sustained attention tasks has confirmed that children with ADHD seem unable to allocate their attention when the demands of the task increased (Jonkman, et al., 2000; Lopez, et al., 2006; Steger, et al., 2000; van der Stelt, et al., 2001). Therefore, arguably a more difficult version of the CPT employed in this study, may elicit similar differences in attention allocation reported previously (Jonkman, et al., 2000; Lopez, et al., 2006; Steger, et al., 2000; van der Stelt, et al., 2001). However, despite these task limitations, there was no between group differences in intervention which is discussed further in the generic limitations section.

Emotion Processing Task

There were no significant effects of omega-3/6 supplementation between on any of the ERP measures at baseline or follow up time points. However, at P2, a significant interaction was observed between condition (e.g., facial expression) and intervention (LC-PUFA versus placebo) with greater activation in the active group to facial expression of happy faces relative to those of fear, implying a relationship between omega-3 supplementation and early ERP responses to happy faces. This is line with previous research published by our team reporting a positive relationship between P3 ERP responses to happy faces relative to sad and fearful facial expressions and EPA (Gow, Matsudaira, Taylor, Rubia, Crawford, Ghebremeskel, Ibrahimovic, Vallee-Tourangeau, et al., 2009). It may be that the omega-3 supplementation effect has more impact on emotional processes as opposed to cognition which would be in line with findings reporting a significant beneficial effect of EPA in depression and mood disorders (Freeman, 2006; Nemets, et al., 2006; Peet & Horrobin, 2002b).

The significant main effect of electrode confirmed that across all time points, frontal scalp regions produced greater activation compared to parietal. Previous research has confirmed that midline electrode sites ERPs are responsive to alteration by facial expressions of emotion

(Carretie & Iglesias, 1995). There is also some evidence that earlier timepoints, that is, within 200 ms of face processing reflect the preliminary torrent of sensory afferents, predominantly in the thalamo-cortical pathways (Williams, et al., 2004). In addition, greater activation at midline frontal scalp regions may reflect anterior cingulate cortex (ACC) activity which in turn is implicated in emotional processing (Etkin, et al., 2011). An immaturity of prefrontal function has been reported in adolescent groups relative to adults and thought to be associated with an imbalance between both control systems and affect processing during this developmental stage (Hare et al., 2008). The enhanced activity to negative emotional stimuli, e.g., to sad faces, observed in this study at frontal scalp regions (Fz) in our ADHD group also lends support to previous research which has demonstrated an association between increased neural activity of the early P1, P2 deflections to negative (e.g., threat related) and neutral facial expressions and anxiety in both children and adults (Bishop, Jenkins, & Lawrence, 2007; Frenkel & Bar-Haim, 2011; Somerville, Kim, Johnstone, Alexander, & Whalen, 2004; Thomas et al., 2001). However, this enhancement was also observed in P2 components to positive stimuli, i.e., to happy faces again reflected by midline frontal and frontal-central (Fz and FCz) scalp regions in this study which perhaps implies perhaps these are more generic augmentation linked to early processing stages. The findings in Chapter ten previously confirmed group differences at P2 with reduced activation at midline frontal and central scalp regions as reflected by Fz, FCz and Cz electrode sites in ADHD compared to controls supporting the hypothesis of hypo-responsivity to facial expressions of emotion (Palm, Elliott, McKie, Deakin, & Anderson, 2011). In Chapter ten we also reported a trend finding for facial expressions with neutral faces producing the greatest activation closely followed by happy and angry faces. Responses to fearful faces were attenuated also at frontal-central midline regions in ADHD compared to controls but this effect was not observed at parietal sites. The combined findings of both Chapter 10 and this study suggest there is a parallel abnormality in the processing of emotional stimuli captured by P2 ERP responses in our ADHD group.

At time point 5, N2 there was a significant interaction between electrode site and faces with sad faces producing positive going responses at parietal sites relative to negative going responses constant at midline frontal and frontal-central regions. The mid-line frontal scalp regions are thought to reflect activity in the ACC (Etkin, et al., 2011) while posterior regions are

associated in the evaluation of facial stimuli and also in selective attention processes (Bush, Luu, & Posner, 2000). The same pattern was observed for happy faces with greater positive going activation at parietal regions. The N2 is linked to face processing and thought to reflect the orienting stage. It is also known to be automatically enhanced to novel or biological stimuli (Balconi, 2005; Balconi & Lucchiari, 2005).

At the late time point N4, there was a trend finding for faces with significantly greater activation produced for sad faces relative to fear which were attenuated. Increased activation to negative facial stimuli and simultaneous lack of reactivity to happy faces has also been observed in other ADHD groups (Herrmann, et al., 2009).

Limitations of the Emotion Processing Task

The main limitation of the emotion processing task was sample size. There were only 9 in the placebo group and 11 in the active (LC-PUFA group). Other studies have examined the effect of laterality and hemisphere (e.g., T5, T6, O1, O2) and the face specific N170 component which are also implicated in emotion processing. This study does not report the effects of these factors in this thesis due to time constraints. However, no significant effects were found for these factors.

Generic Limitations across all three studies

The main limitation across Studies 2A, 2B and 2C were the small sample sizes. Although, it is common for ERP studies to publish with sample sizes of 20 participants, arguably the question of appropriate statistical power is raised. Clearly, increasing the sample size increases both the statistical reliability and power of the study. In a sample size of 20, the chance of seeing an effect of supplementation is also diminished. During the MAAFA study, the ERP/EEG was secondary to the studies main objective and due to time scales and the study end point; follow up assessments for this part of the study had to abandoned which resulted in fewer than anticipated participant numbers.

Another limitation, as previously mentioned, is the issue of diagnosis. Although, the children met criteria for ADHD, very few had a clinical diagnosis prior to recruitment and randomisation. Furthermore, they were recruited from schools with a provision for children with social, emotional and behaviour difficulties and because of these two reasons may not reflect a "pure" ADHD group. As mentioned in previous chapters, there is also some evidence to suggest

that clinically referred patients may be more severely impaired than those from the community (Angold, et al., 1999; Sprafkin, et al., 2007).

Finally, the supplementation period of 12 weeks was likely to be too short to fully impact and/or alter the brain's biochemistry. Animal studies have reported that chronic omega-3 deficiency has been associated with abnormal neurotransmitter systems and that replenishing the diet with omega-3 results in only part recovery of these systems (McNamara & Carlson, 2006b). Therefore, it can be postulated that chronic deficiency in omega-3 in utero can potentially have lasting consequences which are only partly reversible with treatment with omega-3. Intervention with LC-PUFA is likely only to be effective over longer periods of time, possibly over a number of years and only when a complete elimination of refined seed oils rich in saturated fats is stripped from the diet. The continued consumption of saturated fat / refined seed oils throughout a LC-PUFA supplementation trial is arguably a major confound because omega-3 and omega-6 compete for incorporation and synthesis into the red blood cell and higher consumption of omega-6 from refined and manufactured foods (i.e., cakes, biscuits, certain vegetables oils such as safflower) results in not only an under representation of omega-3 in the brain but a higher risk of diet-induced peroxidative obliteration, especially if the child is deficient in Vitamin E which has a protective effect against oxidisation of fatty acids[10]. The continued rise in favour of saturated fats, primarily as a derivative of soybean oil, over omega-3, coupled with the commercial manufacture and refining process of foods, constitutes a major dietary change during the past century which has resulted in the Western diet consumed today and arguably left mankind with very little of the essential omega-3 fatty acids, EPA and DHA in human tissues which are necessary for optimal cognitive and emotional function (Blasbalg, Hibbeln, Ramsden, Majchrzak, & Rawlings, 2011).

