This electronic thesis or dissertation has been downloaded from the King's Research Portal at https://kclpure.kcl.ac.uk/portal/

The Neurocognitive Correlates of Co-occurring Anxiety in Children at Increased Familial Risk for Autism Spectrum Disorder

Milosavljevic, Bosiljka

Awarding institution:
King's College London

The copyright of this thesis rests with the author and no quotation from it or information derived from it may be published without proper acknowledgement.

END USER LICENCE AGREEMENT

Unless another licence is stated on the immediately following page this work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International licence. https://creativecommons.org/licenses/by-nc-nd/4.0/

You are free to copy, distribute and transmit the work

Under the following conditions:

- Attribution: You must attribute the work in the manner specified by the author (but not in any way that suggests that they endorse you or your use of the work).
- Non Commercial: You may not use this work for commercial purposes.
- No Derivative Works - You may not alter, transform, or build upon this work.

Any of these conditions can be waived if you receive permission from the author. Your fair dealings and other rights are in no way affected by the above.

Take down policy

If you believe that this document breaches copyright please contact librarypure@kcl.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

Download date: 12. Jan. 2025

The Neurocognitive Correlates of Co-occurring Anxiety in Children at Increased Familial Risk for Autism Spectrum Disorder

---

Bosiljka Milosavljevic

Institute of Psychiatry, Psychology and Neuroscience

This thesis is submitted to King's College London for the degree of Doctor of Philosophy

2016

Declaration

The data presented in this thesis were collected as part of the British Autism Study of Infant Siblings (BASIS). This study is a large, collaborative project between King's College London and Birkbeck College. I was involved in the follow-up of the phase 1 cohort at the 7-year visit (BASIS-7). I joined the study during the planning stage and contributed a questionnaire measure of anxiety symptoms and an experimental measure of threat bias (presented in Chapter 5). Subsequently, I was involved in recruitment of families, testing of all the participants, as well as processing and analysing data. Measures of temperament, ASD symptoms and developmental level at the 7-, 14-, 24- and 36-month visits used in Chapter 6 were collected by the BASIS team prior to my joining the study. However, I obtained a data/collaboration agreement with the PIs to use this data in my thesis.

A journal article based on the data and information on the threat bias paradigm presented in Chapter 5, on which I am listed as first author, has been published in the *Journal of Autism and Developmental Disorders.*

Milosavljevic, B., Shephard, E., Happé, F. G., Johnson, M. H., & Charman, T. (2017). Anxiety and attentional bias to threat in children at increased familial risk for autism spectrum disorder. *Journal of Autism and Developmental Disorders*, 1-14. doi:10.1007/s10803-016-3012-1

Acknowledgements

I would like to thank my supervisors, Professor Tony Charman and Professor Francesca Happé, for giving me the opportunity to work with them on this incredible study. Their encouragement and guidance over the past 3 years has made this a truly great experience. I have learned so much from you both and could not have hoped for better mentors. I would also like to acknowledge my funders, the Medical Research Council, UK for supporting of my PhD.

I am also very grateful to Dr. Elizabeth Shephard (Lizzie) for teaching me so much and always being there when I needed help or support or just coffee and a chat. I appreciate all the time you spent showing me how to do so many different things, from programming my anxiety task to processing EEG data. It has been such a great experience working with you and I'll never forget all the experiences we had spending weekends together testing!

I am thankful to the incredible families that took part in the BASIS study. Their enthusiasm and dedication to our project has made all of this this possible.

I would also like to acknowledge Professor Jenny Yiend for helping me develop the emotional spatial cueing task. I truly appreciate the time you spent answering my questions and sharing your expertise with me.

I must also acknowledge Anushka Sathiyakeerthy for assisting me in the systematic literature search that was used for the literature review in Chapter 4. I am very grateful for all of your hard work and the care you took to find the relevant literature.

I must say a big thank you to the current and past members of the Autism and Development Team (ADT). Mutluhan Ersoy, Tessel Bazelmans, Julian Tillman, Alex Hendry, Lauren Taylor, Erica Salomone, Isobel Gammer, Antonia San Jose Caceres, Celeste Cheung, Eleanor Janega, Mary Agyapong, Peter Sloan and Sabira Habib – you have been such wonderful people to work with! I will miss our lunches, outings and chats. I'll always be grateful for working with such supportive people who are always willing to help. And you have taught me to make Cappuccino, which I am eternally grateful for! In particular, I would like to thank Greg Pasco and Karen Ashwood for teaching me to administer and code the diagnostic assessments. I really appreciate all the time you took sitting in on my assessments, double coding my videos and answering my many, many questions!

I would like to say a big thank you to my parents, Branko and Mirjana, and my brother Radomir for inspiring me and making me believe that I could achieve whatever I set my mind to. I'm also very grateful to all my friends who have been with me during this journey, keeping my spirits high.

And of course, I have to thank Tobias Hartung for always believing in me, supporting me and never letting me give up – I couldn't have done this without you!

Abstract

Autism Spectrum Disorder (ASD) is a heritable neurodevelopmental condition. In addition to the core symptoms, numerous physical and mental health issues commonly co-occur with ASD, notably anxiety disorders. Despite its high prevalence, the nature of anxiety within ASD remains poorly understood. This thesis investigated the prevalence, neurocognitive correlates and longitudinal predictors of co-occurring anxiety in children at familial high-risk for ASD (HR, *n*=42) and low-risk controls (LR, *n*=37) aged 6-8 years. The HR group was divided into those who met diagnostic criteria for ASD (HR-ASD, *n*=15) and those who did not (HR-non ASD, *n*=27).

This thesis had three broad aims. Primarily, the prevalence of co-occurring anxiety and its association to the core symptoms of ASD was investigated in the HR and LR groups using both parent- and self-report. A further aim was to investigate whether the cognitive correlates of anxiety observed in non-ASD populations (such as increased attentional bias to threat) were also present in the HR-ASD and HR-non ASD groups. The final aim was to examine whether dysregulated temperament (high levels of Negative Affect and low Effortful Control) in infancy and toddlerhood predicted anxiety symptoms in middle childhood in the HR and LR groups.

The HR-ASD group had high levels of parent-reported anxiety, which were associated with the core symptoms of ASD. However, they did not exhibit enhanced bias to threatening stimuli. On the other hand, the HR-non ASD group had somewhat elevated anxiety on specific subscales but did manifest heightened attentional bias to threat. Finally, Negative Affect at the age of 7 months was associated with anxiety at 6-

8 years in all groups. Taken together, these findings suggest that anxiety is highly prevalent in children at high-risk for ASD, but that there may be differential neurocognitive correlates among high-risk children who develop ASD and those who do not.

Table of Contents

Declaration **2**

Acknowledgements **3**

Abstract **5**

List of Tables **17**

List of Figures **19**

List of Acronyms **20**

Chapter 1
General introduction to prospective longitudinal methodology and its application to studying anxiety within Autism Spectrum Disorder **25**

1.1 Introduction **25**

1.2 Operationalisation, characteristics and aetiology of ASD **28**

1.3 Prospective longitudinal methodology to study the development of mental health difficulties **31**

1.3.1 Retrospective and prospective designs to study the development of ASD ... 31

1.3.2 Findings from prospective longitudinal studies in infants at-risk for ASD ... 33

1.4 The prevalence and manifestation of anxiety among individuals with ASD 36

1.5 Prevalence and manifestation of anxiety among relatives of individuals with ASD **42**

1.6 The neurocognitive correlates and longitudinal predictors of anxiety in non-ASD populations **44**

1.6.1 Cognitive theory of anxiety in non-ASD populations 45

1.6.2 Early risk factors and longitudinal predictors of anxiety in non-ASD populations 50

1.7 Neurocognitive correlates and risk factors for anxiety in ASD 53

1.7.1 Cognitive and neural mechanisms associated with anxiety in ASD 54

1.7.2 Longitudinal predictors of anxiety in ASD 58

1.8 Conclusion and aims of thesis 58

Chapter 2
Description of the British Autism Study of Infant Siblings, participant characterisation and general methods 61

2.1 Introduction 61

2.2 Method 62

2.2.1 Participants 62

2.2.2 Measures of ASD symptomatology, adaptive and cognitive functioning 64

2.2.2.1 Measures of ASD symptomatology 65

2.2.2.2 Measures of cognitive skills, adaptive functioning and language ability 68

2.2.3 Assignment to ASD outcome group 69

2.2.3.1 Assignment to ASD outcome group at 36 months 70

2.2.3.2 Assignment to ASD outcome group at 7 years 70

2.2.4 Statistical analyses 71

2.3 Results 72

2.3.1 Diagnostic outcome at the 7-year visit 72

2.3.2 Measures of ASD symptomatology at the 7-year visit 73

2.3.3 Measures of cognitive skills, adaptive functioning and language at 7-years 75

2.4 Discussion **76**

Chapter 3
Parent and Self-Reported Anxiety and its Manifestation in Children at High Familial Risk for Autism Spectrum Disorder **78**

3.1 Introduction **78**

3.1.1 Agreement of parent and child reports on anxiety symptoms in non-ASD populations 80

3.1.2 Child and parent agreement of anxiety symptoms among children with ASD 82

3.1.3 Selecting appropriate measures of anxiety for children with ASD 83

3.1.4 Association between co-occurring anxiety, core ASD symptoms and cognitive/adaptive functioning 86

3.1.5 Sex differences in anxiety symptoms 88

3.1.6 Aims and hypotheses 89

3.2 Method **90**

3.2.1 Measures 90

3.2.1.1 The Spence Children's Anxiety Scale 90

3.2.2 Statistical analyses 91

3.2.2.1 Group differences in anxiety symptoms 91

3.2.2.2 Psychometric properties and inter-rater agreement on SCAS-P and SCAS-C 92

3.2.2.3 Association between anxiety, core symptoms of ASD, cognitive functioning and language ability 92

3.3 Results **94**

3.3.1 Parent- and self-reported anxiety symptoms 94

3.3.2 Psychometric properties and inter-rater agreement on SCAS-P and SCAS-C 97

3.3.3 Association between anxiety, ASD symptoms, cognitive functioning and language 99

3.4 Discussion 102

3.4.1 Prevalence of co-occurring anxiety 103

3.4.2 Inter-rater agreement and psychometric properties 106

3.4.3 Association between anxiety, ASD symptoms and cognitive functioning.. 107

3.4.4 Strengths, limitations and implications for future work 109

Chapter 4
Review of threat bias paradigms used to assess cognitive mechanisms associated with anxiety in young children 113

4.1 Introduction 113

4.1.1 Cognitive mechanisms associated with anxiety 114

4.1.2 Threat bias in childhood anxiety 117

4.1.3 Challenges to evaluating anxiety and cognitive bias in early childhood..... 119

4.1.4 Threat bias and temperament in early development 120

4.1.5 Aims of the present review 121

4.2 Method 122

4.2.1 Search Strategy 123

4.2.2 Inclusion and Exclusion criteria 124

4.2.3 Data Extraction 124

4.3 Results 125

4.3.1 Search results 125

4.3.2 Data extraction 126

4.3.3 Measure used and task characteristics 126

4.3.4 Age differences on task performance 132

4.3.5 Anxiety measures 132

4.4 Discussion 139

4.4.1 Summary 139

4.4.2 Paradigms to measure association between threat bias and anxiety in early childhood 140

4.4.3 Methodological considerations for applying threat bias paradigms to test children with ASD 145

4.4.3.1 Measuring Reaction Time (RT) in Children with ASD 145

4.4.3.2 Measuring threat bias in individuals with ASD and reduced cognitive functioning 147

4.4.3.3 Ethical considerations 149

4.4.4 Conclusion 149

Chapter 5

Anxiety and attentional bias to threat in children at increased familial risk for Autism Spectrum Disorder 151

5.1 Introduction 151

5.1.1 Attentional bias to threat and anxiety 152

5.1.1.1 Distinct components of attention measured in threat bias tasks 153
5.1.1.2 Association between threat bias and anxiety among individuals with ASD 154

5.1.2 Social and non-social threat stimuli 155

5.1.3 The present study 157

5.2 Method 159

5.2.1 Emotional Spatial Cueing task 159
5.2.1.1 Stimuli 160
5.2.1.2 Procedure 161

5.2.2 Measures of anxiety, ASD severity and cognitive functioning 163
5.2.2.1 Anxiety symptoms 163
5.2.2.2 Measure of ASD severity 163
5.2.2.3 Measure of cognitive functioning 164

5.2.3 Statistical analyses 165
5.2.3.1 Group differences in threat bias 165
5.2.3.2 Association between threat bias and anxiety 166

5.3 Results 167

5.3.1 Preparation of RT data 167

5.3.2 Group differences in threat bias 168

5.3.3 Association between threat bias and anxiety symptoms 171

5.4 Discussion 175

5.4.1 Attentional bias to threat in children at high-risk for ASD 176

5.4.2 Threat bias, anxiety symptoms and ASD severity 178

5.4.3 Comparison between parent- and self-report anxiety symptoms and threat bias 180

5.4.4 Strengths, limitations and implications for future research 181

Chapter 6

Dysregulated temperament in infancy and toddlerhood among children at high familial risk for ASD and its association with anxiety symptoms in middle childhood 184

6.1 Introduction 184

6.1.1 The construct of temperament 185

6.1.2 The association between temperament and psychopathology 188

6.1.3 Temperamental dimensions and the development of anxiety disorders 189

6.1.3.1 The taxonomy of Negative Affect and its association to anxiety disorders 190

6.1.3.2 The association between Effortful Control and anxiety 192

6.1.4 Temperament among infants at high-risk for ASD 193

6.1.5 Heightened Negative Affect as an early marker of anxiety in children with ASD 195

6.1.6 Aims and hypotheses 195

6.2 Method 197

6.2.1. Temperament measures 197

6.2.1.1 Inconsistency in factor affiliation across temperament scales 204

6.2.2 Measures of ASD severity, developmental level and anxiety 205

6.2.2.1 Measures of ASD severity 205
6.2.2.2 Measures of developmental level 206

6.2.3 Statistical analyses 207
6.2.3.1 Preparation of temperament data 207
6.2.3.2 Demographic characteristics, ASD severity and developmental level . 207
6.2.3.3 Group differences in temperament across time 208
6.2.3.4 Change in Negative Affect over time and its association with Effortful Control 208
6.2.3.5 The association between Negative Affect, Effortful Control and anxiety 209

6.3 Results 210

6.3.1 Demographic characteristics, ASD severity and cognitive functioning 210

6.3.2 Group differences on temperament factor scores 213

6.3.3 Changes in Negative Affect over time and its association with Effortful Control 217

6.3.4 Association between infant/toddler Negative Affect, Effortful Control and 7-year anxiety and ASD symptoms 218

6.4 Discussion 222

6.4.1 Temperamental characteristics of the HR and LR groups 222

6.4.2 Change in Negative Affect over time and its association to Effortful Control 225

6.4.3 The association between Negative Affect and Effortful Control in early development and anxiety symptoms during middle childhood 227

6.4.4 Strengths, limitations and implications for future work 230

Chapter 7
General Discussion **233**

7.1 Overview of background and aims of thesis **233**

7.2 Summary of main findings **235**

7.2.1 The prevalence of anxiety symptoms among high-risk children and their association with the core ASD symptoms 235

7.2.2 The association between threat bias and anxiety among children at high familial risk for ASD 237

7.2.2.1 Review to identify suitable threat bias tasks for children aged 6-8 years 237

7.2.2.2 Emotional spatial cueing task 238

7.2.3 The association between dysregulated temperament in infancy/toddlerhood and anxiety symptoms in middle childhood 240

7.3 Implications for research and clinical practice **241**

7.3.1 Implications for high-risk research in ASD 242

7.3.2 Implications for research examining co-occurring anxiety within ASD 243

7.3.3 Clinical implications 244

7.4 Limitations **245**

7.5 Targets for future research **248**

7.6 Conclusion **251**

Appendices **252**

Appendix 1: Summary of clinical measures using HR-ASD, HR-Atypical, HR-Typical and LR groups **252**

Appendix 2: Anxiety prevalence when the HR-non ASD group is split into the HR-Atypical and HR-Typically Developing 254

Appendix 3: Results of pilot study of the Emotional Spatial Cueing task 256

Appendix 4: Prevalence of anxiety, when co-varying for FSIQ..... 258

Appendix 5: Additional temperament analyses 260

List of Tables

Table 1: Overview of clinical measures administered to the HR and LR groups..... 69