[10] Written correspondence with Dr Robert Peers, General, Preventative and Nutritional Medical Practitioner, Victoria, Australia, 19th February 2012.

Conclusion

The overall results collapsed across these 3 studies do not support the hypothesis of a beneficial effect of supplementation with omega-3 fatty acids in this subgroup of children/adolescents with ADHD. However, the limitations discussed should be considered and taken into account in the planning of future clinical trials in this area. There were specific task relevant main effects of condition and electrode which lend some support to the existing ERP literature in ADHD but were more apparent during the emotion processing task. On the basis of these findings, the notion of abnormal affect processing in ADHD should be further explored. Future research however should recruit larger sample sizes in order to better validate the null findings observed in these 3 experiments.

Chapter Twelve: General Discussion & Conclusion

Summary of main findings

The investigation into the relationship between LC-PUFA and assessments of brain function in children and adolescents with and without ADHD until now has been largely unexplored. The present study directly addressed a number of novel yet inter-related research questions which had not previously been investigated. Firstly, the study tested whether omega-3/6 levels differed between children and adolescents with ADHD relative to healthy control children in and around the London area. This PhD reports data from two separate groups of children with ADHD, those that took part in the blood and behaviour study (reported in Chapter 7, $n = 29$) and those that took part in the MAAFA trial ($n = 29$ for the Go/NoGo task; $n = 33$ for the CPT and $n = 29$ for the emotion processing task). There was no evidence for group differences in blood levels of omega-3/6 between the first group of children with ADHD ($n =29$) reported in chapter 7 and healthy controls ($n = 43$). Although, prior to correction there was some evidence for lower LC-PUFA levels in the ADHD group compared to controls in plasma PC measures of the omega-3 series, namely, DHA, DPA, EPA and in the omega-6 series, AA. In contrast, there were significant differences following correction in both omega-3 and 6 fatty acid indices in the second group of children/adolescent with ADHD (i.e., the MAAFA group) relative to control children. This was in line with the study's hypothesis and supports previous research reporting abnormalities and/or altered fatty acid metabolism (Antalis, et al., 2006; Stevens, et al., 1996; Stevens, et al., 1995; Young, et al., 2004). It is unclear exactly why there was a discrepancy between the two ADHD groups. The lower levels of both omega-3/6 found in the second may well reflect a lower dietary intake/deficiency of omega-3 fatty acids or indicative of metabolic abnormalities. Above all, the variations in findings reported here have implications in the recruitment of ADHD groups for clinical trials and highlight the necessity to assess dietary baseline levels of fatty acids in blood samples prior to randomisation and dose allocation.

The first study only explored whether relationships in these two groups existed between specific fatty acid indices and clinical symptoms of ADHD and comorbid symptoms employing an extensive battery of nine clinical questionnaires on externalising and internalising symptoms. There were no significant correlations with the exception of a significant relationship between EPA and callous and unemotional traits in children/adolescents with ADHD. This relationship

was further supported by a second significant relationship between low levels of total omega-3 and higher CU scores and finally a trend finding of a negative association between DHA and high CU traits. This suggests for the first time a relationship between omega-3 and specifically EPA/DHA and conduct disorder related CU traits in children with ADHD. Also, in ADHD children, CU traits were also positively associated with CPRS subscale for oppositional behaviour and a trend finding was observed between low omega-3 and high scores of anti-social behaviour as measured by the APSD.

The study advanced to test whether a different group of children with ADHD (that took part in the MAAFA trial) and controls differed in performance and specific indexes of task relevant ERPs measuring brain function, such as the P3 family, during two tasks of executive function measuring inhibitory control (the Go/NoGo), and selective/sustained attention (the CPT) and one emotion processing task. Despite performance deficits of higher omission errors in the Go/NoGo, indicating attention deficits and higher commission errors in the CPT, suggesting impulsiveness, the findings of both EF tasks did not support differences between cases and controls in ERP's linked to inhibitory or selective/sustained attention processes respectively. Rather, in response to Go trials in the Go/NoGo task ADHD children had significantly lower AUC for the earlier portion of the P3 wave (P3a) at the central electrode sites and for the later portion (P3b) at Fz, suggesting that deficits in ERPs in ADHD were specific over fronto-central brain regions during the Go process of the task.

In addition, in the healthy control group, errors of commission reflecting the inhibitory aspect of the Go/NoGo task, were found to be negatively associated with EPA, suggesting the higher the EPA – the lower number of commission errors. During the CPT omission errors, which are thought to reflect selective and sustained attention processes (Brodeur & Pond, 2001; Halperin, et al., 1991) were negatively associated with omega-3 ALA in the control children. On the basis of the role omega-3 has in neurotransmitter functions (Yehuda, et al., 2002), it could be suggested that omega-3 has a beneficial role in the modulation of inhibition and attention in healthy adolescents.

Relationships between ERP measures of brain function and LC-PUFA were not in line with the study's hypothesis but instead confirmed significant negative relationships observed at central and parietal scalp regions with both total omega-3 and DHA in the ADHD group. The relationships suggested that as omega-3 increased P3a amplitude responses to targets (Go

stimuli) decreased. Also, in the ADHD group, P3b responses to Go stimuli were similarly negatively associated with omega-3 indices namely total omega-3 and DHA across fronto-central, central and parietal scalp regions (FCz, Cz and Pz), suggesting that as omega-3 measures increased P3b amplitude responses decreased. In the healthy control groups, omega-3 fatty acids were also negatively associated with P3b amplitude responses to NoGo stimuli. This is discussed in relation to the neuroefficiency theory so that higher omega-3 may be associated with less need to recruit frontal brain regions to achieve the same inhibitory capacity.

The results of the CPT study did not support the hypothesis that omega-3 would be positively related to ERP responses to targets as none of the relationships survived correction for multiple testing. The implications concerning the absence of relationships in this task are further discussed in the task relevant section.

The findings of the emotion processing task, as predicted, confirmed significant group differences in N2 and N4 amplitude responses between ADHD and control children which suggest that children with ADHD are impaired both in the early orienting stage and also in the later evaluation and event integration aspect of emotion processing. The correlational findings in relation to the emotion processing task were in line with the study's prediction, that omega-3 would be negatively related to N4 AUC amplitudes. The first relationship was a negative relationship between N4 responses to happy faces and the total omega-3 ratio. The second was also a negative association between N4 responses to happy faces and the parent omega-3 compound, ALA. N4 is involved in the integration of the attended to event and related cognitive context (Liddell, et al., 2004) and in this study was found be abnormal in ADHD compared to controls. N4 amplitudes were less negative in ADHD in all conditions (i.e., facial expressions) relative to controls, and the relationship suggested that as omega-3 increased N4 responses also decreased, in other words, become more negative, i.e., more similar to that of the healthy control responses. The results then provide evidence that ADHD are impaired in emotion processing relative to controls and furthermore that omega-3 fatty acids are associated with ERP responses which are closer to those of the healthy control group.

Ultimately, the study addressed in a smaller subgroup of children with ADHD whether 12 weeks of supplementation with omega-3/6 would result in increased activation in brain function during the same ERP tasks relative to placebo. The results of both the two EF function tasks did not support the study's hypothesis as there were no significant differences in neuronal activation

between the active and placebo groups at the follow up assessments. However, there was a significant group by intervention interaction in the emotion processing task. This was due to significantly enhanced neuronal activation in the active (omega-3/6) supplementation group in P2 amplitude responses to happy faces at follow up (following 12 weeks of supplementation) relative to placebo, in line with evidence for omega-3 and association with positive emotions. Previous research by Gow and colleagues (2009) found a similar association with omega-3 and P3 responses to happy faces in adolescent boys with ADHD. The previous findings coupled with this finding suggest that omega-3, and specifically EPA, is linked with emotional valence to positive stimuli over and above negative stimuli. This in turn arguably also has implications for the omega-3 fatty acid research in depression.

The collective results of this PhD study provide only limited evidence for the relationship between LC-PUFA, behavioural measures of ADHD and associative clinical symptoms and assessments of brain function in very specific measures, mostly related to emotion processing. There were mixed and somewhat contradictory findings in relation to the biochemistry parameters of LC-PUFA with no group differences in blood levels of fatty acid fractions in the first group of children with ADHD but significant differences between the MAAFA children and HC. Associations were only observed with specific behavioural and functional aspects. Omega-3 was not associated with ADHD typical related behaviour features or EF performance or brain measures as hypothesised but only with very precise aspects of ADHD such as CD related CU traits and emotion processing. While only small associations were found between omega-3 and brain function during cognitive EF tasks, the most pronounced associations between EPA and ERP deficits was during the emotion processing task. Supplementation also only had an effect on brain function to happy faces. Overall the findings suggest an association between low PUFA and clinical measures related to affective or motivational processes such as CU/CD and with brain function measures of emotion processing. In particular PUFA seems to be associated with more positive social and emotion processes, i.e., less CU and anti-social traits and better brain responses to happy faces. Preliminary research in both child (Nemets, et al., 2006) and adult populations (Jazayeri, et al., 2008) have reported a potential modulating role of omega-3 in alleviating symptoms of depression. The findings reported in this study lend some support to the literature and command the necessity for further investigation in ADHD and other clinical groups. Each finding will be discussed in greater detail in the task relevant study sections.

Blood levels of LC-PUFA

As mentioned, the first study found no significant differences between groups in levels of LC-PUFA following correction for multiple testing, while the second study did find differences. Previous research findings of statistically significant differences in blood levels of omega-3/6 between children and young adults with ADHD and controls were therefore not replicated in the first study. This finding was unexpected and not in line with the study's hypothesis. However, the results do support the findings of two other studies reporting no differences in PUFA status in (1) maladjusted children and (2) children with ADHD respectively relative to controls (Mitchell, et al., 1983; Spahis, et al., 2008). Of note and as previously mentioned in Chapter 7, the majority of the published literature reporting significant differences in essential fatty acids between ADHD and healthy controls and in turn, relationships between those indices and behavioural measures, had not reported correcting for multiple testing. This raises the possibility of reliability, given that some of reported relationships may not have survived correction for multiple testing. Nevertheless, an argument could also be made in the opposite direction, that correcting for multiple testing in sensitive measures such as percentage levels of fatty acids may be too stringent. Prior to correction, there were some small group differences between ADHD and control children with lower levels of DHA and higher levels of c22:4n6 (a metabolite of AA) in the plasma cholesterol esters in the ADHD group relative to controls. In the plasma triglycerides measure, there were higher means levels of AA and EPA in the ADHD group relative to controls. Finally in plasma choline phosphoglycerides lower levels of c20:3n3 (eicosatrienoic acid) and DPA were present in the ADHD group relative to controls. This is the first study in the U.K. thus far to report the fatty acid status of children/adolescents with ADHD and healthy controls using venous blood samples which are arguably more reliable (Arab & Akbar, 2002) than for example, buccal cell cheek analyses (Lapillonne, DeMar, Nannegari, & Heird, 2002). A key limitation however was the unforeseen contamination of the red blood cell samples, given that RBC are a more stable and reliable measure of fatty acids status over time (Sun, Ma, Campos, Hankinson, & Hu, 2007).

The LC-PUFA levels in plasma choline phosphoglycerides measures were also contrasted between the ADHD group recruited during the MAAFA trial and the healthy control group recruited in this PhD study. There were significant differences as predicted in key omega-3 and omega-6 fatty acids indices. There was an overall pattern with persistently higher levels of both

omega-3/6 in the healthy control group compared to ADHD. These findings support previous research suggesting altered fatty acid metabolism in ADHD (Antalis, et al., 2006; Stevens, et al., 1996; Stevens, et al., 1995; Young, et al., 2004). It is not entirely clear why the findings differ from those reported in the group of children with ADHD in chapter 7. It is plausible that these differences simply reflect differences in dietary intake of omega-3 but could also be indicative of an altered metabolism in ADHD which should be explored in future studies. However, the two ADHD groups were different in a number of ways. Firstly, the children with ADHD recruited in the MAAFA trial (group 2) had significantly lower scores of both verbal and composite IQ in comparison to the first group of children with ADHD. Evidence reported by Hibbeln et al. (2007) suggests that maternal seafood consumption of less than 340 grams per week during pregnancy is linked to a range of poorer developmental outcomes, including IQ scores in the lowest quartile. Measures of maternal seafood consumption were not collected during this PhD study however future studies could explore further whether low levels of omega-3 are associated with low IQ in children with ADHD. The MAAFA children also presented with significantly lower symptom severity according to the DSM-IV total subscale of the Conner's Teacher Rating scales. Although, the MAAFA children were also recruited from a community sample, they were from a range of specialist residential/day schools with a provision for emotional and behavioural difficulties and therefore diet was likely to be more consistent due to the fact that the majority of meals were provided for them, i.e., at least 5 days a week. School diets have received a lot of negative media attention in the past few years in the U.K. and there have been great efforts to implement changes. It was also evident from the blood levels of LC-PUFA that these schoolchildren were eating very little fish. Another consideration, is the ethnicity of the children in the MAAFA trial was predominantly Caucasian which differed slightly to the ethnicity of the ADHD group reported in chapter 7 of which 17.2% of the group of children were from West Indian or African descent and consumed fish at least once a week. Fish eating habits were not reported in the MAAFA study and therefore a contrast in relation to fish eating habits between the two ADHD groups cannot be made. Future research in this area should include not only records of both ethnicity and fish eating habits but also include an investigation of the FADS2 which in turn has implications for the absorption and synthesis of key HUFA in the omega-3 series such as EPA and DHA from the parent precursor (Brookes, et al., 2006b). As discussed in Chapter 2, the fatty acid desaturase 2 (FADS2) encodes essential enzymes – delta 5 and delta 6

desaturase - for absorption into plasma phospholipids and erythrocyte membranes however the activity of these enzymes is susceptible to metabolic, nutritional and hormonal regulation (Rzehak, et al., 2009). The minor allele of the FADS2 gene is associated also with decreased activity resulting in diminished amounts of PUFA but elevated amounts of the un-metabolised precursors (Steer, et al., 2012). The overall findings concerning both blood studies suggest that there is some evidence for lower levels of LC-PUFA in children with ADHD but the results should be considered with caution and would warrant replication in much larger sample sizes using red blood cell measures and possibly employing stable isotope to map the absorption and synthesis of fatty acids to assess whether or not metabolic problems may exist in ADHD.