Table 2: Demographic characteristics of children in each group..... 73

Table 3: Summary of ASD severity scores for each group..... 74

Table 4: Summary of cognitive ability, adaptive functioning and language scores 76

Table 5: SCAS-P mean scores and group differences for each group 96

Table 6: SCAS-C mean scores and group differences for each group..... 97

Table 7: Intra-class correlations for SCAS-P and SCAS-C scores in HR and LR groups 98

Table 8: Pearson correlation coefficients for the associations between SCAS-P, SCAS-C and measures of ASD symptoms, adaptive functioning and language ability 100

Table 9: Summary of study designs and participant characteristics of records included in review..... 134

Table 10: Scores on the threat bias task in the HR-ASD, HR-non ASD and LR groups 168

Table 11: First-order Pearson correlation coefficients showing the association between threat bias indices, SCAS-P, SCAS-C, SRS-2 t-score and WASI-II FSQI 173

Table 12: Names and definitions of each dimensions measured on the IBQ-R, ECBQ and CBQ..... 200

Table 13: Summary of factor affiliation on each version of the temperament scales (IBQ-R, ECBQ, CBQ) 202

Table 14: Demographic characteristics, ASD severity and cognitive functioning scores in the HR-ASD, HR-non ASD and LR groups at 7, 14, 24 and 36 months..... 211

Table 15: Summary of the temperamental factor scores at each visit for the HR-ASD, HR-non ASD and LR groups 215

Table 16: Correlation coefficients showing association between SCAS-P total score and Negative Affect, Effortful Control and MSEL scores at visits 1-4 220

Table 17: Summary of clinical scores of HR-ASD, HR-Atyp, HR-TD and LR groups 253

Table 18: SCAS-P and SCAS-C scores for the HR-ASD, HR-Atyp, HR-TD and LR groups 254

Table 19: Group differences in 36 month CBQ scores using original factor structure 261

List of Figures

Figure 1. Scatter plot showing association between SCAS-P and SCQ total scores in the HR group 102

Figure 2. Flow-chart summarising stages of the systematic search 125

Figure 3. Sequence of events in a congruent trial (left) and an incongruent (right) trial of the Emotional Spatial Cueing task 162

Figure 4. Threat engagement indices in the HR-ASD, HR-non ASD and LR groups. 170

Figure 5. Threat disengagement indices in the HR-ASD, HR-non ASD and LR groups. 171

Figure 6. Association between the threat-positive engagement index and SCAS-P total score, with data points marked by group (HR-ASD, HR-non ASD and LR) 174

Figure 7. Association between the threat-positive engagement index and SCAS-c total score, with data points marked by group (HR-ASD, HR-non ASD and LR) 175

Figure 8. Mean Negative Affect scores for the HR and LR groups at each visit 217

Figure 9. Association between Negative Affect and Effortful Control, with the HR and LR group scores marked. 218

Figure 10. Scatter plot showing the association between 7-month Negative Affect and SCAS-P total scores with HR-ASD, HR-non ASD and LR groups marked. 221

Figure 11. Child and Adult reaction times in congruent (left) and incongruent (right) trials on the Emotional Spatial Cueing task. 257

List of Acronyms

ABC	Adaptive Behavior Composite
ACC	Anterior Cingulate Cortex
ACT	Acceptance and Commitment Therapy
ADHD	Attention Deficit Hyperactivity Disorder
ADI-R	Autism Diagnostic Interview-Revised
ADIS-C	Anxiety Disorders Interview Schedule-Child Report
ADIS-P	Anxiety Disorders Interview Schedule-Parent Report
ADOS	Autism Diagnostic Observation Schedule
ANX-DOS	Anxiety Dimensional Observation Scale
AOSI	Autism Observation Schedule for Infants
ASD	Autism Spectrum Disorder
BAP	Broader Autism Phenotype
BASIS	British Autism Study of Infant Siblings
BI	Behavioural Inhibition
CASI	Child and Adolescent Symptom Inventory

CBCL	Child Behavior Checklist
CBQ	Child Behavior Questionnaire
CBT	Cognitive Behaviour Therapy
CELF	Clinical Evaluation of Language Fundamentals
CSS	Calibrated Severity Score
DAWBA	Development and Wellbeing Assessment
DSM	Diagnostic and Statistical Manual
ECBQ	Early Childhood Behavior Questionnaire
EEG	Electroencelograph
ELC	Early Learning Composite
ERP	Event Related Potential
FSIQ	Full Scale Intelligence Quotient
GEE	Generalized Estimating Equation
HR	High Risk
IAPS	International Affective Picture System
IBQ-R	Infant Behavior Questionnaire - Revised

ICD	International Statistical Classification of Diseases and Related Health Problems
IQ	Intelligence Quotient
JA	Joint Attention
K-SADS-PL	Kiddie-Schedule for Schizophrenia and Affective Disorders Present and Lifetime version
LLP	Late Positive Potential
LPFC	Lateral Prefrontal Cortex
LR	Low Risk
MASC-C	Multidimensional Anxiety Scale for Children - Child Report
MASC-P	Multidimensional Anxiety Scale for Children - Parent Report
MS	Milliseconds
MSEL	Mullen Scales of Early Learning
NHS	National Health Service
NYLS	New York Longitudinal Study
OCD	Obsessive Compulsive Disorder

ODD	Oppositional Defiant Disorder
PAPA	Preschool Age Psychiatric Assessment
PARS	Paediatric Anxiety Rating Scale
PDD	Pervasive Developmental Disorer
PDD-NOS	Pervasive Developmental Disorer-Not Otherwise Specified
PRI	Perceptual Reasoning Index
RCADS	Revised Child Anxiety and Depression Scale
RMCAS	Revised Children's Manifest Anxiety Scale
RRB	Restricted and Repetitive Behaviour
RT	Reaction Time
SA	Social Affect
SCARED	Screen for Child Anxiety Related Disorders
SCAS-C	Spence Children's Anxiety Scale - Self Report
SCAS-P	Spence Children's Anxiety Scale - Parent Report
SCQ	Social Communication Questionnaire
SD	Standard Deviation

SE	Standard Error
SLI	Specific Language Impairment
SOA	Stimulus Onset Asynchrony
SOR	Sensory Over Responsivity
SRS	Social Responsiveness Scale
SS	Standard Scores
TD	Typically Developing
VCI	Verbal Comprehension Index
WASI	Wechsler Abbreviated Scales of Intelligence
WS	Williams Syndrome

Chapter 1
General introduction to prospective longitudinal methodology and its application to studying anxiety within Autism Spectrum Disorder

1.1 Introduction

Autism Spectrum Disorder (ASD) is a heritable neurodevelopmental disorder that is characterised by a set of core symptoms, namely atypicalities in social interaction, communication, the presence of restricted and repetitive patterns of behaviour (American Psychological Association, 2013). There is evidence of increased familial risk for ASD, as higher prevalence has been observed among family members of individuals with a clinical diagnosis than in the general population (e.g. Constantino, Zhang, Frazier, Abbacchi, & Law, 2010). Within the general population, the prevalence of ASD is reported to be approximately 1% (Baird et al., 2006). However, within families, rates are much higher; studies examining siblings of children with ASD report that in ~10% of families, an additional sibling has a clinical diagnosis of autism. Additionally, among 'non-diagnosed' siblings, up to 20% actually meet diagnostic criteria for ASD or exhibit elevated subclinical traits of ASD and/or language atypicalities associated with autism (Charman et al., 2016; Messinger et al., 2013; Ozonoff et al., 2011). Taken together, these findings suggest that there is substantial familial risk for ASD.

A clinical diagnosis of ASD is rarely made before the age of 24 months (e.g. Valicenti-McDermott, Hottinger, Seijo, & Shulman, 2012), limiting the opportunity to identify atypicalities in early neurocognitive functioning that may lead to the

development of ASD. Prospective longitudinal designs use the basis of increased familial risk for ASD to identify infants that are at high-risk for the condition (due to having an older sibling with a community clinical diagnosis) and examine the emergence of ASD in early development.

In addition to the core symptoms of ASD, individuals with the condition present with numerous mental health issues, among the most notable being anxiety disorders (Salazar et al., 2015; Simonoff et al., 2008). The prevalence of co-occurring anxiety among young people with ASD has been reported to be up to 84% (White, Oswald, Ollendick, & Scahill, 2009) and can cause substantial impairment to daily life. There is also evidence of increased anxiety symptoms among first-degree relatives of individuals with ASD (Lainhart, 2009). More recent research (Hallett, Ronald, et al., 2013) suggests that anxiety is particularly elevated among family members who themselves have ASD or manifest aspects of the Broader Autism Phenotype (BAP), subclinical ASD traits observed in family members (Bolton et al., 1994).

Despite the high prevalence of co-occurring anxiety among individuals with ASD and their relatives, there is presently a dearth in research examining the neurocognitive correlates of anxiety in this population. Such research is highly relevant as the aetiology of this high overlap remains poorly understood, thus limiting effective diagnosis and treatment options. At present, it remains unclear whether the presence of co-occurring anxiety symptoms represents a true comorbidity (the presence of two distinct disorders in an individual) or if it is an artefact of symptom overlap and challenges in self- and caregiver-report (Wood & Gadow, 2010). An alternative, and likely, possibility is that the difficulties in daily life that result from ASD, or from

living with an individual who has ASD, lead to the experience of heightened anxiety (Shivers, Deisenroth, & Taylor, 2013). Further research is needed to examine how the neural and cognitive processes commonly associated with anxiety in non-ASD populations map on to reports of anxiety symptoms within ASD.

Using a prospective longitudinal design of siblings at high-risk for ASD provides a unique opportunity to address multiple issues currently unresolved in research on co-occurring anxiety in ASD populations. Firstly, this design enables the comparison of the manifestation and neurocognitive correlates of anxiety symptoms among high-risk siblings who themselves meet diagnostic criteria for ASD and those who do not. It also provides the opportunity to examine the association between subclinical traits of ASD and anxiety severity. Finally, and perhaps most importantly, a prospective longitudinal design allows for the examination of the early predictors and developmental trajectories of anxiety symptoms among infants at high-risk for ASD. The present thesis aims to use a prospective longitudinal design to examine the neurocognitive correlates and longitudinal predictors of co-occurring anxiety in high-risk siblings during middle childhood (aged 6-8 years), who have been studied since infancy.

The aims of this chapter are to describe the prevalence and manifestation of anxiety symptoms among individuals with ASD and their family members. Furthermore, the cognitive mechanisms and longitudinal predictors associated with anxiety in non-ASD populations will be reviewed to provide a basis for the measurement of the neurocognitive correlates that will be examined in this thesis. I will also give evidence of the extent to which these mechanisms have been studied among

individuals with ASD and how the use of a prospective longitudinal design can help fill gaps in current literature.

1.2 Operationalisation, characteristics and aetiology of ASD

The concept of autism was first noted over a century ago by Bleuler (1950), who used the term 'autistic thinking' to describe the egocentric way of thinking that was observed among individuals with Schizophrenia. Autism was later described in detail by Kanner (1943), who provided an account of the behaviours that were observed among eleven children who he deemed to have "inborn autistic disturbances of affective contact" (pg. 250). Kanner (1943) emphasised that all these children exhibited extreme seclusion from social contact, unusual interests and a preoccupation with objects, an obsessive desire for the maintenance of sameness, and atypical language ability (e.g. delayed onset of speech, presence of echolalia). Shortly after Kanner's account of autism was published, Asperger (1944) described a group of children who manifested very similar symptoms, including atypical social interaction and unusual interests. However, Asperger's description included an account of the heterogeneous language skills and motor coordination abilities also observed among these children.

Autism was first formally operationalised in the third edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-3; American Psychiatric Association, 1980). This operationalisation was heavily influenced by the seminal work of Rutter (1978) and characterised autism as a set of symptoms involving atypical social and communicative development, insistence on sameness and early onset (prior to 30 months of age). Over the past three decades, revisions of the DSM have included

various adjustments to the operationalisation of autism. Prior to the publication of DSM-5 (American Psychological Association, 2013), ASD was considered an umbrella term describing a set of neurodevelopmental conditions, including autistic disorder, Asperger's syndrome, Pervasive Developmental Disorder Not Otherwise Specified (PPD-NOS), among others (American Psychiatric Association, 1994). The most recent edition, DSM-5 (American Psychological Association, 2013), has removed the different subtypes and describes ASD as a single disorder, with markedly varying severity, that is characterised by atypical social communication and the presence of restricted and repetitive behaviours. In addition, sensory processing atypicalities, such as sensory hyper- and hypo-sensitivity, as well as unusual interests in the sensory features of objects, have been included. Finally, the instead of allocating individuals to various sub-groups of ASD, the DSM-5 uses *specifiers* to denote the severity of the core symptoms, language and intellectual ability, age of onset and concurrent conditions (Lai, Lombardo, Chakrabarti, & Baron-Cohen, 2013).

Despite being characterised as a single condition, there is vast heterogeneity in symptom presentation, functional level, and cognitive and linguistic abilities among individuals with ASD (Lai, Lombardo, & Baron-Cohen, 2013). There is a high prevalence of intellectual disability (~45%) among individuals with ASD and ~30% are reported to have experienced regression (the loss of previously acquired skills) in functional ability (Barger, Campbell, & McDonough, 2013; Fombonne, Quirke, & Hagen, 2011; Lai, Lombardo, & Baron-Cohen, 2013). Linguistic ability is also highly varied among individuals with ASD, with some exhibiting clinically normal language while others have atypicalities in language development and production (Boucher,

2012). Furthermore, there are a vast array of conditions that co-occur with ASD, ranging from genetic disorders (e.g. Fragile X, Tuberous Sclerosis), medical conditions (e.g. Epilepsy), and other mental health or emotional difficulties (Lai, Lombardo, & Baron-Cohen, 2013; Salazar et al., 2015; Simonoff et al., 2008).

Given the vast heterogeneity and complex nature of ASD, identifying the aetiological pathways of the condition has been challenging. It is widely accepted that ASD is a neurobiological condition with strong genetic underpinnings. Evidence from twin research reports that concordance of autism and broader ASD traits is substantially higher among monozygotic twin pairs than dizygotic twin pairs (Bailey et al., 1995; Hallmayer, Cleveland, Torres, & et al., 2011). There is also evidence to suggest that some of the genetic components associated with ASD are also associated with other conditions that commonly co-occur with ASD (Ronald & Hoekstra, 2011). Certain environmental factors, such as older parental age and exposure to harmful substances have also been implicated in the development of ASD (Lai, Lombardo, & Baron-Cohen, 2013).

In addition to the wide heterogeneity of ASD, the nosology and diagnostic criteria of the condition are also constantly undergoing revisions. For example, a vast body of research suggests that ASD is more highly prevalent in males than females (Lai, Lombardo, Pasco, Ruigrok, Wheelwright, Sadek, & Baron-Cohen, 2011). However, recent research suggests that this could be because ASD manifests somewhat differently in females and that contemporary diagnostic criteria do not capture this well enough (Lai, Lombardo, Pasco, Ruigrok, Wheelwright, Sadek, Chakrabarti, et al., 2011; Lai, Lombardo, Auyeung, Chakrabarti, & Baron-Cohen, 2015). Furthermore,

while ASD is most commonly diagnosed at ~36 months of age, there is growing evidence that symptoms of the condition are observable much earlier, within the first year of life (Jones, Gliga, Bedford, Charman, & Johnson, 2014). While there has been substantial improvement in the characterisation of ASD since its first description, further research attempting to understand the vast heterogeneity of ASD and prevalence of co-occurring conditions is currently highly relevant.

1.3 Prospective longitudinal methodology to study the development of mental health difficulties

Investigation aimed at identifying early predictors and developmental pathways of psychiatric conditions is highly relevant to both research and clinical practice. Elucidating the atypical developmental pathways associated with psychiatric outcomes has high scientific value, as it improves understanding of human development. From a clinical perspective, such investigation may help in the earlier identification of psychiatric difficulties in children and, consequently, the development of targeted interventions earlier in life. Targeted interventions that can be administered during a critical time of neural plasticity (typically infancy/early childhood) may result in more lasting, lifelong changes (Cramer et al., 2011).

1.3.1 Retrospective and prospective designs to study the development of ASD

For many years, information about the early development of ASD was obtained through retrospective research. Retrospective studies most commonly rely on parent-report, where parents are asked to describe their initial concerns about the child's development or relevant behaviours in early life, through interviews and questionnaires

(e.g. De Giacomo & Fombonne, 1998). Additionally, researchers analyse pre-diagnostic home videos of children with ASD to monitor for the presence of behaviours associated with autism in early development (e.g. Mars, Mauk, & Dowrick, 1998). Such research has provided valuable insights into the early behavioural atypicalities in children with ASD and suggested that symptoms could be observed as early as the first 12-18 months of life (Gillberg et al., 1990; Stone, Hoffman, Lewis, & Ousley, 1994). Atypicalities in Joint Attention (JA) behaviours, such as reduced eye contact, gaze monitoring and response to name (e.g. Adrien et al., 1993; Werner, Dawson, Osterling, & Dinno, 2000), as well as affect sharing (Osterling & Dawson, 1994) were considered to be the most robust early signs of ASD (Charman, 2003). There was also some evidence of heightened sensory processing atypicalities and increased restricted and repetitive behaviours during early development (Rogers, 2000).

While retrospective research has the advantage of being both cost and time efficient (Euser, Zoccali, Jager, & Dekker, 2009), there are multiple important limitations to this methodology. Accounts of early behaviour are prone to errors and distortions of recall, particularly of events that occurred many years ago, and parents may endorse early behaviours that are consistent with their child's diagnosis (Zwaigenbaum et al., 2007). Furthermore, parents may be less aware of subtler social and communicative atypicalities, which may be more readily observable through systematic assessment by a trained clinician (Stone, Hoffman, Lewis, & Ousley, 1994). Analysis of home videos captures behaviour within a limited context and may not be representative of the child's conduct in daily life. Finally, retrospective methodology does not allow for experimental measurement of neural and cognitive functioning during early development.

To account for these limitations, research has turned to the use of prospective longitudinal methodology, where individuals are tested longitudinally prior to receiving a diagnosis (Euser, Zoccali, Jager, & Dekker, 2009). One way of doing this is through population studies, where ASD symptoms are studied in infants drawn from the general population, who are followed-up several years later when a diagnosis can be made (e.g. Baird et al., 2000). Because the population prevalence of ASD is generally low, such research requires substantially large sample sizes and is rarely feasible to conduct. On the other hand, genetic heritability of ASD has been found to be moderate (Hallmayer, Cleveland, Torres, & et al., 2011) and recurrence rates in families are higher than in the general population (Constantino, Zhang, Frazier, Abbacchi, & Law, 2010). Therefore, studying infants that are at familial risk for developing ASD provides a unique opportunity to prospectively study young children before the age of diagnosis, but reduces the need for very large sample sizes that would be required in population studies.

1.3.2 Findings from prospective longitudinal studies in infants at-risk for ASD

Over the past decade, prospective longitudinal studies of infants at high-risk for ASD, most frequently younger siblings of a proband with a clinical diagnosis, have elucidated the behavioural, cognitive and neural manifestations of ASD in early development (Elsabbagh & Johnson, 2010; Jones, Gliga, Bedford, Charman, & Johnson, 2014). Prospective studies following infants to the age of 36-months report that ~20% meet diagnostic criteria for ASD, suggesting that recurrence may be higher than previously thought (Messinger et al., 2015; Ozonoff et al., 2011). Among HR siblings who do not meet diagnostic criteria at 36-months, a further ~20% exhibit

heightened subclinical ASD symptoms and/or reduced developmental and language ability (Messinger et al., 2013; Messinger et al., 2015). In a study of the manifestation of BAP in early development, Ozonoff et al. (2014) report that over a quarter of high-risk infants are not considered 'typically developing' by the age of 36 months and are distinguishable from low-risk controls on multiple domains as early 12 months of age. Among these children, one third manifest aspects of BAP (increased socio-communicative atypicalities), while others show signs of developmental delay, reduced speech and language ability, and attention-deficit/hyperactivity disorder (ADHD). Furthermore, Schwichtenberg et al. (2013) report heightened anxiety, depression and aggression scores among high risk children who do not have ASD at 36-months.

Prospective longitudinal studies have advanced their scope to not only report on emerging symptoms in high-risk infants, but to also characterise the developmental mechanisms that lead to these symptoms (for review see Jones, Gliga, Bedford, Charman, & Johnson, 2014). Potential early markers of ASD have been identified that distinguish high-risk infants who meet diagnostic criteria for ASD at 36-months from both high-risk infants that have non-ASD outcomes and low-risk controls. For example, difficulty in flexibly disengaging attention (Elsabbagh et al., 2013; Zwaigenbaum et al., 2005), attenuated neural sensitivity to eye gaze (Elsabbagh et al., 2012), and reduced partaking in joint attention (Bedford et al., 2012; Sullivan et al., 2007; Yoder, Stone, Walden, & Malesa, 2009) in the first year of life are among the atypicalities reported, which are characteristic of infants that later go on to meet diagnostic criteria for ASD. Additionally, Bedford et al. (2014) report that there is an additive effect of infant social and non-social attentional atypicalities, whereby they have independent and cumulative effects on the severity of ASD symptoms at 36-months of age. This finding contradicts

previous models suggesting a single underlying cause of ASD (Baron-Cohen, Leslie, & Frith, 1985; Dawson, Meltzoff, Osterling, Rinaldi, & Brown, 1998). Early temperamental differences, including heightened Negative Affect and reduced Effortful Control, are also present among those who go on to meet diagnostic criteria for ASD (Clifford et al., 2013; Del Rosario, Gillespie-Lynch, Johnson, Sigman, & Hutman, 2014; Garon et al., 2009) and complement earlier retrospective studies where parental report indicated that children with ASD had more 'difficult' temperament in infancy (Watson et al., 2007).

Recent studies have begun to report on outcomes of high-risk children beyond the age of 36 months and into middle childhood. One study has reported on the stability of diagnostic outcome (Brian et al., 2015), suggesting that ~12% of high-risk children who did not meet diagnostic criteria for ASD at 36 months did so at age 7-12 years, while very few (only 1) lost diagnosis. Other studies have largely focused on the developmental outcomes of children who did not meet diagnostic criteria for ASD. Multiple reports of language difficulties, particularly within the "semantic-pragmatic" domain, and reduced cognitive functioning in non-ASD high-risk siblings have emerged from school-age follow-ups (Drumm, Bryson, Zwaigenbaum, & Brian, 2015; Gamliel, Yirmiya, Jaffe, Manor, & Sigman, 2009; Warren et al., 2012). Miller et al. (2015) are among the only studies to report on other forms of psychopathology among high-risk siblings at school-age (5-9 years). Almost 40% of 'non-ASD' siblings met criteria for some type of clinical concern, compared to only 13% in the low-risk group. These clinical concerns included elevated ASD symptomatology, as well as higher prevalence of internalising (anxiety/depression) and externalising (e.g. ADHD) symptoms.

Taken together, prospective longitudinal studies suggest higher recurrence of ASD among high-risk siblings, as well as increased prevalence of language and cognitive functioning difficulties, and vulnerability for psychopathology among those who do not have ASD. However, there are multiple important limitations to the evidence provided. Firstly, school-aged follow-ups have thus far had a very narrow scope in terms of the type of psychopathology/outcome measured, focusing primarily on cognitive functioning and language development. Additionally, these studies have largely explored outcomes in high-risk infants without ASD and excluded those with ASD from analyses. Such an approach has provided a useful account of BAP-related features, but does not enhance knowledge of outcomes that may be specific to those who have ASD. This is particularly relevant to the study of the development of co-occurring psychopathology within ASD, as elevated rates of difficulties such as ADHD and anxiety are expected to largely occur among children who have ASD (Salazar et al., 2015; Simonoff et al., 2008). Finally, no research to date has examined the longitudinal neurocognitive predictors of co-occurring psychopathology in high-risk children. This is of particular relevance as such studies have a unique opportunity to examine developmental pathways and early factors that place children with ASD at heightened risk for developing a range of co-occurring mental health problems that cause significant impairment throughout life.

1.4 The prevalence and manifestation of anxiety among individuals with ASD

Comorbid diagnoses of psychiatric conditions are a frequent occurrence; under DSM-IV criteria (American Psychiatric Association, 1994), over 20% of individuals with a diagnosis of one condition were diagnosed with an additional mental health

problem, most commonly mood or substance misuse issues (Kessler, Chiu, Demler, & Walters, 2005). The prevalence of co-occurring mental health problems in both adults and children with ASD is highly elevated, with reports suggesting that up to 70% of individuals with ASD have one additional psychiatric diagnosis and 40% have two or more (Mattila et al., 2010; Simonoff et al., 2008). Among these, anxiety, ADHD and Oppositional Defiant Disorder (ODD) are the most frequently observed (Simonoff et al., 2008). The study of comorbidity is of great importance as individuals that present with co-occurring conditions generally have more severe symptoms, greater functional impairment and prolonged illness course compared to those with a single diagnosis (e.g. Cerda, Sagdeo, & Galea, 2008).

Co-occurring anxiety symptoms are highly prevalent and cause significant impairment among individuals with ASD (Simonoff et al., 2008; White, Oswald, Ollendick, & Scahill, 2009). A systematic review of 40 studies reports that the estimated prevalence of anxiety in ASD ranges from 11-84% (White, Oswald, Ollendick, & Scahill, 2009). The high rate of variability likely reflects difficulty in self and caregiver reports of anxiety symptoms in this population (Mazefsky, Kao, & Oswald, 2011). The type of anxiety reported among young people with ASD ranges across various subtypes (Simonoff et al., 2008). A meta-analysis including over 2000 young people, below the age of 18, with ASD suggests that specific phobias (29.8%), OCD (17.4%) and social anxiety disorder (16.6%) are the most common forms of anxiety in this population (van Steensel, Bogels, & Perrin, 2011). These forms of anxiety are also highly prevalent among preschool and young children (4-9 years) with ASD (Salazar et al., 2015). However, other studies report that separation anxiety is more common and highly prevalent among children with ASD, below the age of 12,

(Gillott, Furniss, & Walter, 2001), while social anxiety is more readily reported in adolescent samples (Bellini, 2004). These findings are consistent with the reported prevalence of separation and social anxiety in non-ASD populations, suggesting that they are more frequently observable in children and adolescents, respectively (Weems & Costa, 2005). Among young people who meet diagnostic criteria for an anxiety disorder, symptoms typically persist from childhood into adulthood (Pine, Cohen, Gurley, Brook, & Ma, 1998). While no study to date has examined the progression of anxiety symptoms from childhood into adulthood among individuals with ASD, research suggests that anxiety symptoms are highly elevated among adults with ASD, as they are in children (Joshi et al., 2013).

While the estimated prevalence of anxiety differs across studies, the general pattern of findings consistently suggests that anxiety symptoms are elevated among individuals with ASD compared to the general population. On the contrary, the nature and function of anxiety among individuals with ASD remains poorly understood (Kerns & Kendall, 2012; Wood & Gadow, 2010). It is unclear whether the co-occurrence of anxiety within ASD presents a true comorbidity, where it could be classified as a separate disorder that manifests in the same way as it does in its monomorbid form (Kerns & Kendall, 2012; Wood & Gadow, 2010).

Research to date largely contradicts the notion that the presentation of anxiety within ASD is a true comorbidity. Firstly, the nature and underlying mechanisms of anxious symptoms among individuals with ASD differ from those observed among non-ASD individuals with anxiety disorders. For example, the fears and phobias among children with ASD have substantially distinct content from those reported in non-ASD

individuals with specific phobias. Children with ASD are reported to exhibit extreme distress to unusual or commonplace objects (e.g. water, mechanical objects, beards) or sensory input, rather than objects that pose actual threat or danger (Evans, Canavera, Kleinpeter, Maccubbin, & Taga, 2005; Ozsivadjian, Hollocks, Southcott, Absoud, & Holmes, 2016). Furthermore, while individuals with ASD exhibit behaviours associated with social phobia, such as avoidance of or distress during social encounters, they do not appear to be concerned with negative social evaluation, which is a core feature of social phobia (Leyfer et al., 2006). Kerns et al. (2014) examined the prevalence of 'traditional' and 'atypical' anxiety among young people with ASD. Traditional anxiety included the measurement of anxious symptoms that are reported among non-ASD individuals with anxiety disorders. Atypical anxiety was characterised by stressors and fears frequently observed among individuals with ASD (e.g. atypical phobias), which are not considered diagnostic features of anxiety disorders. The findings suggest that 17% of the ASD sample presented with traditional anxiety, 15% with atypical anxiety and 31% with both.

Additionally, a multitude of studies have reported significant associations between anxiety and the core symptoms of ASD. The interplay between anxious and ASD-like symptoms were first observed by Kanner (1943), who noted that children with ASD showed an anxious desire for the maintenance of sameness. These observations have been supported by more recent empirical evidence suggesting that anxiety is associated with RRBs over and above social and communication difficulties, among children with ASD (Hallett, Lecavalier, et al., 2013; Sukhodolsky et al., 2008). This association has been narrowed down further to suggest that, among RRBs, anxiety is specifically associated with the factor of insistence on sameness and not with sensory

motor behaviours (Lidstone et al., 2014; Rodgers, Glod, Connolly, & McConachie, 2012). Furthermore, there is evidence of an association between anxiety and sensory over-responsivity (SOR) among children with ASD (Ben-Sasson et al., 2008; Liss, Saulnier, Fein, & Kinsbourne, 2006; Mazurek et al., 2013). SOR in toddlers with ASD has been shown to predict anxiety in later childhood over and above other difficulties, such as ASD severity or cognitive functioning, but anxiety does not predict sensory modulation later in life (Green, Ben-Sasson, Soto, & Carter, 2012). Recent evidence also suggests that RRBs may serve as a mechanism to reduce the heightened arousal and anxiety that results from sensory sensitivity, among individuals with ASD (Lidstone et al., 2014; Wigham, Rodgers, South, McConachie, & Freeston, 2014). The findings from studies reporting on these associations will be discussed in greater detail in Chapter 3.

On the contrary, the association between the atypicalities in social cognition that characterise ASD and anxiety symptoms is less clear. While research suggests that reduced social competence is associated with elevated anxiety symptoms among individuals with ASD (Bellini, 2004), atypicalities in social cognition (such as reduced Theory of Mind ability) have not been found to contribute to anxiety symptoms (Hollocks et al., 2014). Both individuals with ASD and those with social phobia (who do not have ASD) have reduced accuracy on tasks that measure mentalising ability (Hezel & McNally, 2014; Hoogenhout & Malcolm-Smith, 2014). However, individuals with social phobia have poorer accuracy because they attribute more hostile or negative mental states to characters or objects, while misattributions among individuals with ASD are more random (Hezel & McNally, 2014). Furthermore, in a study measuring

physiological responding to a psychosocial stress test, adolescent males with ASD and co-occurring anxiety exhibited attenuated heart and cortisol responses to the stressor compared to ASD participants without anxiety and TD controls (Hollocks, Howlin, Papadopoulos, Khondoker, & Simonoff, 2014). These results are unexpected, as individuals with anxiety disorders present with heightened physiological arousal when faced with stressors (e.g. Schmitz, Kramer, Tuschen-Caffier, Heinrichs, & Blechert, 2011). However, the authors suggest that the reduced arousal may have been observed because individuals with ASD are less likely to anticipate social stressors, limiting their ability to plan and use effective coping strategies.

Finally, multiple studies have examined the association between intellectual functioning and co-occurring anxiety symptoms among individuals with ASD. It has been suggested that anxiety is more highly prevalent among individuals with ASD who have normative IQ, compared to those with intellectual disability (Hallett, Lecavalier, et al., 2013; Sukhodolsky et al., 2008). However, there is contradictory evidence, suggesting that, among ASD individuals with normative cognitive ability, anxiety is associated with lower IQ (van Steensel, Bogels, & Perrin, 2011). Findings from these studies will also be discussed in greater detail in Chapter 3.