LC-PUFA and Behavioural Questionnaires/Clinical Data

Furthermore, against predictions, there was no evidence that LC-PUFA levels were associated with (i) IQ as measured by the Kaufman Brief Intelligence test, (ii) scores of fatty acid deficiency as measured by the Essential Fatty Acid Deficiency Questionnaire, (iii) subscales scores of both teacher and parent Conner's Rating Scales, (iv) behavioural measures of impulsivity as measured by the Barratt Impulsivity Scale, aggression as measured by the Buss-Perry Aggression scale, (v) depression as measured by the Depression, Stress and Anxiety Scale, and finally (vi) depression, anxiety, anger, disruptive behaviour and self concept subscales as measured by the Becks Youth Inventory, second edition. The absence of additional relationships between self-rated scores of behaviour measures and LC-PUFA in ADHD and typically developing control children was unexpected and warrant some discussion. There are some factors which may have contributed to these findings including the relationship between diet and ethnicity which has been already discussed in Chapter 7. However, to summarise, approximately 17.2% of the group of children with ADHD recruited in the blood and behavioural study were from West Indian or African descent and consumed fish at least once a week. This fish eating ADHD subgroup is likely to have impacted the overall percentage levels of fatty acids in the total ADHD group. Future research could control better for fish eaters versus non-fish eaters when investigating fatty acids and behaviour between ADHD and typically developing children to establish whether these findings can be replicated. However as the sample size was already relatively small in this group, such calculations would significantly reduce the power of the study and therefore lower the reliability of the results.

There was evidence however for a relationship between EPA and callous and unemotional traits in children/adolescents with ADHD. This relationship was further supported by a second relationship between low levels of total omega-3 and higher CU scores and finally a third relationship (trend finding) which was a negative association between DHA and high CU traits. In additional support of these associations in the ADHD group, were (1) a significant positive relationship between the CU traits and CPRS subscale for oppositional behaviour and (2) trend associations between low omega-3 and high scores of anti-social behaviour as measured by the APSD. The ADHD group recruited in this study consisted of 16 boys with a clinical diagnosis of ADHD and 13 from the community of which just under half (44.4%) were at medium or high risk of conduct disorder as assessed by the Strengths and Difficulties Questionnaire. As the ICU also measures the presence of conduct disorder related CU traits, the evidence presented in this study strongly suggests a link between ADHD; conduct disorder related CU related symptoms and omega-3 fatty acids, in particular with EPA. Previous literature has suggested that dietary supplementation of omega-3 essential fatty acids is associated with a decrease in anti-social and aggressive type behaviours (Gesch, et al., 2002; Zaalberg, et al., 2010). In contrast, positive associations have been reported between elevated levels of omega-6 in the diet and homicide rates, neuroticism, suicidal and depressive behaviour (Conklin, Manuck, et al., 2007; Hibbeln, 2001, 2007). Comparatively, a positive association was also observed between self-rated scores of anti-social behaviour and higher plasma levels of omega-6 in the ADHD group, lending support to the potential negative effects of diets rich in omega-6.

The CU and EPA association lends additional support to the role of omega-3 fatty acids in anti-social behaviour and arguably has implications for public health and therapeutic interventions. The mechanistic action of omega-3 fatty acids in the activation of molecular systems that are involved in synaptic plasticity has previously been reported, (Gomez-Pinilla, 2008). Furthermore, the early nutritional status of children and later development of anti-social and delinquent behaviours is an area of current scientific and government interest and specific recommendations have been made by the associate parliamentary food and health forum (2008) for further research in this area (Associate Parliamentary Food and Health Forum, 2008[11]). The relationship between ADHD and comorbid disorders has long been established with estimates of

[11] http://www.fhf.org.uk/meetings/inquiry2007/FHF_inquiry_report_diet_and_behaviour.pdf

comorbidity between ADHD and CD/ODD between circa 42.7% to 93.0% (Jensen, Martin, & Cantwell, 1997). Children with ADHD and CU traits often engage in risky and dangerous behaviour (including promiscuous sexual behaviour and substance misuse) (Biederman, et al., 2006; Colledge & Blair, 2001; Lahey, et al., 2000; Wilens, 2004). In addition, children with ADHD and CU traits are at greater risk in terms of their developmental pathway (Barry, et al., 2000). This is especially relevant given that callous and unemotional personality traits represent a distinct vulnerability to persistent antisocial behaviour and are furthermore at the core of psychopathy (Viding, 2004; Viding, Jones, Frick, Moffitt, & Plomin, 2008). The relationship observed in this study between the omega-3, EPA and CU traits in ADHD, coupled with previous literature suggesting that low omega-3 potentially represents a risk factor for the later development of psychopathy including anti-social, aggressive/depressive/suicidal behaviours and higher incident of homicide (Conklin, Manuck, et al., 2007; Hallahan, Hibbeln, Davis, & Garland, 2007; Hibbeln, 2007, 2009; McNamara & Carlson, 2006a) warrant further investigation. This is particularly relevant given that some supplementation trials have found a reduction in anti-social and conduct disorder related behaviours in incarcerated individuals (Gesch, et al., 2002; Zaalberg, et al., 2010). The fact that almost half of the boys with ADHD in this study were at medium or high risk for CD, and that higher scores of CU traits were in turn negatively associated with low EPA has implications for the design of future clinical trials in this area. Future research could contrast clinically diagnosed groups of (1) children with ADHD and CU traits (2) children with CD and CU traits alone and (3) typical developing control children, while simultaneously controlling for fish consumption, would be recommended. This will be expanded upon in the future research section.

Studies 1A, 1B and 1C: LC-PUFA and Associations with Performance Measures

In the two executive function tasks reported in chapters 8 (study 1A) and 9 (study 1B) the results, only partially support the hypothesis of higher commission and omission errors in ADHD compared to control groups in the Go/NoGo and CPT tasks respectively. In the Go/NoGo task as reported in Chapter 8, the ADHD group made significantly greater omission errors compared to the control children which was in line with the study's hypothesis and support previous findings in this area (Rubia, Smith, Brammer, et al., 2007; Tamm, et al., 2004). There were no significant differences between cases and controls in the mean number of commission errors which was

unexpected and not consistent with prior research (Fisher, et al., 2011; Halperin, et al., 1991; Rubia, Smith, Brammer, et al., 2007; Willcutt, et al., 2005). In the second EF task, the CPT, children with ADHD made significantly more errors of commission compared to the control children, which again was in line with the study's hypothesis and supported previous findings in this area (Boonstra, Kooij, Oosterlaan, Sergeant, & Buitelaar, 2005). There were no significant differences in the mean number of omission errors during the CPT task. This finding was not in line with the study's predictions nor supported previous research showing deficits in the attention process of the task (Epstein et al., 2003; Losier, et al., 1996a; Nazari et al., 2010; Oades, 2000).

There were significant associations between performance data and LC-PUFA in the healthy control group only. Specifically, errors of commission during the Go/NoGo task were negatively associated with EPA in the healthy control group, suggesting the higher the EPA – the lower number of commission errors. Commission errors reflect the inhibitory aspect of the task and the relationship observed here suggests that EPA may be associated with inhibitory control processes. During the CPT, a similar pattern was observed also in the healthy control group, a negative association between omega-3 (ALA) and omission errors, suggesting as omega-3 increased, the number of omission errors decreased. Omission errors are thought to reflect sustained and selective attention (Brodeur & Pond, 2001; Halperin, et al., 1991). On the basis of the role omega-3 has in neurotransmitter functions (Yehuda, et al., 2002), it could be suggested that omega-3 has a beneficial role in the modulation of attention. The finding of altered activation in attention networks including dorsolateral prefrontal regions in healthy school children following 8 weeks of DHA supplementation has been reported by McNamara and colleagues (2010). McNamara and colleagues (2010) also reported an inverse relationship between DHA and RT at baseline and endpoint only. One other study by Hirayama et al (2004) found an improvement in commission errors in their healthy control group following two months of intervention with DHA enriched fortified foods relative to ADHD. However, aside from the McNamara and Hirayama studies, there are no previous studies which have tested baseline fatty acids with behavioural measures during CPT and Go/NoGo tasks. Therefore, further research is needed in larger sample sizes to replicate the findings in typically developing children and to further test for associations within ADHD patients.