In summary, there is substantial evidence to suggest that co-occurring anxiety in ASD differs qualitatively to anxiety in non-ASD populations. Within community samples, there is only a modest association between sub-clinical internalising symptoms and ASD traits (Hallett, Ronald, & Happé, 2009). As outlined above among individuals with ASD, there is high overlap between the core features of ASD (particularly RRBs) and anxiety symptoms. It is plausible that symptoms of ASD and

the stressors associated with these symptoms contribute to internalising difficulties (Hallett, Ronald, & Happé, 2009). However, the high symptom overlap could also mean that it is difficult to truly differentiate symptoms of anxiety from the core features of ASD (Wood & Gadow, 2010). Although, to counter this, Hallett, Ronald, Rijsdijk, and Happé (2010) suggest that ASD traits in childhood predict later internalising symptoms, while internalising symptoms predict ASD traits to a lesser degree, implying a degree of causality in this association. It is, therefore, important to understand the mechanisms that underlie these symptoms to be able to differentiate them.

1.5 Prevalence and manifestation of anxiety among relatives of individuals with ASD

Numerous forms of psychopathology have been reported among first degree relatives of individuals with ASD (Hodge, Hoffman, & Sweeney, 2011). As outlined above, studies examining infants at high-risk for ASD report a number of ASD-related, cognitive, internalising and externalising difficulties among younger siblings who do not themselves meet diagnostic criteria for ASD (e.g. Miller et al., 2015). Beyond the scope of high-risk studies, there has been a substantial body of research examining adjustment and psychosocial functioning among “unaffected” siblings. Heightened prevalence of internalising symptoms has been reported in both parents and siblings of children with ASD, and is higher than among relatives of children with other disabilities, such as Down syndrome (Bolton, Pickles, Murphy, & Rutter, 1998; Fisman et al., 1996). These early findings have important implications as there is much greater familial risk for both clinical level symptoms and sub-clinical traits in ASD than in

Down syndrome (Seltzer, Abbeduto, Krauss, Greenberg, & Swe, 2004). Therefore, these studies allude to the possibility that internalising symptoms may also be part of the broader symptom manifestation of ASD in family members.

However, other research has contradicted these findings, suggesting that siblings of children with ASD go on to have normative development without adjustment difficulties (e.g. Kaminsky & Dewey, 2002). To account for the discrepant findings, more recent research has proposed that increased adjustment difficulties may be specific to siblings who themselves have a disability or features of the BAP (Benson & Karlof, 2008; Meyer, Ingersoll, & Hambrick, 2011). A number of environmental factors also play an important role in the psychological wellbeing of siblings of children with ASD. For example, more severe challenging behaviours in the ASD proband, maternal depression, and family stress have all been associated with worse outcomes for siblings (Hastings & Petalas, 2014; Petalas et al., 2012). Orsmond and Seltzer (2009) suggest that the presence of internalising symptoms among siblings may, at least partially, be accounted for by a diathesis-stress model, where characteristics such as the BAP interact with familial stressors to place these children at increased risk for maladjustment.

While general adjustment and internalising difficulties have been studied in relatives, the prevalence of anxiety disorders in particular is not fully clear. Some studies report elevated anxiety among both parents and siblings across multiple subtypes, including generalised anxiety, social phobia, panic disorder, specific phobia and OCD (Mazefsky, Folstein, & Lainhart, 2008; Piven et al., 1991). Others suggest that the prevalence of anxiety in family members of children with ASD is equivalent to

those observed in community samples (Bolton, Pickles, Murphy, & Rutter, 1998). However, Bolton, Pickles, Murphy, and Rutter (1998) examined OCD separately from other anxiety disorders and suggested that the prevalence of OCD is elevated in family members. Hallett, Ronald, et al. (2013) were the first to examine the association between anxiety symptoms and BAP characteristics among twins of children with ASD. Anxiety symptoms were compared across probands with ASD, twins who manifested aspects of the BAP, TD twins and controls. Anxiety was most highly prevalent among probands with ASD and twins with BAP. Within this sample of probands and twins, anxiety symptoms were significantly associated with the core symptoms of ASD, particularly RRBs. Additionally, Tick et al. (2015) suggest that, among twins of children with ASD, genetic overlap accounts almost fully for the presence of internalising symptoms, while environmental factors do not.

1.6 The neurocognitive correlates and longitudinal predictors of anxiety in non-ASD populations

One of the approaches used in this thesis will be to examine whether the neurocognitive correlates and infant/early childhood predictors of anxiety in non-ASD populations are also associated with anxiety among children at high-risk for ASD. This approach may contribute to better understanding whether anxiety within ASD functions similarly as it does in other populations. In the next two sections, I will provide an overview of the cognitive theories of anxiety disorders and the longitudinal predictors of anxiety that have been examined thus far.

1.6.1 Cognitive theory of anxiety in non-ASD populations

Prominent theories of anxiety disorders are centred on to contribution of biased cognitive mechanisms to the aetiology and maintenance of the condition (Beck & Clark, 1997; Beck, Emery, & Greenberg, 1985; Eysenck, 1992; e.g. Eysenck, Derakshan, Santos, & Calvo, 2007). Beck, Emery, and Greenberg (1985) proposed that anxiety is characterised by the presence of maladaptive cognitive schemas that predispose anxious individuals to biasedly interpret stimuli as threatening or dangerous. A range of stimuli, including both external events and internal thoughts and feelings, can activate these cognitive structures, endorsing the processing of schema-congruent information about the threat value of a stimulus, and interfere with positive information processing that is schema-incongruent (Beck, 1991; Beck & Clark, 1997; Beck, Emery, & Greenberg, 1985; Clark & Beck, 2010). Continued biased processing of information that contributes to the schema-congruent subjective experience of threat and vulnerability results in the development of negative automatic thoughts, images and memories that maintain an anxious state (Clark & Beck, 2010). Furthermore, individuals with more severe or clinical-level anxiety overestimate the presence of danger, in a manner that is inconsistent with reality, while those with less severe symptoms tend to have a more objective perception of threat (Beck & Clark, 1997).

Ellis (1984) formulated the ABC model of anxiety, which is complementary to Beck's schema theory. The model outlines that *Activating events (A)*, which are stressors or threatening stimuli, have *Consequences (C)* for an individual's emotional wellbeing, largely due to their *Beliefs (B)* about the negative value of these events.

Therefore, individuals with anxiety tend to interpret stressful life events as more harmful and have less perceived control, contributing to their anxious state.

Finally, Eysenck, Derakshan, Santos, and Calvo (2007) proposed the *Attentional Control* theory of anxiety, which is similar to Beck and Ellis's models in that suggests that individuals with anxiety have biased processing of threatening information, but focuses on how anxiety interferes with task performance. The model suggests that threatening stimuli, such as worrisome thoughts or external events, act on executive functioning systems to disrupt the balance between goal-directed, top-down information processing in favour of bottom-up sensory driven mechanisms. As a result, attentional resources are disproportionately allocated to the processing of threat-relevant stimuli, and interfere with performance on other tasks. The model further suggests that such a processing style results in reduced ability to inhibit responding or flexibly shift attention from the threatening stimulus. Essentially the individual places their cognitive resources into managing or avoiding the perceived stressor, thus preventing them from using more effective coping mechanisms that are derived from executive functions.

Cognitive theories of anxiety have received widespread support from experimental research, demonstrating that individuals with elevated anxiety have cognitive biases to threat. Experimental research has largely focused on attentional and interpretation biases that are associated with anxiety. Attentional bias is most commonly measured using paradigms that compare reaction times (RTs) to threatening and non-threatening stimuli. Such tasks demonstrate that individuals with elevated anxiety show faster RTs to threatening, compared to neutral, stimuli (Bar-Haim, Lamy,

Pergamin, Bakermans-Kranenburg, & van IJzendoorn, 2007). Research into the attentional control theory suggest that elevated anxiety is associated with reduced ability to inhibit responding to threatening stimuli (Coombes, Higgins, Gamble, Cauraugh, & Janelle, 2009). Furthermore, the presence of threat relevant stimuli is thought to have an impact on *performance efficiency* (the relationship between performance effectiveness and the use of resources), as the presence of threatening, task-irrelevant distractors, slows responding to task-relevant stimuli, in individuals with anxiety. Interpretation bias paradigms measure perception of ambiguous information, suggesting that individuals with anxiety interpret ambiguous scenarios as more negative and think that dangerous events are more likely to occur (Castillo & Leandro, 2010). These findings have been consistently reported in hundreds of studies in both adults and school-aged children (Dudeney, Sharpe, & Hunt, 2015). Furthermore, cognitive biases are not only present among individuals with clinical-level anxiety but have also been observed in participants with heightened sub-clinical anxiety from community samples (Fox, Russo, Bowles, & Dutton, 2001), albeit less consistently than in clinical samples (Bar-Haim, Lamy, Pergamin, Bakermans-Kranenburg, & van IJzendoorn, 2007).

There is also evidence of an association between anxiety and reduced executive functioning ability, even in the absence of threatening stimuli, particularly among individuals with OCD (Airaksinen, Larsson, & Forsell, 2005). Participants with OCD are reported to have reduced performance on paradigms assessing cognitive set-shifting (e.g. the Wisconsin Card Sorting Task) and inhibitory control (e.g. the go/no go task), especially inhibition of motor responses (for review see Olley, Malhi, & Sachdev, 2007). These cognitive atypicalities have been proposed as endophenotypic markers of

OCD and are thought to contribute to difficulty in regulating and disengaging from intrusive thoughts and from inhibiting performance of motor rituals (Chamberlain, Blackwell, Fineberg, Robbins, & Sahakian, 2005; Harsanyi et al., 2014). Evidence of reduced executive functioning ability in other anxiety disorders has been limited and less conclusive. However, there is some evidence suggesting reduced set-shifting ability among individuals with social phobia (Cohen et al., 1996; Fujii et al., 2013) and difficulty in performing tasks that require selective attention among participants with panic disorder (Lautenbacher, Spernal, & Krieg, 2002). There is also some evidence of reduced cognitive control ability among individuals with anxiety disorders (Paulus, 2015).

While the cognitive correlates of anxiety have been studied widely, there is also a growing body of research examining the neural mechanisms associated with anxiety and threat processing (Pergamin-Hight, Naim, Bakermans-Kranenburg, van, & Bar-Haim, 2015). Evidence from fMRI studies supports findings from cognitive research and suggests that there is an interplay between the neural networks associated with emotion processing and attentional regulation, including the amygdala and prefrontal cortex (Bishop, 2008). The amygdala is involved in the automatic processing of emotional information and serves an adaptive function by promoting the detection of danger (LeDoux, 2000). Individuals with anxiety exhibit hypersensitive amygdala activation in response to threatening stimuli (Etkin & Wager, 2007) and the degree of amygdala activation to threat is significantly associated with anxiety severity (Monk, Telzer, Mogg, & et al., 2008). Furthermore, increased connectivity between the amygdala and dorsomedial prefrontal cortex (which has a role in endorsing attentional allocation to relevant stimuli) has also been observed among individuals with

heightened anxiety, suggesting that once threat has been detected, there is also higher neural activity endorsing attentional allocation to the threatening stimulus (Robinson, Charney, Overstreet, Vytal, & Grillon, 2012). Regions of the prefrontal cortex associated with attentional control, particularly the lateral prefrontal cortex (LPFC) and the anterior cingulate cortex (ACC), are thought to be involved in later emotion regulation processes (Bishop, Duncan, Brett, & Lawrence, 2004) by modulating amygdala activation in response to threat (Pine, Helfinstein, Bar-Haim, Nelson, & Fox, 2009; Quirk & Mueller, 2008). Attenuated LPFC and ACC activity was found to be associated with increased threat bias (Monk et al., 2006) and reduced ability to inhibit responding to threatening stimuli (Bishop, Duncan, Brett, & Lawrence, 2004; Forster, Nunez Elizalde, Castle, & Bishop, 2015).

Taken together, there is consistent evidence suggesting that cognitive processing atypicalities, namely hypersensitivity to threatening stimuli and reduced ability to regulate responding to stress, contribute to the aetiology of anxiety disorders. Prevalent clinical interventions for anxiety, such as Cognitive Behaviour Therapy (CBT) and Acceptance and Commitment Therapy (ACT), work to help the individual recognise and restructure the cognitive distortions associated with anxiety and form adaptive strategies to coping with stress (Arch & Craske, 2008). Newer interventions, such as Cognitive Bias Modification, train individuals to regulate attention to threatening stimuli and to generate positive interpretations of ambiguous scenarios (MacLeod & Mathews, 2012). Such methods have shown promise in reducing anxiety symptoms among both adults and children (Lau, 2013).

1.6.2 Early risk factors and longitudinal predictors of anxiety in non-ASD populations

Childhood and adolescence are considered to be the core risk phases for the development of anxiety disorders (Beesdo, Knappe, & Pine, 2009). Many adult anxiety disorders have an onset in childhood (Kessler et al., 2005) and children that have one type of anxiety disorder are likely to develop new psychiatric conditions later in life, primarily another anxiety disorder (Last, Perrin, Hersen, & Kazdin, 1996). Because anxiety disorders begin to emerge early in life, it is important to characterise early behavioural and neurocognitive risk factors of these conditions to assist in earlier diagnosis and intervention. Among the earliest predictors that have been examined, temperament and heightened reactivity to novelty in infancy and toddlerhood are thought to be indicators of anxiety disorders in childhood and adolescence (Pahl, Barrett, & Gullo, 2012). However, environmental factors, such as parental psychopathology, insecure attachment and family stress also contribute substantially to child anxiety symptoms (Bogels & Brechman-Toussaint, 2006; Pahl, Barrett, & Gullo, 2012). Taken together, childhood predictors of anxiety suggest a diathesis-stress model, where the early predispositions, such as inhibited temperament, interact with environmental stressors, resulting in heightened anxious symptoms (Brozina & Abela, 2006).

Several distinct, but interrelated, aspects of temperament have been identified as early childhood predictors of anxiety disorders (Lonigan, Vasey, Phillips, & Hazen, 2004). Temperament describes how an individual engages with their surroundings and consists of reactive and self-regulatory traits, including Negative Affect, which is

characterised by high levels of distress and displeasure in engaging with the environment, and Effortful Control, the ability to regulate responses to stimuli (Rothbart, 2007; Thomas & Chess, 1977). Additionally, the related construct of Behavioural Inhibition (BI) has widely been studied as a risk-factor for the development of anxiety, particularly social withdrawal and social phobia (Fox, Henderson, Marshall, Nichols, & Ghera, 2005). BI refers to the tendency of some children to become distressed or withdraw from novel situations, people or environments (Kagan, Reznick, & Snidman, 1987). While Negative Affect is typically measured using parent-report questionnaires (e.g. Rothbart, Ahadi, Hershey, & Fisher, 2001) and BI is often measured using observational techniques (Fox, Henderson, Rubin, Calkins, & Schmidt, 2001), there is great overlap among the two constructs, with both being characterised by displeasureable engagement with the environment (Lonigan, Vasey, Phillips, & Hazen, 2004).

In some respect, these early temperamental factors are analogous to the heightened threat bias and reduced attentional control mechanisms outlined in the cognitive models of anxiety (Beck & Clark, 1997; Eysenck, Derakshan, Santos, & Calvo, 2007). There is evidence of a direct association between emerging Negative Affect and BI in infancy/early childhood and the development of anxiety symptoms in toddlerhood (e.g. Gartstein et al., 2010; Lahat et al., 2014). However, not all children with negative temperament and inhibition go on to develop anxiety disorders. To account for this, developmental models have posited that Effortful Control serves to moderate the development of anxiety symptoms, whereby children with high Effortful Control are able to regulate their responses to distressing stimuli, while those low on

the trait cannot effectively exercise attentional control and are at heightened risk for anxiety (Lonigan, Vasey, Phillips, & Hazen, 2004).

There has been substantial empirical support for these models implicating early temperament as a risk factor for the development of anxiety. Dysregulated temperament within the first two years of life has been associated with heightened anxiety in adolescence (Schwartz, Snidman, & Kagan, 1999). Observational assessments of temperament support parent-report measures, suggesting that young children that are described by parents are more emotionally reactive or inhibited, exhibit fearful reactions and withdrawal when faced with novel objects or people (Rubin, Burgess, & Hastings, 2002). Furthermore, infants with high parent-reported Negative Affect exhibit heightened attentional bias to threatening stimuli, which is associated with anxiety in later childhood (Nakagawa & Sukigara, 2012). There is also evidence that infants with dysregulated temperament exhibit atypical visual attention, even in the absence of threatening stimuli. For example, 9-month old infants with high levels of BI exhibit reduced sustained attention and prolonged monitoring of novel stimuli, which is potentially analogous to hypervigilance and poorer attentional control (Perez-Edgar, McDermott, et al., 2010).