Chapter 11 reported the results of two EF tasks (study 2A and 2B) from the ADHD subgroup intervention studies - the Go/NoGo and CPT respectively. There was no significant

effect of active intervention relative to placebo on the mean number of commission and omission errors between baseline and endpoint. This was not in line with the hypothesis of inverse relationships between omega-3 and errors of commission and omission at the follow up assessments. The null findings suggest that intervention with omega-3 was not effective in reducing errors of commission or omission in this ADHD subgroup. There are however several limitations of note. The first is that the sample size was small; this was due to drop out at follow up and also due to the deadline for completing the MAAFA study and the need to close the dataset according to MHRA guidelines. The EEG/ERP part of the MAAFA study was secondary to the primary objective which sought to examine the efficacy of omega-3 intervention in reducing symptoms of ADHD according to the Teacher Conner's Rating scale (reported in the PhD thesis of Toshiko Matsudaira) and therefore that data collection took priority. This meant however a subsequent loss of power and a higher number of baseline assessments than follow up. The second limitation surrounds the diagnostic status of the sample. Unlike, the ADHD sample reported in Chapter 7 in which over 50% (55.2%) had a clinical diagnosis, very few of the sample in the MAAFA trial had a clinical diagnosis of ADHD. Although, all children recruited during the MAAFA study met research criteria for ADHD according to the DSM-IV via the process of a semi-structured interview (ChIPS) and completion of both Conner's Parent and Teacher Rating Scales Long Version ensuring *t* scores were above 65 on both forms, there is some evidence that clinically referred patients present with higher symptom severity than those from the community (Angold, et al., 1999; Sprafkin, et al., 2007) . This raises the question as to whether the ADHD group recruited in the MAAFA trial were a "pure" and clinical ADHD group especially as the majority were recruited from specialist schools with a provision for children with emotional and behavioural difficulties. From this perspective, one could speculate that some of the presenting ADHD symptoms which met criteria for a diagnosis of ADHD may have been present as a result of either an attachment disorder or exposure to neglect/maltreatment or similar developmental trauma. A clinician when assessing a child for ADHD would have screened for the possibility of an attachment disorder as a potential reason for over-activity and inattention (Hill, 2008). However, the screening criteria in this study did not control for this possibility. Another consideration was that as this was not a "pure" ADHD group, the children's symptoms of ADHD were not severe enough to capture the group differences reported in other studies. This will be discussed further in the limitation and recommendations for future research sections.

Electrophysiological Measures for Go/NoGo task (Study 1A) and CPT (Study 1B)

The overall findings of the Go/NoGo task do not support previous research reporting alterations in N2 and P3 responses in ADHD compared to control children during NoGo trials in Go and Stop tasks (Fallgatter, et al., 2004; Johnstone & Barry, 1996; Karayanidis, et al., 2000; Pliszka, et al., 2000a; Smith, et al., 2004). Nevertheless, the absence of between group differences is consistent with some previous studies reporting no significant group differences in N2 components (Overtoom, et al., 1998; van Leeuwen, Steinhausen, Overtoom, Pascual-Marqui, van't Klooster, et al., 1998). The only group effects were a significant finding for the 3-way interaction between group, condition and electrode site for P3a and P3b deflections respectively. This was as a result of increased positivity in the healthy control group during Go trials at frontal-central sites (Fz and Cz respectively) relative to ADHD, suggesting differences in the executive processes involving selective attention, response selection and motor response execution (Eagle, et al., 2008). Responses to Go trials reflect the executive process of the task involved in attention allocation and response selection (Rubia, Russell, et al., 2001). The series of Go trials appear more frequently than NoGo, allowing a prepotency to develop towards response execution (Johnstone, et al., 2007). Some studies have reported greater activation in P3 during NoGo trials relative to Go trials (Bokura, et al., 2001; Falkenstein, Hoormann, & Hohnsbein, 2002), while another study has reported enhanced ERP responses to Go in ADHD (Fisher, et al., 2011). The results of this study do not support enhanced P3 to NoGo and this is likely to be due to the block task design which minimises the inhibitory load. A consideration is that that the midline P3a responses in healthy controls were significantly associated with composite IQ scores which suggest the possibility that this group difference may be associated with the lower IQ in ADHD cases rather than the ADHD pathology itself.

As previously discussed in chapter 8, a major limitation appears to be the block design of the task which meant that the inhibitory load was smaller than in other previous event-related potential tasks. NoGo trials were presented together in blocks and therefore only the first NoGo in a block had a high inhibitory load while all subsequent NoGo trials may have caused adaptation to the inhibitory process. Future research should employ a Go/NoGo task with a greater inhibitory load which presents Go and NoGo trials in random order with 50% probability of each occurring to fully capture group differences.

The electrophysiological findings of the CPT as presented in chapter 9 (study 1B)

were unexpected as there was no main effect of group (ADHD versus control children) for any of the mean AUC amplitude ERP responses across time points 5 – 8 (P2, N2, P3a and P3b), suggesting that sustained / selective attention was not impaired in ADHD during this CPT task. Therefore, the findings of this study do not support previous ERP literature which suggest children with ADHD are impaired in selective and/or sustained attention processes as indexed by smaller P3 ERP amplitudes during both target and non-target conditions of the CPT (Banaschewski, et al., 2004; Barry, Johnstone, et al., 2003b; Brandeis, Banaschewski, et al., 2002; Jonkman, Kemner, Verbaten, Koelega, Camfferman, v.d. Gaag, et al., 1997; Kratz, et al., 2011; Liotti, et al., 2007; Seifert, et al., 2003). There were also no group differences in the earlier deflections (i.e., P2, N2) which have also been reported to be reduced in ADHD compared to controls during tasks of visual attention (Perchet, et al., 2001). Of note, is that the sustained / selective attention deficit theory in child ADHD can be quite controversial with some studies reporting no group differences in P3 waves (Lazzaro, et al., 1997; Tucha, et al., 2009; Zamorano, et al., 2008). A dissociation between sustained and selective attention capabilities have also been proposed based on findings that children with ADHD are able to perform as well as control children on selective attention tasks while their performance during sustained attention tasks demonstrate impairment (DeShazo Barry, et al., 2001).

Emotion Processing Task (Study 1C)

The results confirmed significant group differences in N2 responses between ADHD and control children as predicted. There were also significant group differences for N4 waves and a trend finding for the early P2 waves between ADHD and control children. In all cases the ADHD group had significantly less negative activation to facial expressions of emotion as well as neutral faces compared to the healthy control group. The findings confirm deficits in the processing of emotion stimuli in ADHD and further extend earlier research suggesting altered ERPs during face processing studies in other clinical child populations including maltreated children and those with psychopathy (Herrmann, et al., 2009; Ibanez, et al., 2011; Pollak, et al., 2001). The results also support behavioural and neuropsychological studies in ADHD groups reporting specific deficits in the ability to correctly evaluate and identify facial emotions of fear, anger and sadness (Pelc, et al., 2006; Singh, et al., 1998; Yuill & Lyon, 2007).