While the attentional and behavioural mechanisms associated with infant temperament have received substantial empirical support, the associated neural structures have not been widely studied. This is likely due to methodological challenges of performing brain imaging techniques with very young children. However, studies with adults and adolescents offer promising evidence to suggest that dysregulated temperament is associated with heightened amygdala and reduced anterior cingulate

cortex activity to novelty, particularly unfamiliar faces or pictures of individuals showing fearful facial expressions (Clauss, Cowan, & Blackford, 2011; Perez-Edgar et al., 2007). Furthermore, adults that were reported to have been highly behaviourally inhibited before the age of 2 years, also show heightened amygdala activity to unfamiliar faces in adulthood, suggesting infant temperament is predictive of adult reactivity to novelty (Schwartz, Wright, Shin, Kagan, & Rauch, 2003).

Taken together, research on early temperamental predictors parallels cognitive theories of anxiety. Heightened reactivity to threat (unfamiliarity for very young children) and reduced ability to regulate attention in infancy and toddlerhood are associated with increased anxiety later in life. These factors interact with environmental stressors and jointly contribute to the development of anxiety in later development.

1.7 Neurocognitive correlates and risk factors for anxiety in ASD

While there is a multitude of empirical evidence for the cognitive models that describe the underlying mechanisms of anxiety disorders, there has been very little investigation examining whether these same processes are associated with co-occurring anxiety in ASD. Despite this, there is a growing body of research that has begun to describe some of the cognitive manifestations and risk factors of ASD, beyond the association between anxiety and the core ASD features. There are certain features, including reduced executive functioning ability, heightened physiological arousal, and dysregulated temperament, which may place individuals with ASD and their first-degree relatives at increased risk for anxiety disorders.

1.7.1 Cognitive and neural mechanisms associated with anxiety in ASD

Firstly, reduced executive functioning abilities have been reported among individuals with ASD and, to a lesser extent, among their first-degree relatives (Hill, 2004; Hughes, Plumet, & Leboyer, 1999; Pellicano, 2012). Ability within the domains of cognitive flexibility, set shifting and inhibition in particular are reduced among individuals with ASD (Poljac & Bekkering, 2012). Set shifting refers to the ability to flexibly modify attention and behaviours to meet the demands of changing rules or situations (Miyake et al., 2000). Individuals with ASD generally exhibit perseverance of a particular response style, even when feedback indicates that the response is inaccurate (e.g. Maes, Eling, Wezenberg, Vissers, & Kan, 2011). They also have more difficulty performing tasks that require shifting attention from one type of response to another or tasks that require different modalities to make a response, such as a combination of auditory and visual cues (Reed & McCarthy, 2012). High-risk studies, including one examining the present cohort, report difficulty in flexibly disengaging attention among infants who go on to meet diagnostic criteria for ASD at 36 months (Elsabbagh et al., 2013). Individuals with ASD also have reduced performance on tasks measuring inhibitory control, such as the go/no go task, where participants are instructed to withhold responding upon the presentation of a particular stimulus (Solomon, Ozonoff, Cummings, & Carter, 2008; Uzefovsky, Allison, Smith, & Baron-Cohen, 2016).

Hollocks et al. (2014) were the first to report on the association between the executive functioning atypicalities outlined above and anxiety symptoms among individuals with ASD. Within the sample of adolescents with ASD, difficulties in

flexibly switching attention, set shifting and inhibitory control were all associated with symptoms of anxiety, but not depression. While these findings suggest that executive function has a unique association with anxiety, it is also important to note that difficulties within this domain are observed among individuals with other mental health conditions. For example, individuals with ADHD manifest challenges in inhibitory control (Woltering, Liu, Rokeach, & Tannock, 2013), while reduced cognitive flexibility is observed in eating disorders (Gillberg et al., 2010). As outlined above, among individuals with anxiety symptoms, executive functioning abilities become particularly disrupted in the presence of perceived threat or danger. However, it is possible that having pre-existing difficulties with certain domains of executive functioning may place individuals with ASD at risk of poorer self-regulation in distressing situations.

There is presently a dearth in empirical investigation of threat perception and anxiety among individuals with ASD. Findings from threat bias tasks in ASD samples will be reviewed in greater detail in Chapters 4 and 5. In summary, findings from studies examining attentional bias to threat have been inconclusive. Two studies found no association between parent- or self-reported anxiety symptoms and attentional allocation to threatening faces and words (Hollocks, Ozsivadjian, Matthews, Howlin, & Simonoff, 2013). White, Maddox, and Panneton (2015) found that adolescents with ASD and increased fear of negative social evaluation (a construct linked to social phobia) spend more time viewing angry and disgusted faces, than happy ones. Finally, Isomura, Ogawa, Shibasaki, and Masataka (2015) report that children with ASD, but without co-occurring clinical anxiety diagnoses, show delayed disengagement from non-social threatening images (snakes). Finally, one study found evidence of an

association between heightened interpretation bias and anxiety among young people with ASD (Hollocks, Pickles, Howlin, & Simonoff, 2016; Sharma, Woolfson, & Hunter, 2014). To date, threat bias has not been studied among relatives of individuals with ASD. More investigation is needed to better understand the root of these discrepant findings and to better characterise the cognitive correlates of anxiety among individuals with ASD and their relatives.

A number of studies have examined fear conditioning among individuals with ASD (Gaigg & Bowler, 2007; Top et al., 2016). Gaigg and Bowler (2007) report that individuals with ASD show similar levels of arousal when faced with both threatening and non-threatening cues. However, ASD participants did have a residual level of fear acquisition, suggesting that the equivalent responding to threat and non-threat cues was not due to a failure in acquiring a fear association. The authors propose, instead, that individuals with ASD have difficulty in fear discrimination. Top et al. (2016) report that individuals with ASD show similar amygdala activity to threat and safety cues during acquisition and decreased amygdala activity during extinction. These findings suggest that individuals with ASD may have difficulty distinguishing safety from threat, resulting in heightened levels of arousal and anxiety. However, in this study, amygdala activity was not associated with self-reported anxiety symptoms.

On a neural level, there also appear to be discrepancies in the mechanisms associated with anxiety disorders and with ASD. While hyperactive amygdala activity is thought to contribute to the maintenance of threat responses in anxiety disorders (e.g. LeDoux, 2000), reduced activity in the amygdala has been associated with socio-emotional atypicalities in ASD (Baron-Cohen et al., 2000; Pelphrey, Shultz, Hudac, &

Vander Wyk, 2011). In particular, social-motivation theories posit that activity in the network (which includes the amygdala) associated with social attention and reward are compromised in ASD (for review see Gaigg, 2012). As such, individuals with ASD experience less reward from social interactions and orient less to social stimuli, thereby limiting opportunities to learn socially relevant information. Contrary to this model, there is evidence that individuals with ASD exhibit heightened amygdala activity, similar to TD controls, when viewing emotional faces, which is associated to symptoms of social anxiety (Kleinhans et al., 2010; Monk, 2010). Recent research has aimed to consolidate these discrepant findings and suggests that individual differences in amygdala function among ASD samples contribute to both symptoms of ASD and anxiety (Herrington, Miller, Pandey, & Schultz, 2016; Kleinhans et al., 2016). A study examining the neural mechanisms of face processing among children with ASD suggests that reduced amygdala activity is observed only among those with low levels of anxiety. Furthermore, heightened amygdala activity was associated with higher levels of anxiety and reduced ASD severity. Kleinhans et al. (2016) suggests that, within the amygdala itself, specific nuclei and their connectivity to other areas are associated with social difficulties (e.g. the laterobasal subregion), while others are associated with anxiety symptoms (e.g. centromedial subregion, superficial subregion), among individuals with ASD.

Taken together, findings from cognitive and MRI studies suggest that the association between fear processing and anxiety within ASD is not as straightforward as it is in non-ASD populations. However, evidence suggests that individual differences in threat perception may contribute to anxiety symptoms in ASD. For example, there is promising evidence outlined above to suggest that some young people with ASD do

show attentional and interpretation biases and that amygdala activity is associated with anxiety in some individuals with ASD. This warrants further investigation and refining of experimental methods to further explore these associations. Furthermore, the association between fear processing and anxiety has not been studied among first-degree relatives of individuals with ASD.

1.7.2 Longitudinal predictors of anxiety in ASD

To date, there have been no published studies that have examined the developmental trajectories or early-childhood risk factors for co-occurring anxiety among individuals with ASD. However, high-risk studies suggest that there may be an overlap in the temperamental profiles of children who are at risk for ASD and anxiety. In particular, infants at high-risk for ASD are characterised as having dysregulated temperament in the first 2 years of life (Clifford et al., 2013; Garon et al., 2009; Zwaigenbaum et al., 2005). High-risk infants manifest heightened Negative Affect and reduced Effortful control, which have been implicated in the development of anxiety in non-ASD children. Furthermore, there is evidence that heightened Negative Affect is observed specifically in the infants who meet diagnostic criteria for ASD at 36 months (Clifford et al., 2013). However, the association between temperament and anxiety symptoms in children at high-risk for ASD have not yet been investigated.

1.8 Conclusion and aims of thesis

In summary, there is evidence of heightened anxiety among individuals with ASD and their first-degree relatives. However, the nature of anxiety symptoms in this population is not fully understood. Evidence suggests that anxiety may function

differently within ASD than in other populations, as it is associated with some of the core symptoms of ASD. However, this could also mean that the high prevalence of anxiety in ASD is due to an artefact of symptom overlap. It is, therefore, essential to characterise the mechanisms that underlie anxiety among individuals with ASD to be able to truly differentiate symptoms.

In this thesis, I will examine the prevalence of anxiety symptoms, associated cognitive correlates and longitudinal predictors among school-aged children at high familial risk for ASD. In chapter 3, I will examine parent- and self-reported anxiety symptoms and their association to the core features of ASD in the sample. In chapter 5, I will use a threat-bias task to examine whether children at high-risk for ASD manifest heightened attending to threatening stimuli and if this is associated with anxiety severity. I will also examine the longitudinal predictors of anxiety in this sample, by investigating the association between infant temperament and school-aged anxiety symptoms.

Overall, this aim of this thesis is to investigate anxiety symptoms among high-risk children and to examine whether the neurocognitive correlates associated with anxiety in non-ASD populations are also present among children with ASD and those with sub-clinical traits. To address this broad aim, I will investigate the following questions:

1. Are anxiety symptoms elevated among children at high-risk for ASD compared to low-risk controls? Given the literature, it can by hypothesised that anxiety

will be most highly elevated among high-risk children who meet diagnostic criteria for ASD.

2. Do children at high-risk for ASD exhibit heightened bias to threatening stimuli and is this associated with anxiety?
3. Do longitudinal predictors of anxiety observed in non-ASD populations also predict anxiety within ASD? To address this, I will examine the association between Negative Affect and Effortful Control in infancy and anxiety at 6-8 years of age.

Chapter 2
Description of the British Autism Study of Infant Siblings, participant characterisation and general methods

2.1 Introduction

As outlined in Chapter 1, only several high-risk studies have followed participants beyond the age of 36-months and into middle childhood. The studies that have conducted school-aged follow-ups (Brian et al., 2015; Drumm, Bryson, Zwaigenbaum, & Brian, 2015; Gamliel, Yirmiya, Jaffe, Manor, & Sigman, 2009; Miller et al., 2015; Warren et al., 2012) tended to exclude high-risk children with ASD from analyses and have focused on a somewhat narrow range of outcomes, such as cognitive and language development only. The aim of the present study was to conduct a middle childhood (age 6-8 years) follow-up of a cohort of children at-risk for ASD, who were tested in infancy and toddlerhood, and to include children with ASD in analyses. A variety of measures were used to assess ASD symptomatology, cognitive and language functioning, and the presence of additional psychopathology (e.g. Anxiety and ADHD). This thesis focuses specifically on anxiety symptoms and associated neurocognitive correlates. In subsequent chapters, anxiety symptoms and performance on experimental tasks will be compared across 3 outcome groups (HR-ASD, HR-non ASD and LR). Therefore, in this methodological chapter, I aim to describe the broader methodology of the study and the process by which participants were assigned to these groups.

In this chapter, I will give an overview of the British Autism Study of Infant Siblings (BASIS). All cross-sectional data used in this thesis was collected as part of the BASIS mid-childhood follow-up and the longitudinal data (used in Chapter 6) was collected at the infant and toddler visits. In particular, this chapter will focus on describing the clinical measures used and the process by which diagnostic decisions about children in the high-risk group were reached. I will also characterise the sample and provide information about ASD symptomatology, cognitive ability, language ability and adaptive functioning.

2.2 Method

2.2.1 Participants

One hundred and four infants were recruited from the British Autism Study of Infant Siblings (BASIS; www.basisnetwork.org). This is a prospective longitudinal study of infants at increased familial risk for ASD. At baseline, 54 infants (21 males, 33 females) at high-risk for ASD (HR) were recruited on the basis of having an older sibling (hereafter "proband") with a community clinical diagnosis of ASD (half-siblings in 4 cases). Fifty low-risk (LR) control infants (21 males, 29 females) with no family history of ASD and a typically developing older sibling were also recruited from the Birkbeck Centre for Brain and Cognitive Development volunteer database.

Presence of ASD in probands of the HR infants was confirmed by two expert clinicians (from the research team). The clinicians used parent-report of symptoms from the Development and Wellbeing Assessment (DAWBA; Goodman et al. 2000)[1]

and the Social Communication Questionnaire (SCQ; Rutter et al. 2003)[1]. The DAWBA is a web-based parent-report questionnaire, asking respondents to rate their child's symptoms and provide a narrative account of their behaviour. The SCQ is described in the Methods section (below). Parents were also asked to report on their family's medical history to screen for conditions related to ASD (e.g. Fragile X syndrome, Tuberous Sclerosis). None of the children, probands or extended family members were reported to have any relevant conditions. Parents of LR infants also provided family medical history, which were screened to confirm that the infants were born at full-term[2] and that there was no family history of ASD. Absence of ASD in older siblings of the LR infants was confirmed using the SCQ, with no child scoring above cut-off (≥15)[3].

The families participated in research visits at the ages of 7-months, 14-months, 24-months, 36-months and 6-8 years (hereafter the '7-year follow-up'). At the 7-year follow-up, 44 HR and 37 LR children took part. Two HR children did not complete research visits and their parents only completed questionnaires. In the absence of information from clinical measures, it was not possible to assign these children to an ASD outcome group. Therefore, these 2 children were excluded from analyses. The final sample consisted of 42 HR (15 males, 27 females) and 37 LR (15 males, 22 females) participants. The participants who were retained at the 7-year follow-up did not differ from non-retained participants on measures of ASD symptoms (ADOS, SRS,

---

[1] Five DAWBA and 5 SCQ missing

[2] One infant was not born at full-term

[3] One SCQ missing

SCQ, ADI-R), adaptive functioning (Vineland-II) or developmental level (MSEL), all *ps*≥.40.

At the 7-year follow-up, family medical histories were attained using parent-report. None of the children had ever been diagnosed with conditions relevant to ASD (as outlined above). Four HR children did have seizures in early childhood, these had occurred prior to the age of 5 years and had ceased in all cases. Five HR children had more than one sibling with a community clinical diagnosis of ASD and were from 'Multiplex families'. The remainder only had one older sibling with a community clinical diagnosis of ASD.

Ethical approval was obtained from the NHS National Research Ethics Service (NHS RES London REC 14/LO/0170). Parents provided written informed consent. Children provided written informed assent wherever possible given developmental level.

2.2.2 Measures of ASD symptomatology, adaptive and cognitive functioning

Various measures of ASD symptoms, adaptive and cognitive functioning were administered at each of the visits. Results from these assessments were reviewed by experienced researchers, who conducted the assessments, and the lead clinician at the 36-month and 7-year visits. Consensus best estimate research diagnoses were assigned to children in the HR group at 36-months and 7-years, as described below. Table 1 provides an outline of the clinical measures used at each visit and details about each measure are provided below.

2.2.2.1 Measures of ASD symptomatology

Autism Diagnostic Observation Schedule – Second Edition (ADOS-2; Lord et al., 2012) was administered at the **7-year visit**. ADOS-2 is a standardised, semi-structured observational assessment of ASD symptoms, focusing particularly on communication, social interaction, play and restricted and repetitive behaviours. Behaviours are coded on a scale of 0-2 or 0-3 (depending on the item), a score of 0 indicates typical behaviour and higher scores indicate increasingly severe ASD-type features. Pre-selected items constitute the final algorithm scores, from which two domains are derived – social affect and restricted and repetitive behaviours, as well as a total score. Age-normed cut-off scores are provided for the total score in each module, indicating values for 'autism spectrum' and 'autism'. Calibrated Severity Scores (CSS) for Social Affect (SA), Restricted and Repetitive Behaviours (RRB) and total score were computed and provide standardised ASD severity based on the module administered and the participant's age and verbal ability (Gotham et al. 2009; Hus et al. 2014). Within our sample, Module 3 was used for 73 children, Module 2 for one child, Module 1 for one child, and 3 LR controls did not complete the assessment.

The Autism Diagnostic Observation Schedule – Generic (ADOS-G; Lord et al., 2000) is an older version of the ADOS-2 assessment described above and was administered at the **24- and 36-month visits**. Similar to ADOS-2, the ADOS-G is a semi-structured observational assessment of ASD symptoms, focusing on language, gestures, eye-contact, creativity and repetitive or stereotyped behaviours. The ADOS-G also uses a 0-2 or 0-3 coding scheme (depending on the item), with higher scores indicating more severe ASD-type behaviours. Algorithm scores are calculated for

domains of social ability, communication, creativity and repetitive/stereotyped behaviours. Age-normed 'autism spectrum' and 'autism' cut-off scores are provided for the social and communication domains and children must score above threshold for autism spectrum/autism on both domains and the combined total score to meet criteria for ASD. ADOS-G scores were converted to ADOS-2 equivalent scores and Calibrated Severity Scores (CSS) for Social Affect (SA), Restricted and Repetitive Behaviours (RRB) and the total score were computed as described above (Gotham, Pickles, & Lord, 2009). At the 24-month visit, the ADOS-G was administered to children in the HR group only.

The Autism Observation Scale for Infants (AOSI; Bryson, Zwaigenbaum, McDermott, Rombough, & Brian, 2008) was administered at the **7- and 14-month visits**. AOSI is a semi-structured, observational assessment examining the emergence of ASD-related behaviours in infants aged 6-18 months. The assessment consists of 5 activities, each consisting of presses for specific behaviours, and 2 periods of free-play. The scale consists of 19 items and behaviours are coded on a scale of 0-2 or 0-3 (depending on the item), with 0 indicating typical responses and increasing scores denoting more severe ASD-like behaviours. A total score is derived by summing all the items and can range from 0-44.

Autism Diagnostic Interview-Revised (ADI-R; Lord, Rutter, & Couteur, 1994) was administered at the **24-month, 36-month and 7-year visits**. ADI-R is a standardised, semi-structured clinical interview that is administered to parents. The ADI-R provides a diagnostic algorithm for autism based on both ICD-10 (World Health Organisation, 1993) and DSM-IV (American Psychiatric Association, 1994) criteria.

The interview measures ASD symptoms across three domains: Reciprocal Social Interaction, Communication, Restricted, Repetitive and Stereotyped patterns of behaviour, as well as onset of symptoms. The ADI-R was administered only to children in the HR group at each visit.

Social Responsiveness Scale – Second Edition (SRS-2; Constantino, 2012) was administered at the **36-month and 7-year visits**. SRS-2 was used as a measure of the severity of social difficulties associated with ASD and was completed by parents. The questionnaire consists of 65 items, asking about the child's social difficulties over the last 6 months, with a yes/no response format. The measure provides a total score of ASD severity and scores can range from 0-65, with higher scores indicating greater severity of impairment. Age- and sex-normed T-scores (M=50; SD=10) were used in analyses.

The Social Communication Questionnaire (SCQ; Rutter, Bailey, & Lord, 2003) lifetime version was completed by parents at the **36-month and 7-year visits**. The SCQ was designed as a companion measure to the ADI-R and consequently parallels the interview closely. The questionnaire consists of 40 items and asks respondents to indicate whether their child engages in a variety of ASD-related behaviours (e.g. 'Has he/she ever gotten his/her pronouns mixed up?') and responses are recorded in a yes/no format. The total score ranges from 0-40 and a total of ≥15 indicates the presence of ASD. In addition to the total score, domain scores assessing Reciprocal Social Interaction, Communication and Restricted, Repetitive and Stereotyped patterns of Behaviour (RRB) can be calculated by summing specific items.

2.2.2.2 Measures of cognitive skills, adaptive functioning and language ability

The Wechsler Abbreviated Scales of Intelligence – Second Edition (WASI-II; Wechsler, 2011) was administered at the **7-year visit**. WASI-II was used as a measure of cognitive functioning. The test provides standardised, age-normed intelligence quotients (*M*=100; *SD*=15) for perceptual reasoning (PRI), verbal comprehension (VCI) and a full-scale IQ quotient (FSIQ). One child in the HR group was unable to complete this measure due to intellectual disability.

The Mullen Scales of Early Learning (MSEL; Mullen, 1995) scale were administered at the **7-, 14-, 24- and 36-month visits**. MSEL is a standardised assessment of motor and cognitive development from birth to 68 months. It assesses development across 5 domains, including gross motor skills, fine motor skills, visual reception, receptive language ability and expressive language ability. Items are scored on a scale of 0-5, with higher scores indicating higher levels of functioning. A total scaled score (*M*=100; *SD*=15), the Early Learning Composite (ELC), is computed based all subscales, except for the gross motor scale.

The Vineland Adaptive Behavior Scales – Second Edition (Vineland-II; Sparrow, Cicchetti, & Balla, 2005) were administered at **all visits**. Vineland-II is semi-structured interview and was used to assess current level of adaptive functioning. The Vineland evaluates an individual's personal and social abilities from birth to adulthood. Age-normed standard scores (*M*=100; *SD*=15) were derived for the domains of Communication, Daily Living Skills, Socialisation and Motor Skills. The measure also produces an overall Adaptive Behaviour Composite (ABC) standard score, which will be used in analyses.

The Clinical Evaluation of Language Fundamentals – Fourth Edition UK (CELF-4; Semel, Wiig, & Secord, 2006) was administered at the **7-year visit**. CELF-4 is a standardised instrument used to assess general language ability. Participants completed the subtests of *Concepts and Following Directions* and *Recalling Sentences*. These two subtests assess receptive and expressive language, respectively, and age-normed standard scores are provided for each domain (*M*=10, *SD*=3).

Table 1: Overview of clinical measures administered to the HR and LR groups

Measure	**7-months**		**14-months**		**24-months**		**36-months**		**7-years**	
	HR	**LR**	**HR**	**LR**	**HR**	**LR**	**HR**	**LR**	**HR**	**LR**
ADOS-2									√	√
ADOS-G					√		√	√		
AOSI	√	√	√	√						
ADI-R						√		√		√
SRS-2							√	√	√	√
SCQ							√	√	√	√
WASI-II									√	√
MSEL	√	√	√	√	√	√	√	√		
Vineland-II	√	√	√	√	√	√	√	√	√	√
CELF-4									√	√

ADOS-2 indicates Autism Diagnostic Observation Schedule – 2nd edition; ADOS-G Autism Diagnostic Observation Schedule-Generic; AOSI Autism Observation Schedule for Infants; ADI-R Autism Diagnostic Interview – Revised; SCQ Social Communication Questionnaire; SRS-2 Social Responsiveness Scale – 2nd Edition; WASI-II Wechsler Abbreviated Scales of Intelligence – 2nd Edition; MSEL Mullen Scales of Early Learning; CELF-4 Clinical Evaluation of Language Fundamentals-4th Edition; HR High Risk; LR Low Risk.

2.2.3 Assignment to ASD outcome group

Diagnostic outcome was evaluated at two times; at the 36-month visit and the 7-year visit. As outlined above, experienced researchers involved in testing and the lead

clinician reached a consensus best estimate diagnostic decision for each child in the HR group. The ADI-R was not administered to children in the LR group and, in the absence of a full developmental history, a research diagnosis was not sought. However, none of the children in the LR group had a clinical community diagnosis of ASD at either the 36-month or 7-year visits. For this thesis, the diagnostic outcome groups derived at the 7-year visit will be used in all analyses. Neither the categorisation derived at the 36-month visit nor the change in outcome from 36-months to 7-years will be used in any of the analyses in this thesis.

2.2.3.1 Assignment to ASD outcome group at 36 months

At the 36-month visit, clinical measures from both the 24- and 36-month visits (including ADOS-G, ADI-R, MSEL and Vineland-II) were reviewed. ICD-10 (World Health Organisation 1993) criteria were used to make a consensus best estimate research diagnosis of ASD for children in the HR group. The HR group was divided into children who met diagnostic criteria for ASD (HR-ASD), children who scored above threshold on at least one clinical measure and/or had reduced cognitive functioning and formed the 'atypical' group (HR-Atyp) and children who exhibited normative development (HR-TD).

2.2.3.2 Assignment to ASD outcome group at 7 years

Clinical measures administered at the 7-year visit, including information on ASD symptomatology (ADOS-2, ADI-R, SRS, SCQ) and adaptive functioning (Vineland-II), as well as information from all previous visits, were reviewed to establish ASD consensus best estimate diagnostic outcomes for children in the HR

group, according to DSM-5 criteria (American Psychological Association 2013). Subsequently, children in the HR group were divided into those who met diagnostic criteria for ASD (HR-ASD) and those who did not (HR-non ASD). Additionally, among the HR children, two additional groups were formed: the HR-Atypical (HR-Atyp; *n*=7) and HR-Typically developing (HR-TD; *n*=20) groups. Given the small sample size of these groups, they were not used in the main analyses. However, a full description of these groups and their scores on the clinical measures are provided in Appendix 1. None of the 37 LR children met DSM-5 criteria for ASD and none had a community clinical ASD diagnosis.

2.2.4 Statistical analyses

In this chapter, I will present results from the clinical measures administered at the 7-year visit. The ADOS-G, AOSI and MSEL will be used for analyses in Chapter 6, and scores from these measures will be presented there. All data reduction and statistical analyses were carried out in SPSS version 20.0 (IBM Corp., 2011). Variables with ratios (such as gender ratio) will be analysed using the chi-square (X^2) statistic. Group differences on each of the clinical measures of ASD symptoms (ADOS-2 CSS, ADI-R, SRS-2 T-score, SCQ domain and total scores), cognitive functioning (WASI-II subscales and FSIQ), adaptive functioning (Vineland-II ABC) and language ability (CELF-4 subscale standard scores) will be analysed using ANOVA/MANOVA. Assessments with more than one subscale will be analysed using MANOVA, while those with just a total score will be analysed with ANOVA, across the HR-ASD, HR-non ASD and LR groups. Where significant group differences emerge, planned comparisons will be run between each pair of groups, with Bonferonni adjustment

applied to correct for multiple testing. Cohen's d and η^2 will be used as indicators of effect size (Cohen, 1973).

2.3 Results

2.3.1 Diagnostic outcome at the 7-year visit

As described above, assignment to diagnostic outcome group was conducted at the 7-year visit. Of the total number of HR participants (42), 15 met DSM-5 criteria for ASD and formed the HR-ASD group. Twenty-seven HR children did not meet diagnostic criteria for ASD and these children formed the HR-non ASD group. None of the LR controls met criteria for ASD (American Psychological Association, 2013).

Within the HR group, 5 children were "late diagnosed", meaning that they did not meet diagnostic criteria for ASD at 36-months but did so at 7-years. However, due to the modest size of this group, no further analyses will be done based on a late ASD diagnostic outcome and these children were included in the HR-ASD group. Three children who did meet diagnostic criteria for ASD at 36-months no longer met criteria at 7-years. Again, due to the very small size of this sample, no further analyses could be performed using this grouping. However, since ASD status in these 3 children was not fully clear, they were excluded from further analyses. Thus, the final HR-non ASD sample was n=24. There were no differences among the HR-ASD, HR-non ASD and LR groups in age ($F(2, 71)=1.16$, $p=.321$, $\eta^2=.032$) or gender ratio ($X^2(2)=3.16$, $p=.206$), details of which are presented in Table 2.

Table 2: Demographic characteristics of children in each group

Characteristic	HR-ASD (*n*=15)	HR-non ASD (*n*=24)	LR (*n*=37)
Age (months)	89.13 (6.53)	91.42 (6.28)	89.26 (4.86)
Sex (M:F)	7:8	5:19	14:23

2.3.2 Measures of ASD symptomatology at the 7-year visit

There were significant group differences on measures of ASD severity, group means for each measure are presented in Table 3. There were significant group differences on the ADI-R, where the HR-ASD group scored higher than the HR-non ASD group on all domains, including Social Interaction ($p<.001$, $d=1.78$), Communication ($p<.001$, $d=1.33$), and RRBs ($p<.001$, $d=2.03$).

On the ADOS-2, the HR-ASD group had higher scores on all domains compared to both the HR-non ASD and LR groups. On the total CSS, the HR-ASD group scored higher than both the HR-non ASD ($p<.001$, $d=1.69$) and LR groups ($p<.001$, $d=2.08$). On the Social Affect CSS, the HR-ASD group scored significantly higher than both the HR-non ASD ($p<.001$, $d=1.69$) and LR groups ($p<.001$, $d=2.02$). Finally, for the RRB CSS, the HR-ASD group scored significantly higher than both the HR-non ASD ($p<.001$, $d=1.12$) and LR ($p<.001$, $d=2.54$) groups. Furthermore, within this domain, the HR-non ASD group also scored significantly higher than the LR group ($p=.003$, $d=.93$).

On the SRS-2, the HR-ASD group scored significantly higher than both the HR-non ASD ($p<.001$, $d=1.24$) and LR ($p<.001$, $d=1.77$) groups. The HR-ASD group

scored highly on all domains of the SCQ. The HR-ASD group had a significantly higher total score than both the HR-non ASD group ($p<.001$, $d=1.41$) and the LR group ($p<.001$, $d=1.59$). On the social domain, the HR-ASD group scored significantly higher than both the HR-non ASD group ($p<.001$, $d=1.21$) and the LR group ($p<.001$, $d=1.54$). On the communication domain, the HR-ASD group scored significantly higher than both the HR-non ASD group ($p<.001$, $d=1.48$) and the LR group ($p<.001$, $d=1.26$). Finally, on the RRB domain, the HR-ASD group scored significantly higher than both the HR-non ASD group ($p<.001$, $d=1.28$) and the LR group ($p<.001$, $d=1.33$).

Table 3: Summary of ASD severity scores for each group

Measure	HR-ASD	HR-non ASD	LR	ANOVA/MANOVA
ADI-R	***N*=14**	***N*=24**	**N/A**	
ADI - Social	13.14[a] (4.69)	4.04[b] (5.48)	N/A	$F(1, 36)=27.00$, $p<.001$, $\eta^2=.429$
ADI - Communication	10.43[a] (4.59)	4.25[b] (4.67)	N/A	$F(1, 36)=15.70$, $p<.001$, $\eta^2=.304$
ADI - RRB	3.57[a] (1.74)	0.58[b] (1.41)	N/A	$F(1, 36)=33.33$, $p<.001$, $\eta^2=.481$
ADOS-2 CSS	***N*=15**	***N*=24**	***N*=34**	
ADOS Total	6.33[a] (2.92)	2.46[b] (1.41)	1.70[b] (1.19)	$F(2, 69)=37.61$, $p<.001$, $\eta^2=.522$
ADOS SA	6.60[a] (2.59)	2.96[b] (1.60)	2.18[b] (1.70)	$F(2, 69)=29.11$, $p<.001$, $\eta^2=.458$
ADOS RRB	6.13[a] (2.70)	3.04[b] (2.84)	1.12[c] (0.70)	$F(2, 69)=29.80$, $p<.001$, $\eta^2=.463$
SRS-2	***N*=13**	***N*=19**	***N*=35**	
SRS T-score	74.85[a] (22.77)	52.37[b] (11.74)	45.49[b] (5.820	$F(2, 64)=26.59$, $p<.001$, $\eta^2=.454$

SCQ	***N*=14**	***N*=22**	***N*=37**	
SCQ total score	14.50[a] (10.57)	2.64[b] (5.52)	2.27[b] (2.51)	$F(2, 70)=24.99$, $p<.001$, $\eta^2=.417$
SCQ Social	5.57[a] (4.75)	1.14[b] (2.10)	.32[b] (.75)	$F(2, 70)=24.62$, $p<.001$, $\eta^2=.413$
SCQ communication	4.64[a] (2.87)	.95[b] (2.04)	1.51[b] (2.01)	$F(2, 70)=13.44$, $p<.001$, $\eta^2=.278$
SCQ RRB	3.07[a] (2.64)	.41[b] (1.30)	.43[b] (.93)	$F(2, 70)=17.43$, $p<.001$, $\eta^2=.332$

Group sizes are smaller for some variables due to missing data. Groups denoted with different subscript letters (a, b, c) differed significantly with Bonferonni correction applied (p<.05). HR/LR indicates high-risk or low-risk group; ASD autism spectrum disorder; SD standard deviation; ADI Autism Diagnostic Interview – Revised; RRB Restricted Repetitive Behaviour; ADOS Autism Diagnostic Observation Schedule; CSS Calibrated Severity Score; SA Social Affect; SRS Social Responsiveness Scale; SCQ Social Communication Questionnaire

2.3.3 Measures of cognitive skills, adaptive functioning and language at 7-years

The groups differed significantly on FSIQ, where the HR-non ASD group's performance was significantly lower than the LR group's (p=.05, d=.75), but there were no significant differences on either of the individual IQ subscales. There were also no significant differences on either of the CELF-4 subscales. There were significant group differences on the Vineland-II ABC, the LR group scored significantly higher than the HR-ASD (p<.001, d=1.69) and HR-non ASD groups (p=.02, d=.81). Likewise, the HR-non ASD group had higher adaptive scores than the HR-ASD group (p=.01, d=.85). A summary of group means and statistical analyses is presented in Table 4.