A significant interaction at P2 was also observed between faces and group, this was due to attenuated activation in ADHD to a variety of emotional expressions including fear, neutral and

angry faces compared to healthy control children. This suggests that ADHD are impaired in the ability to process different emotional facial expressions of emotion at this early time point (P2), which in turn is related to basic structural encoding and configural recognition of faces (Ashley, et al., 2004; Eimer & Holmes, 2002; Pizzagalli, et al., 2002). It also lends support to generic deficits in early information processing in ADHD as the lower activation observed was not specific to any one type of emotion (Banaschewski, et al., 2006; Engert & Pruessner, 2008; Nigg, 2005).

Associations between LC-PUFA and ERPs in Studies 1A, 1B and 1C

There were several main hypothesis related to the relationships between LC-PUFA and ERP responses in ADHD and control children in the two EF tasks and emotion processing task reported in chapters 8, 9 and 10. The Go/NoGo task, predicted that P3 responses to Go trials would be significantly positively related to omega-3 fatty acids in both healthy control children and ADHD. The results were not in line with the study's hypothesis but instead confirmed significant negative relationships observed at central and parietal scalp regions with both total omega-3 and DHA in the ADHD group. The relationships suggested that as omega-3 increased P3a amplitude responses to targets (Go stimuli) decreased. Also, in the ADHD group, P3b responses to Go stimuli were similarly negatively associated with omega-3 indices namely total omega-3 and DHA across fronto-central, central and parietal scalp regions (FCz, Cz and Pz), suggesting that as omega-3 measures increased P3b amplitude responses decreased. The frontal-central regions were found to be reduced in activation in ADHD and furthermore associated with fatty acid measures. In the healthy control groups, omega-3 fatty acids were also negatively associated with P3b amplitude responses to NoGo stimuli. A plausible theory, given there were no group differences in neuronal activation, could be that higher levels of omega-3 are associated with more efficient and hence decreased activation. This is in line with previous published research which describes a neuronal efficiency hypothesis in males suggesting that reductions in neuronal activation is linked with a more efficient utilisation of the cortex (Neubauer & Fink, 2009). This would be supported by the fact that the same association was observed in healthy controls. Therefore, higher omega-3 may be associated with less need to recruit frontal brain regions to achieve the same inhibitory capacity.

There was no support for the hypothesis of positive associations between omega-3 and P3 responses to target stimuli during the CPT. The results of this aspect of the CPT study did not

support the hypothesis that omega-3 would be positively related to ERP responses to targets as none of the relationships survived correction for multiple testing.

There was some support for the study's prediction, that omega-3 would be negatively related to N4 AUC amplitudes during the emotion processing task. The first relationship was a negative relationship between N4 responses to happy faces and the total omega-3 ratio. The second was also a negative association between N4 responses to happy faces and the parent omega-3 compound, ALA. N4 is involved in the integration of the attended to event and related cognitive context (Liddell, et al., 2004) and in this study was found be abnormal in ADHD compared to controls. N4 amplitudes were less negative in ADHD in all conditions (i.e., facial expressions) relative to controls, and the relationship suggested that as omega-3 increased N4 responses also decreased, in other words, become more negative, which is in line with the healthy control responses. Both N2 and N4 are significant indexes of face processing and demonstrate that omega-3 is involved in the modulation of emotional responses. Further research would be needed to better understand these relationships ideally using both temporal and spatial measures such as simultaneous fMRI and ERPs. These combined techniques would provide both a temporal resolution (in order to assess the precision of the timing of the response) and spatial (in order to better assess the location of the response) following a supplementation trial with high dose of omega-3 supplementation to assess differences in activation between baseline and study end points. The investigation of omega-3 and emotion processing in children in ADHD is virtually unexplored and these findings provide support for the first time relationships between specific ERP measures and omega-3.

In contrary to the hypotheses there was no support for a relationship between omega-3 and P3 responses to facial expressions of happiness or for a relationship between omega-6 and N4 responses to negative stimuli (i.e., facial expressions of fear, anger and sad faces) in both cases and controls.

MAAFA Studies 2A, 2B and 2C

Chapter 11 presented the findings of the intervention study in a small subgroup of children with ADHD only using the same EF tasks reported in chapters 8, 9, 10 - the Go/NoGo, CPT and emotion processing tasks respectively. In relation to Study 2A and 2B, it was predicted that those children with ADHD in the LC-PUFA intervention arm of the study would display increased

neuronal activation in both N2 and P3 deflections during the Go/NoGo task and in P3 waves during the CPT task at the follow up testing time assessment relative to the placebo arm. The results of both the two EF function tasks did not support either hypothesis as there were no significant differences in neuronal activation between the active and placebo groups at the follow up assessments. Overall, there was no support for an effect of LC-PUFA intervention in selective/sustained attention processes in ADHD. Finally, Study 2C reported the findings from the emotion processing task. The hypothesis predicted significant differences in EPR amplitude responses to both positive (i.e., happy faces) and negative (i.e., sad and fearful facial expressions) facial stimuli indexed by greater activation in N2 and N4 amplitude responses in the active group compared to placebo at follow up. The results confirmed that contrary to the predictions, there were no significant main effects of intervention on any of the ERP measures between baseline and follow up assessments in this ADHD subgroup. However there was a significant group by condition interaction with increased activation in P2 amplitude responses to happy faces in the intervention group relative to placebo at follow up. This finding lends some support to previous research in our group reporting positive associations between EPA and a cognitive bias as measured by P3 amplitudes responses to happy faces relative to both fear and sad facial stimuli in children with ADHD (Gow et al., 2009) and shows a potential modulating effect of omega-3 in emotional responses as characterised by enhanced P2 amplitudes following supplementation. This finding has implications for future studies in emotion processing and warrants replication in other clinical populations including child and adolescent groups with depression, mood disorders, CD and/or young adults in the prodromal phase of schizophrenia. It also lends some support to the literature in depression which suggests (1) that fish consumption cross culturally is associated with lower incident of depression (Hibbeln, Nieminen, et al., 2006), and (2) that supplementation trials have found significant effects of omega-3 in alleviating symptoms in adult patients (Jazayeri, et al., 2008; Peet & Horrobin, 2002b), (3) improving mood and vigour in healthy adults (Fontani, Corradeschi, Felici, Alfatti, Migliorini, et al., 2005) and finally (4) improving symptoms of depression in children (Nemets, et al., 2006). The absence of an overall group effect between active and placebo in other ERP measures may be explained by the fact that this MAAFA group of children had lower levels of omega-3 fatty acids compared to healthy control children at baseline and therefore both a higher dose and a longer supplementation period may have been necessary to correct a deficiency. The finding however

does demonstrate some preliminary promise for the first time that omega-3 supplementation may alter brain function and influence emotion processing in children with ADHD. The collective results of both emotion processing tasks (1C and 2C) suggest some abnormality in emotion processing in ADHD relative to controls as captured by the group differences in N2 and N4 deflections and in addition some involvement of omega-3 fatty acids as demonstrated by the association between LC-PUFA and N4 responses to happy faces and finally by the significant condition by intervention interaction in study 2C.

There are some general limitations in relation to the both EF studies including task duration, selection of sample (i.e., community versus clinic), and duration of supplementation which will be discussed further in the limitations section.