Table 4: Summary of cognitive ability, adaptive functioning and language scores

Measure	HR-ASD	HR-non ASD	LR	ANOVA/MANOVA
WASI-II	***N*=14**	***N*=24**	***N*=35**	
full-scale	109.79 (21.36)	107.96[a] (12.76)	117.06[b] (11.61)	$F(2, 70)=3.25$, $p=.045$, $\eta^2=.085$
verbal	110.14 (25.87)	110.83 (14.94)	119.77 (13.93)	$F(2, 70)=2.65$, $p=.078$, $\eta^2=.070$
perceptual	109.57 (18.26)	102.71 (9.97)	110.34 (12.05)	$F(2, 70)=2.70$, $p=.074$, $\eta^2=.072$
CELF-4	***N*=11**	***N*=20**	***N*=24**	
Receptive	11.27 (3.17)	11.85 (3.05)	12.71 (2.20)	$F(2, 52)=1.18$, $p=.314$, $\eta^2=.044$
Expressive	12.55 (2.77)	11.30 (2.13)	12.79 (3.01)	$F(2, 52)=1.82$, $p=.173$, $\eta^2=.065$
Vineland-II	***N*=15**	***N*=23**	***N*=34**	
ABC	90.27[a] (15.46)	102.22[b] (12.67)	110.53[c] (6.98)	$F(2, 69)=17.67$, $p<.001$, $\eta^2=.339$

Group sizes are smaller for some variables due to missing data. Groups denoted with different subscript letters (a, b, c) differed significantly with Bonferonni correction applied (α=.05/3=.02)

HR/LR indicates high-risk or low-risk group; ASD Autism Spectrum Disorder; SD standard deviation; WASI-II Wechsler Abbreviated Scales of Intelligence-II; CELF-4 Clinical Evaluation of Language Fundamentals – 4th Edition; ABC Adaptive Behavior Composite

2.4 Discussion

The aim of this chapter was to provide an overview of the general methodology of the prospective longitudinal design used in the BASIS study, to describe how assignment to diagnostic outcome group was conducted and to provide participant characterisation across the three groups. Among the HR group, a proportion of children met DSM-5 (American Psychological Association, 2013) for ASD at the 7-year visit and were assigned a research diagnosis of ASD (the HR-ASD group), while children

who did not meet criteria were considered to be 'non-ASD' (HR-non ASD group). None of the LR controls showed signs of ASD or had a community clinical diagnosis.

A variety of ASD measures were used at the 7-year visit, including a clinical interview (ADI-R), semi-structured observational assessment (ADOS-2) and questionnaire measures (SRS-2, SCQ). Children in the HR-ASD group scored significantly higher than both the LR and HR-non ASD groups on all of these measures. There was no evidence of heightened ASD severity among the HR-non ASD group, except for increased prevalence of RRBs reported on the ADOS-2. We also administered a variety of measures of cognitive ability (WASI-II), adaptive functioning (Vineland-II) and language skills (CELF-4). Overall, there were no significant group differences on measures of cognitive and language ability, except that the HR-non ASD group scored significantly lower on overall IQ, but none of the children were in the range of intellectual disability. On the other hand, all HR children exhibited lower adaptive functioning than the LR group. This finding is somewhat surprising, as prior high-risk studies suggest that HR children who do not have ASD exhibit social and communicative atypicalities at school-age (e.g. Miller et al., 2015). It is possible that such difficulties are observable among a subset of HR children who manifest heightened features of the BAP. In this study, there was an attempt to examine such a group (the HR-atypical group), but the very small sample size (n=7) meant that it was difficult to detect significant effects in this group. Furthermore, elevated ASD traits and functional difficulties within this group may not have been observable when their data were analysed with the broader HR-non ASD group.

Chapter 3
Parent and Self-Reported Anxiety and its Manifestation in Children at High Familial Risk for Autism Spectrum Disorder

3.1 Introduction

In Chapter 1, the prevalence of co-occurring psychopathology and, in particular, the elevated rates of anxiety symptoms among individuals with ASD were discussed (Simonoff et al., 2008; White, Oswald, Ollendick, & Scahill, 2009). It was also noted that there is evidence of increased anxiety symptoms among parents and siblings of children with ASD (Lainhart, 2009). However, there is wide heterogeneity in the rates of anxiety reported and the true prevalence of anxiety symptoms among individuals with ASD and their relatives is not fully clear. As White, Oswald, Ollendick, and Scahill (2009) note, between 11% and 84% of young people with ASD are reported to experience a certain degree of impairment resulting from anxiety, but only about half actually meet criteria for an anxiety disorder (de Bruin, Ferdinand, Meester, de Nijs, & Verheij, 2007; Simonoff et al., 2008). Furthermore, some studies report that approximately 29% of first-degree relatives of children with ASD exhibit heightened anxiety (Mazefsky, Folstein, & Lainhart, 2008), while others suggest that the prevalence is much lower (~8%) and not elevated compared to rates observed in community samples (Bolton, Pickles, Murphy, & Rutter, 1998). The number of siblings that actually reach cut-off for clinical level anxiety is only 4% (Shivers, Deisenroth, & Taylor, 2013). Hallett, Ronald, et al. (2013) examined the prevalence of anxiety among children with ASD, their co-twins who manifested aspects of BAP, TD twins and non-ASD controls. Anxiety was most highly elevated among ASD probands and co-twins

with features of BAP, but not among TD twins. This suggests that anxiety may not be elevated among all first-degree relatives, but particularly those who also have heightened traits of ASD themselves. Further investigation of the factors that contribute to the wide heterogeneity in anxiety symptoms within ASD populations is highly relevant to improving measurement of anxiety symptoms and understanding their manifestation.

While a multitude of issues potentially contribute to the disparity in reports of anxiety rates, there are several prominent factors that are consistently implicated. Among these are challenges in accurately measuring anxiety symptoms in individuals with ASD (e.g. Mazefsky, Kao, & Oswald, 2011), difficulties in disentangling symptoms of anxiety and the core features of ASD (Kerns & Kendall, 2012), and capability of ascertaining information about symptoms from individuals with reduced intellectual functioning (Sukhodolsky et al., 2008). Using a high-risk design, the present study is well placed in addressing some of these issues. In particular, we are able to compare anxiety symptoms among high-risk siblings who meet diagnostic criteria for ASD and those who do not, helping to elucidate whether anxiety is unique to family members who themselves have ASD or if it extends to non-ASD siblings. Furthermore, the design allows us to examine the association between anxiety symptoms and features of ASD, even among children who do not actually reach clinical cut-off for an ASD diagnosis.

3.1.1 Agreement of parent and child reports on anxiety symptoms in non-ASD populations

It has long been recognised that there is significant discrepancy among child- and parent-reports of both internalising and externalising symptoms, even among non-ASD populations (Achenbach, 2011; Achenbach, McConaughy, & Howell, 1987). With regard to measurement of anxiety symptoms, parent and child agreement is generally low on both clinical interviews and questionnaire measures, with children reporting higher severity than parents do (Choudhury, Pimentel, & Kendall, 2003; for review see Foley et al., 2005; Nauta et al., 2004). Longitudinal studies suggest that discrepancies in parent- and child-report remain consistent from childhood through to adolescence and persist even after children undergo treatment (Safford, Kendall, Flannery-Schroeder, Webb, & Sommer, 2005). On the contrary, there is high agreement among parent- and teacher-reports of child anxiety symptoms (Miller, Martinez, Shumka, & Baker, 2014).

A substantial body of research has been dedicated to understanding the source of disagreement in child and informant reports of anxiety, with a multitude of factors being implicated. To date, no single child characteristic has been found to moderate agreement in self- and parent-reported anxiety (Hamblin et al., 2016). Although some studies report that older children have somewhat higher agreement with parents, likely because they have better language and ability to communicate information about symptoms (Choudhury, Pimentel, & Kendall, 2003; Grills & Ollendick, 2003). On the other hand, parent psychopathology, particularly anxiety and depression, is consistently associated with greater discrepancy in reports of anxiety symptoms. Parents who

themselves have a form of psychopathology generally report more severe symptoms for their children (Becker, Jensen-Doss, Kendall, Birmaher, & Ginsburg, 2016). There is also evidence to suggest that child and parent agreement varies depending on the type of symptoms reported and the type of anxiety in question. For example, there is higher inter-rater agreement on observable or overt behaviours associated with anxiety, but low agreement on physiological or internal sensations and cognitions (March, Parker, Sullivan, Stallings, & Conners, 1997). There is no definitive way to determine whose account of symptoms is more accurate and clinicians are instructed to consider both child- and parent-report to gain a comprehensive understanding of the child's well-being and functioning (Achenbach, McConaughy, & Howell, 1987). However, evidence suggests that clinicians tend to favour parent-report in forming a diagnosis (Grills & Ollendick, 2003).

In spite of this, it is widely believed that children are able to reflect on their own internal states and provide useful information about internalising symptoms (Luby, Belden, Sullivan, & Spitznagel, 2007). A majority of self-report anxiety questionnaires have been validated and widely used to evaluate symptoms among children of school age and above (e.g. Spence, 1998). Younger, preschool aged, children have also demonstrated the ability to report on internalising symptoms when the measure used did not rely on reading ability (Ablow et al., 1999; Luby, Belden, Sullivan, & Spitznagel, 2007). Taken together, evidence suggests that children are able to effectively provide information about anxiety symptoms, but that discrepancy with parent-reported symptoms complicates clinical decisions. As a result, it is more informative to use information from multiple informants.

3.1.2 Child and parent agreement of anxiety symptoms among children with ASD

Measuring anxiety symptoms using self-report among children with ASD presents several challenges additional to those described in non-ASD populations. In particular, individuals with ASD are suggested to have reduced introspection and ability to identify and express emotions (Berthoz, Lalanne, Crane, & Hill, 2013), as well as higher prevalence of communication and language difficulties (Ricketts, Jones, Happé, & Charman, 2013). Mazefsky, Kao, and Oswald (2011) compared four self-report measures of anxiety symptoms with a parent interview in adolescents with ASD and found poor inter-rater agreement. While participants who met clinical cut-off for anxiety disorders did report more symptoms than those who were below cut-off, they reported fewer symptoms than were ascertained through parent-report, suggesting that the self-report measures had low sensitivity and specificity in ASD populations. Correspondingly, similar research suggests that adolescents with ASD under-report on anxiety symptoms compared to both parents and clinicians (White, Schry, & Maddox, 2012). In a younger age group (8-12 years), Gillott, Furniss, and Walter (2001) report reduced agreement in reports of social worries among ASD parent-child dyads compared to dyads of TD children and those with Specific Language Impairment (SLI). In particular, among ASD dyads, parents reported a higher level of social worries than children did, while this pattern was reversed for the TD and SLI dyads, where children reported more social worries than parents. This suggests that discrepancies in child- and parent-report in ASD populations are not solely due to ASD children's language abilities.

However, these conclusions should be treated with some caution as multiple other studies with adolescents with ASD report high inter-rater agreement (Farrugia & Hudson, 2006; Ozsivadjian, Hibberd, & Hollocks, 2014). Furthermore, recent research suggests that measures of salivary cortisol correspond more closely to self-reported, than parent-reported, anxiety symptoms in adolescents with ASD (Bitsika, Sharpley, Andronicos, & Agnew, 2015). Kaat and Lecavalier (2015) suggest that parent and child agreement is higher among older children and those with less severe ASD, elucidating the discrepant findings in previous literature.

Hallett, Ronald, et al. (2013) are the first to report on inter-rater agreement in anxiety symptoms among siblings (twins) of children with ASD, including those who had ASD, manifested aspects of BAP and had typical development. Within this population, there was significant correspondence between parents and children, including probands with ASD, twins with BAP and unaffected twins, across anxiety subtypes. In some cases, agreement in the control group was lower than in the ASD twin groups. While these findings offer promising evidence of the reliability of anxiety measures in siblings of children with ASD, this study tested an adolescent sample and there is presently a scarcity in research examining inter-rater agreement in siblings from younger age groups.

3.1.3 Selecting appropriate measures of anxiety for children with ASD

While there are a multitude of anxiety scales that have been well validated and widely used with school aged children, it is not clear whether these measures are equally adept at evaluating symptoms among children with ASD (for review see Grondhuis & Aman, 2012). Two reviews (Lecavalier et al., 2014; Wigham &

McConachie, 2014) have evaluated the use of child anxiety questionnaires to measure outcomes of children with ASD after treatment for anxiety. Lecavalier et al. (2014) identified four measures that were considered appropriate, including two clinical interviews, the Anxiety Disorders Interview Schedule (ADIS-P/C; Silverman & Albano, 1996) and the Paediatric Anxiety Rating Scale (PARS; The Research Units On Pediatric Psychopharmacology Anxiety Study, 2002), a parent- and teacher-questionnaire, the Child and Adolescent Symptom Inventory (CASI; Gadow & Sprafkin, 2002), and one questionnaire measure that includes both parent- and child-report, the Multidimensional Anxiety Scale for Children (MASC-P/C; March, Parker, Sullivan, Stallings, & Conners, 1997). On the contrary, Wigham and McConachie (2014) suggest the use of three questionnaire measures, which all include parent- and self- report, including the Spence Children's Anxiety Scale (SCAS-P/C; Spence, 1998), Revised Child Anxiety and Depression Scale (RCADS; Chorpita, Moffitt, & Gray, 2005), and the Screen for Child Anxiety Related Disorders (Birmaher et al., 1997).

Multiple studies have examined the psychometric properties of the questionnaire measures outlined above. In general, self-report measures of anxiety have high internal consistency and specificity among young people with ASD (e.g. White, Schry, & Maddox, 2012). However, these measures have poor sensitivity for detecting clinical cases of anxiety among individuals with ASD. For example, White, Schry, and Maddox (2012) assessed young people with ASD who all had clinical diagnoses of anxiety disorder, but only 23% reached cut-off for clinical-level anxiety on the MASC-C. This perhaps highlights the tendency of young people with ASD to under-report anxious symptoms. Parent-report measures exhibit equally good psychometric properties and improved sensitivity. For example van Steensel, Deutschman, and

Bogels (2013) suggest that the SCARED parent-report has excellent sensitivity (α=.95) among children with ASD.

The Spence Children's Anxiety Scale (Nauta et al., 2004; Spence, 1998) has thus far been the most widely used measure of anxiety symptoms among young people with ASD (Grondhuis & Aman, 2012). Gillott, Furniss, and Walter (2001) reported that children with ASD reported higher anxiety symptoms on the SCAS-C, compared to TD controls and children with SLI. They also scored higher than the mean of the non-clinical sample in the Spence standardisation trials (Gillott, Furniss, & Walter, 2001; Gillott & Standen, 2007; Spence, 1998). Magiati, Chan, Tan, and Poon (2014) suggest that the SCAS exhibits satisfactory parent-child agreement among non-referred children (mean age 12 years) with ASD, particularly on the subscales measuring Separation anxiety, Generalised anxiety, and Physical Injury Fears.