Limitations

There were some specific task relevant limitations during this clinical research project which have already been discussed in the individual chapters. However, to summarise, the blocked design of the Go/NoGo restricted the inhibitory load which meant that only the first NoGo would be a true measure of inhibitory control. The CPT task employed in this study was arguably short in duration (8 minutes) compared to other sustained attention tasks such as the Test of Variables of Attention and the Conners' CPT which are 14 to 21 minutes, age dependent. In addition, the difficulty of the CPT is questionable; it is likely to have been too easy for both groups of children and a more demanding version may have been more effective in distinguishing groups. As previously mentioned, there is the consideration that the group of ADHD employed in this study did not represent a "true" ADHD sample based on the premise they were in the main recruited from the community. Previous research has demonstrated that clinically referred cases with ADHD, which have been recruited in most of the cited previous ERP studies, are likely to have more severe symptoms of ADHD compared to community ADHD patients (Angold, et al., 1999; Sprafkin, et al., 2007). In relation to the MAAFA intervention study, there are a number of reasons retrospectively that supplementation did not work, the main being that there was some evidence for deficiency in specific LC-PUFA fractions in both the omega-6 series (c20:2n6; c20:3n6; c20:4n6 (AA), and total omega-6) and in the omega-3 series (c18:3n3 (ALA); c20:5n3 (EPA); c22:5n3 (DPA); c22:6n3 (DHA) and total omega-3) in the ADHD group compared to control children at baseline (prior to supplementation). Therefore, in order to correct a deficiency a longer supplementation period is

likely to be necessary. However, another factor of consideration is that despite the lower LC-PUFA fractions in ADHD there were no apparent group differences in brain function during the two EF tasks and despite the fact that ADHD showed poorer performance on the executive and inhibitory measures (omission and commission errors) compared to controls. The performance measures were related to omega-3 levels in the control group only suggesting that omega-3 may have a modulating effect in cognition in typically developing children/adolescents.

The absence of overall group differences in ERP measures of brain function may also be simply due to the absence of impairment since the ADHD group performed in line with the healthy control children during the two EF tasks at least with some evidence for impairment in the emotion processing task only. Future research should investigate the fatty acid desaturase genes in addition to the neuropsychological and imaging profile of children with ADHD relative to healthy control children to fully extrapolate these findings further.

Conclusion and Future Research

The collective findings of this PhD raise fundamental questions in relation to the translational implications of both the clinic and future research. In relation to the clinic there are several levels of discussion. Firstly, the question as to whether LC-PUFA levels should be measured in children/adolescents clinic attendees with ADHD and/or subgroups of children with ADHD who score high for CU traits and/or have emotion processing deficits. This also raises the question as to whether supplementation should ever be advised by the clinician and moreover whether there are grounds for NHS prescription. Of note, the measure of CU traits does not form part of the clinical assessment for ADHD. However, CU traits are also a reliable indicator of emotional instability. For example, children who score high for such traits often lack empathy and emotional constrictedness (Wootton, Frick, Shelton, & Silverthorn, 1997). The emotion lability aspect of ADHD is widely unexplored in both the LC-PUFA and ERP literature despite the debilitating effect that emotional dysregulation can have in these children (Schlochtermeier, et al., 2011). Furthermore, the current diagnostic process is in essence clinical and computerised or ERP assessments of attention, inhibition or emotion processing are seldom used (Hill, 2008). Previous literature suggests that high CU traits, poor emotion recognition and low omega-3 are risk factors for the later development of psychopathy, susceptibility to violence and offending behaviour (Blair, 1995, 2005; Blair, Peschardt, Budhani, Mitchell, & Pine, 2006). This PhD provides preliminary evidence for the first time to support relationships between EPA and CU

traits exist in ADHD and therefore provide a novel contribution to the existing research which in turn has implications for the development of knowledge in this area. The screening of CU traits and fatty acids levels in the clinic may have implications for emotion recognition based treatments and recommendations for LC-PUFA supplementation, in particular with EPA, in the future. However, the current findings reported here will need replicating in much larger sample sizes and better powered studies before any firm conclusions can be drawn.

Nutritional intervention with omega-3 supplementation has proven to be an inexpensive and safe adjunct treatment with some efficacy in reducing symptom severity in a variety of clinical populations including depression/mood disorders (Freeman, et al., 2006; Jazayeri, et al., 2008), schizophrenia (Peet & Horrobin, 2002b), and ADHD (Bloch & Qawasmi, 2011). However, there are many inconsistencies in the literature which leave the question as to whether omega-3/6 can directly benefit child/adolescent ADHD groups unanswered. The main conclusion drawn from the findings reported in this research project coupled with previous research in ADHD populations is that current knowledge at this stage does not allow firm answers for translation to the clinic or NHS prescription. It does however raise many further research questions and commands the necessity for larger RCTs with a range of biochemical (i.e., RBC measures), genetic (to assess the delta 5 and delta 6 desaturase which are an index of synthesis and absorption of DHA) and neurophysiological markers (such as emotion related ERPs) in children with ADHD with CU traits and/or CD. It is recommended that initial screening for the omega-3 index (i.e., the sum of EPA and DHA) is conducted prior to randomisation to establish those that may be deficient so high or low doses can be allocated accordingly. Furthermore, future studies should be better powered with much larger sample sizes and tasks preselected for sensitivity in distinguishing clinical groups from controls.

The combined results of this PhD study suggest that relationships with omega-3 fatty acids exist in ADHD on a number of levels. Firstly, on a behavioural level, but only with a very specific behavioural domain of CU (as documented by the relationship between EPA and higher CU traits), secondly on a biochemistry level as evidenced by the differences in blood measures of LC-PUFA between the MAAFA group of ADHD children and control children and finally on a neurophysiological level but only in the emotion processing domain (as evidenced by the significant interaction between intervention and condition, which showed that P2 amplitude responses were enhanced to happy faces in the active group (LC-PUFA) at follow up during the

emotion processing task in MAAFA subgroup intervention study) as well as the significant associations between omega-3 and N4 amplitude responses. Overall, the relationships with omega-3/6 fatty acids reported in this PhD study appear to be more closely linked with emotion than cognition as evidenced by (1) the relationships between high CU traits and EPA in ADHD, (2) the associations between N4 responses to happy faces and total omega-3 ratio as well as ALA and (3) the significant condition by intervention with omega-3 effect which confirmed that 12 weeks supplementation with omega-3 resulted in enhanced P2 responses to happy faces compared to placebo. The enhanced response to happy faces lends some support for a role in omega-3 in the processing of positive emotions relative to negative during early emotion processing. The emotion processing study also provided evidence for abnormalities in ERP face processing as captured by the flat affect to emotional expressions in the ADHD group relative to controls. The finding of lower levels in both omega-3/6 fatty acids in children/adolescents with ADHD compared to controls supports existing literature but warrants replicating in larger sample sizes. Above all, the findings reported in this clinical project represent a sizeable contribution to the field of fatty acids in ADHD and healthy control children. Furthermore, they unveil for the first time a relationship between emotion processing, conduct disorder related CU traits and LC-PUFA in child/adolescent groups with ADHD which have significant implications for the future. For example, in the development of treatment based interventions, in the identification of potential risk factors and more importantly in the prevention of symptoms which may be detrimental to both the individual and society.

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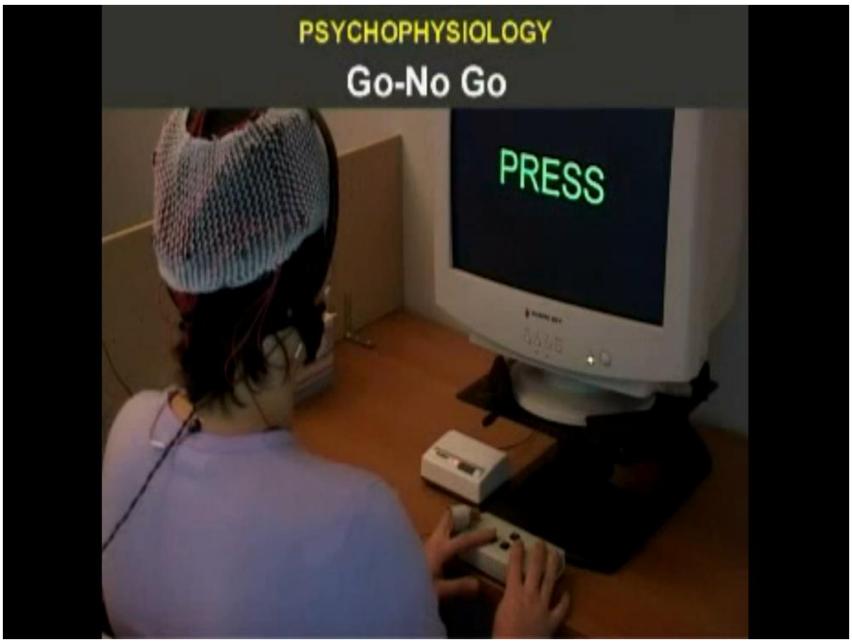
Appendices

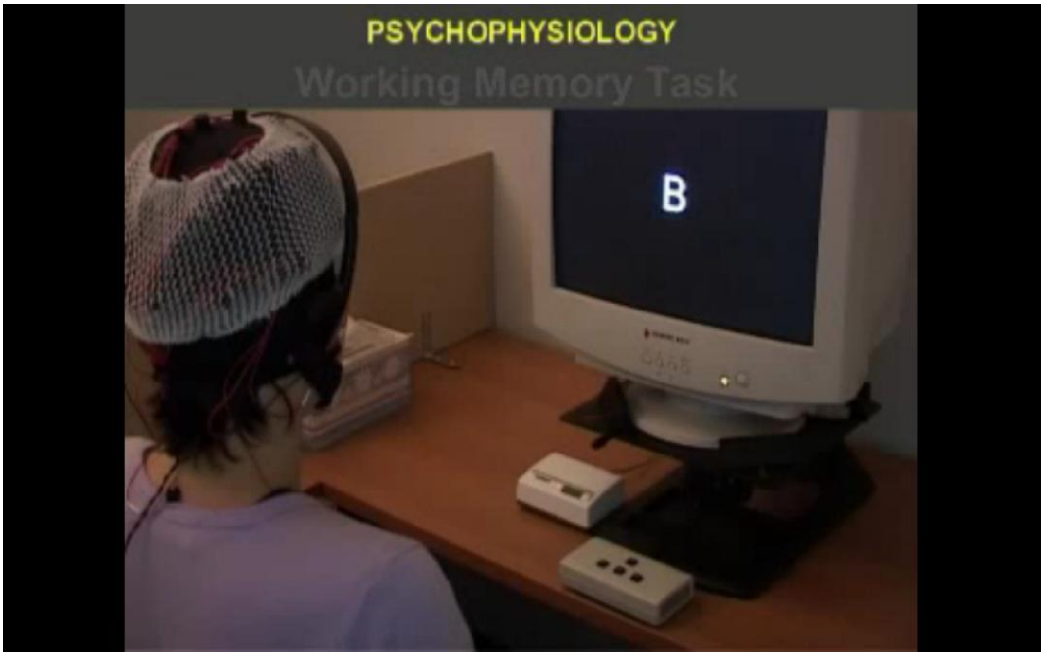
Appendix 1: Conflict response inhibition (Go/NoGo) task[12]



The word, 'PRESS' was flashed alternately in green and red colours. The Go stimuli ("PRESS" in green letters appeared in the centre of a black screen) and the NoGo stimuli ("PRESS" in red in the centre of a black screen) were presented to participants for 500 milliseconds (ms) with an interstimulus interval (ISI) of 1143 ms. In order to build up a prepotent-response tendency. Seven blocks of 6 No/Go each (i.e., in total 42 NoGo stimuli = 25% or trials) were interspersed between 7 blocks of Go stimuli (i.e., 3 blocks containing 12, two 18, one 24 and one 30 Go stimuli (i.e., in total 126 stimuli, 75% of trials). All participants received standardised visual and auditory instructions to tap a response box by pressing left and right buttons simultaneously upon the appearance of the Go stimuli and to withhold response upon the appearance of the NoGo stimuli. All participants completed a quick practice test prior to performance to ensure that the task instructions were understood. In total the test lasted 7 minutes. The dependent variables of the Go process of the task were mean reaction time and omission errors to Go stimuli. The dependent variables for the inhibitory process were commission errors (responses to NoGo stimuli).

[12] Pictures with permission from the Brain Resource Company : **www.BRAINnet.net**

Appendix 2: Continuous Performance Task



This task consists of a series of letters (B, C, D or G which are in white Arial font on a black background) presented to the participant on a computer screen for 500 milliseconds, separated by an interstimulus interval of 1 second. The participant is requested to press two buttons with the index finger of each hand to the target stimuli. The speed and accuracy of response are equally stressed in the task instructions. There are 125 stimuli presented in total. These include 85 non-target letters (i.e., background letters); 20 pseudo-randomly presented, "1-back", target letters (that is repetitions of the previous letter), and 20 distracter stimuli consisting of checkerboard patterns (black and white 1 x 1 cm checkerboards). The checkerboards were interleaved randomly with the letter stimuli. Participants were asked to ignore the "checkerboards". A brief practice was given at the start to ensure understanding of the task instructions. Participants were advised the test would last for 8 minutes. This task measures the processes involved in the orienting reflex, categorisation, contextual updating, sustained attention and working memory. Behavioural measures include reaction time to targets, errors of omission and errors of commission.

Appendix 3: Emotion Processing Task

EEG data were recorded during an emotion perception task containing 48 grey scale stimuli of facial expressions. The stimuli represented 3-D facial expressions of happiness, sadness, anger, fear and disgust relative to neutral faces and were chosen from a standandised set of stimuli (Gur, Sara, Hagendoorn, Marom, Hughett et al., 2002). The stimuli were made up of eight different individuals representing each expression. The images were tailored for orientation (i.e., so that the eyes of each image were at the central horizontal in all cases) and equivalent luminance. A maximum of 192 stimuli (8 different individuals representing each expression recurred four times) were shown pseudo-randomly under both covert (to measure non-conscious, automatic processing) and overt (to measure controlled processing) conditions.

In the covert condition, the facial expressions were shown for 10 ms, followed by a neutral mask for 150 ms. An ISI of 1,100 ms between target and mask was used to make sure that the total length of stimulus plus ISI was the same across conditions (1, 267 ms). The duration of each stimulus was 500 ms, with ISI of 767 ms. The mask was spatially offset somewhat (randomly, 1 degree in the course of the four diagonals) to control for the possible perceptual priming effects (in other words, the perceptual difference due to the arrangement of fear-neutral opposed to neutral-neutral target-mask pairs). Participants were advised to focus on each face in preparation for post-test questions to ensure that attention was paid.

Appendix 4: Statement of Authorship

Some of the blood data from the ADHD participants reported in Chapter's 8-11 of this PhD thesis was collected during the MAAFA trial and is reported also in the PhD thesis of Dr Toshiko Matsudaira but in a different context. Specifically, this PhD reports blood data from a proportion of the MAAFA trial participants to assess relationships with the EEG/ERP data which is not reported in the thesis of Dr Matsudaira. The data from the EEG recordings belonging to the ADHD participants only reported in Studies 1A, 1B, 1C and 2A, 2B and 2C were collected during the MAAFA trial. During the MAAFA trial, I also assisted in the collection of this data alongside colleagues: Almira Ibrahimovic, Dr Matsudaira and Dr Sumich.