Zainal et al. (2014) examined the use of the SCAS as a screening tool for anxiety among children with ASD, aged 6-18 years (mean age ~10 years), when compared to the Kiddie-Schedule for Schizophrenia and Affective Disorders Present and Lifetime version (K-SADS-PL; Birmaher et al., 2009), a standardised DSM-IV-TR based clinical interview. Overall, SCAS-P exhibited excellent internal consistency (α=.88), had satisfactory sensitivity for detecting clinical cases (>.70), and good convergent validity with K-SADS-PL. Taken together, the evidence suggests SCAS is a robust measure of anxiety symptoms among children with ASD and has improved sensitivity compared to similar measures.

3.1.4 Association between co-occurring anxiety, core ASD symptoms and cognitive/adaptive functioning

Given the high prevalence of co-occurring anxiety in individuals with ASD, multiple studies have examined the association between anxiety, core ASD symptoms and cognitive functioning. Sukhodolsky et al. (2008) found that, among children with Pervasive Developmental Disorder (PDD), those with IQ<70, which signifies the presence of intellectual disability, were less likely to meet criteria for an anxiety disorder than those with IQ>70. Furthermore, among this cohort with PDD, anxiety was also associated with more challenging behaviours and adaptive difficulties. Using a large sample of children with ASD (*N*=415), Hallett, Lecavalier, et al. (2013) also found increased anxiety symptoms were more prevalent among children with IQ>70. However, the association between IQ and anxiety symptoms in children with ASD has yielded equivocal results. For example, in a meta-analysis of anxiety manifestation in children with ASD, van Steensel, Bogels, and Perrin (2011) report that increased anxiety severity is associated with lower IQ. The association between anxiety and lower IQ was more evident for some subtypes of anxiety (social and overall anxiety) than others (OCD, separation anxiety). The discrepancy in findings likely reflects difficulty in caregiver reports of anxiety symptoms in individuals with reduced cognitive functioning.

Core symptoms of ASD have also been associated with increased anxiety symptoms in children with PDD and ASD (Hallett, Ronald, et al., 2013; Sukhodolsky et al., 2008). In particular, stereotyped behaviours are associated with increased anxiety over and above social and communication symptoms (Sukhodolsky et al., 2008).

However, more recent evidence suggests that different subtypes of anxiety may be differentially associated with ASD core symptoms. For example communication difficulties are associated with higher separation anxiety and OCD and lower Social anxiety, while Restricted and Repetitive Behaviours (RRBs) are associated with increased symptoms of OCD and panic disorder (Hallett, Ronald, et al., 2013). Evidence also suggests that insistence on sameness, one domain of RRBs, is more strongly associated with anxiety than other aspects of RRBs (Rodgers, Glod, Connolly, & McConachie, 2012).

While elevated anxiety has also been observed in siblings of children with ASD, there is presently little investigation into how anxious symptoms associate with ASD traits in this population. While Hallett, Ronald, et al. (2013) reported on associations between anxiety and core symptoms of ASD in probands with a diagnosis of ASD, their twins with BAP and unaffected twins, the association was not analysed separately for each group. This makes it difficult to discern the impact of subclinical ASD traits on anxiety symptoms among siblings who do not meet diagnostic criteria. However, among twins of children with ASD, emotional difficulties are accounted for entirely by genetic overlap (Tick et al., 2015). Furthermore, non-ASD children with anxiety disorders are reported to have more sub-clinical ASD symptoms compared to typically developing controls, both in early development and current scores (van Steensel, Bogels, & Wood, 2013). There is presently a need for further examination of the correlates of anxiety symptoms among siblings of children with ASD, as this could help elucidate the pathways that lead to high prevalence of anxiety among this population.

3.1.5 Sex differences in anxiety symptoms

Sex differences in the prevalence of anxiety disorders have been widely established in the general population, with females reported to have higher rates of anxiety across subtypes (McLean & Anderson, 2009). McLean, Asnaani, Litz, and Hofmann (2011) suggest that women are also more likely to develop multiple, comorbid, anxiety disorders and that they experience greater burden due to the condition than men do. Additionally, multiple studies suggest that sex differences in anxiety can be observed in early childhood, but that they become more readily observable during adolescence, reaching ratios of up to 3:1 (for review see Beesdo, Knappe, & Pine, 2009; Costello, Egger, & Angold, 2005).

Sex differences in the prevalence of co-occurring anxiety among individuals with ASD have not been studied widely. However, it is possible that potentially higher rates of anxiety among females would present challenges in accurately diagnosing girls with ASD (Lai, Lombardo, Auyeung, Chakrabarti, & Baron-Cohen, 2015). For example, Trubanova et al. (2014) suggest that "diagnostic overshadowing" may be a prevalent problem for females with ASD, whereby females who have both ASD and co-occurring anxiety are more likely to be diagnosed with an anxiety disorder alone, rather than with ASD. However, research investigating whether females with ASD actually have higher levels of anxiety symptoms has yielded equivocal results. For example, Lai, Lombardo, Pasco, Ruigrok, Wheelwright, Sadek, Chakrabarti, et al. (2011) report no sex differences among high functioning adults with ASD. Solomon, Miller, Taylor, Hinshaw, and Carter (2012) found that adolescent (aged 12-18 years) girls with ASD are reported by parents to have more internalising difficulties than boys are. However,

this difference was not observed among children (aged 8-11 years) with ASD in the same study. On the contrary, May, Cornish, and Rinehart (2014) report that, among school aged children (7-12 years) with ASD, girls exhibit heightened anxiety symptoms. Finally, in early childhood (1.5-3.9 years), girls diagnosed with ASD manifest heightened anxious behaviours and more sleep problems than boys (Hartley & Sikora, 2009).

3.1.6 Aims and hypotheses

The aim of this chapter is to examine the prevalence of anxiety symptoms among children at high risk for ASD. In particular, to compare anxiety levels among high-risk children who meet diagnostic criteria for ASD (HR-ASD) at age 6-8 years, those who do not (HR-non ASD) and low-risk controls (LR). A further objective is to examine factors that contribute to the measurement of ASD symptoms in this cohort. Parent- and child-reported anxiety symptoms and psychometric properties of the scale will be examined in the HR and LR groups. Finally, I aim to examine the association between anxiety and core symptoms of ASD within the HR group. Given the previous literature, the following hypotheses will be investigated:

1) On parent-reported anxiety, HR children with exhibit elevated anxiety compared to LR controls. A pattern is expected to emerge whereby the HR-ASD group will have the highest levels of anxiety, followed by the HR-non ASD group, while LR controls will have lowest anxiety levels.
2) Given the mixed findings on self-reported anxiety symptoms among individuals with ASD and the young age of the children in this sample, there is some difficulty in predicting whether the self-report measure of anxiety used is fully

able to capture anxiety symptoms in the HR sample. However, in light of the evidence that children with ASD tend to under-report on anxiety symptoms (Mazefsky, Kao, & Oswald, 2011) and this is particularly problematic among young children with ASD (Kaat & Lecavalier, 2015), I hypothesise that group differences will be less readily observable on the self-report, compared to the parent-report, measure.

3) Given previous evidence of heightened anxiety among both girls with ASD (May, Cornish, & Rinehart, 2014) and in non-ASD populations (McLean, Asnaani, Litz, & Hofmann, 2011), girls are predicted to have higher anxiety scores than boys across groups.
4) Low inter-rater agreement on anxiety symptoms is expected across groups, but will be more highly pronounced in the HR group than the LR group.
5) Within the HR group, parent-reported anxiety symptoms will be associated with increased ASD severity, particularly the domain of RRBs and higher cognitive functioning.
6) Within the HR group, self-reported anxiety symptoms will be associated with higher cognitive functioning and language ability.

3.2 Method

3.2.1 Measures

3.2.1.1 The Spence Children's Anxiety Scale

The Spence Children's Anxiety Scale - Parent (SCAS-P; Nauta et al., 2004) and Self –report (SCAS-C; Spence, 1998) versions were used to assess anxiety symptoms across 6 domains, including Separation Anxiety, OCD, Social Phobia, Physical Injury

Fears, Panic/Agoraphobia, Generalised Anxiety and a total anxiety score. The parent-version consists of 38 items and asks parents to report how frequently their child exhibits a range of anxiety related behaviours (e.g. 'My child worries about things'). Responses are recorded on a 4-point Likert scale (Never, Sometimes, Often, Always). Total scores range from 0 to 112 and higher scores indicate more severe anxiety.

The SCAS-C consists of 38 items asking the child to report how frequently they experience a range of anxiety related thoughts and feelings (e.g. 'When I have a problem, I have a funny feeling in my stomach') and an additional 5 filler items asking about positive cognitions. Responses are recorded on a 4-point Likert scale and total scores can range from 0-112. The SCAS-C provides age and sex normed t-scores for each domain and the total score. However, because no such equivalent is available for the parent version, raw scores on both scales were used in analyses to ensure comparability between the two measures.

3.2.2 Statistical analyses

3.2.2.1 Group differences in anxiety symptoms

All data reduction and statistical analyses were carried out in SPSS version 20.0 (IBM Corp., 2011). Group and sex differences in parent- and self-reported anxiety were assessed using a 3 (group: HR-ASD, HR-non ASD, LR) by 2 (sex: male, female) multivariate ANOVA on total anxiety score and each of the 6 subscales. Two separate MANOVAs were run to examine differences on SCAS-P and SCAS-C scores. Where significant group differences emerged, planned comparisons were run between each pair of groups, using Bonferonni correction to account for multiple testing. If a significant group by sex interaction emerged, follow-up independent samples t-tests

were run separately in each group, with Bonferonni adjustment applied to the *p*-value to account for family-wise error resulting from multiple testing (.05/6=.008).

To assess anxiety symptoms in the HR-Atyp group described in Chapter 2 (Appendix 1), the two MANOVAs described above were run again, splitting up the HR-non ASD group into HR-Atyp and HR-TD. Therefore, two 4 (group: HR-ASD, HR-Atyp, HR-TD and LR) by 2 (sex: male, female) MANOVAs were run on each subscale and the total score using both SCAS-P and SCAS-C scores. Because of the modest sample size and the small number of participants that fell into the HR-Atyp group (*n*=7), these MANOVAs will not be included in the main analysis but are instead presented in Appendix 2.

3.2.2.2 Psychometric properties and inter-rater agreement on SCAS-P and SCAS-C

Chronbach's alpha statistic was used to measure internal consistency of the SCAS-P and SCAS-C measures. Internal consistency is reported for both questionnaires in the whole sample and separately within the HR and LR groups. To compare parent- and self- reported anxiety, intra-class correlations were performed on SCAS-P and SCAS-C total scores and scores from each of the 6 domains for the HR and LR groups separately.

3.2.2.3 Association between anxiety, core symptoms of ASD, cognitive functioning and language ability

Pearson correlation was used to evaluate the associations between SCAS-P and SCAS-C total scores and measures of ASD symptomatology (SCQ Social Interaction, Communication, RRB domains and total SCQ score), IQ (WASI FSIQ, WASI PRI, and WASI VCI) and language ability (CELF-4 subtests of Concepts and Following

Directions and Recalling Sentences), which are described in detail in Chapter 2. Because these analyses were exploratory, the *p*-value was not adjusted to correct for multiple testing and $p<.05$ was used to identify statistically significant associations.

As significant associations between SCAS-P and multiple SCQ domains were found and there were significant sex differences on anxiety symptoms (see results), a linear regression was performed within the HR group to examine the unique contribution of different aspects (social, communication and RRB) of ASD severity and sex to total anxiety. Following the method of Sukhodolsky et al. (2008), the domains of social interaction, communication and RRBs were all entered into the regression as independent variables to determine the unique contribution of each domain. SCAS-P total score was entered as the dependent variable and SCQ social, SCQ communication, SCQ RRB scores and sex were entered as predictors. The SCQ variables were mean centred to reduce the risk of multicollinearity. In order to ascertain whether significant associations were driven by the HR-ASD group, follow-up correlations between SCQ scores and SCAS-P total score were run for the HR-ASD and HR-non ASD group separately.

All confidence intervals are for the 95% significance level and Cohen's *d*, η^2 and r^2 were used as an indication of the effect size where appropriate (Cohen, 1973). Given the modest size of the sample, post hoc power analyses were carried out for all analyses described above using G*Power (Faul, Erdfelder, Buchner, & Lang, 2009; Faul, Erdfelder, Lang, & Buchner, 2007), to determine whether there was sufficient power to detect significant effects.

3.3 Results

3.3.1 Parent- and self-reported anxiety symptoms

Tables 5 and 6 summarise the SCAS-P and SCAS-C scores for each group. Parent-report of anxiety symptoms, SCAS-P total score, revealed significant differences between groups. The HR-ASD group had substantially higher total SCAS-P scores than the LR group ($p<.001$, $d=.89$), whereas the HR-non ASD group did not differ from either the HR-ASD ($p=.23$, $d=.52$) or LR ($p=.08$, $d=.72$) groups. The HR-ASD group had higher scores than LR on multiple subscales, including separation anxiety ($p=.001$, $d=1.00$), OCD ($p=.02$, $d=.64$), Panic/Agoraphobia ($p=.001$, $d=.72$), generalised anxiety ($p<.001$, $d=.96$) and had higher Panic/Agoraphobia scores than the HR-non ASD group ($p=.03$, $d=.66$). The HR-non ASD group also had significantly higher separation anxiety scores than the LR group ($p=.02$, $d=.77$).

A post hoc power analysis was conducted to determine the power that the current sample had to achieve a medium effect size of $\eta^2=.06$, which corresponds to a power of $f=.25$ (Cohen, 1973). The present sample ($n=74$) had a power of $(1-\beta)=.45$, critical $F(2, 71)=3.13$, to achieve a medium effect. Furthermore, post hoc power analysis was conducted to determine the power that each group (HR-ASD, HR-non ASD, LR) had to detect a significant difference from one of the other groups with a medium effect size ($d=.50$). To achieve a significant difference between the HR-ASD ($n=15$) and HR-non ASD ($n=23$) groups, there was a power of $(1-\beta)=.31$, critical $t(36)=2.03$. To achieve a difference between the HR-ASD and LR ($n=36$) groups, there was a power of $(1-\beta)=.36$, critical $t(49)=2.01$. Finally, to achieve a difference between

the HR-non ASD and LR groups, the present sample had a power of (1-β)=.45, critical $t(57)=2.00$.

There were significant sex differences in total anxiety levels ($F(1, 68)=11.08$, $p=.001$, $\eta^2=.14$), where females ($M=18.50$, $SD=13.96$) had higher anxiety than males ($M=13.65$, $SD=8.55$), $d=.42$. There was also a significant group by sex interaction on the total anxiety score ($F(2, 68)=10.64$, $p<.001$, $\eta^2=.24$) and to follow up on this interaction, independent samples t-tests were run within each group to examine sex differences on total anxiety. Bonferonni correction was applied to the p-value to account for family wise error related to multiple testing ($\alpha=.05/6=.008$). The only significant difference emerged in the HR-ASD group, where females ($M=38.88$, $SD=21.50$) had significantly higher anxiety levels than males ($M=11.71$, $SD=4.11$), $t(13)=-3.28$, $p=.001$, $d=1.76$, but there were no sex differences in the LR or HR-non ASD groups.

On the contrary, there were no significant group differences on self-reported anxiety symptoms. A significant sex difference emerged on the SCAS-C OCD subscale, where females ($M=2.76$, $SD=2.25$) reported more symptoms than males ($M=1.96$, $SD=2.44$), $F(1, 59)=4.37$, $p=.04$, $\eta^2=.08$, $d=.34$. However, there was no significant group by sex interaction.

A post hoc power analysis was conducted to determine the power the current sample of children who had completed SCAS-C had to achieve a medium effect size of $\eta^2=.06$, which corresponds to a power of $f=.25$ (Cohen, 1973). The present sample ($n=66$) had a power of (1-β)=.41, critical $F(2, 63)=3.14$, to achieve a medium effect. Furthermore, post hoc power analysis was conducted to determine the power that each

group (HR-ASD, HR-non ASD, LR) had to be significantly different from one of the other groups with a medium effect size (d=.50). To achieve a significant difference between the HR-ASD (n=11) and HR-non ASD (n=23) groups, there was a power of (1-β)=.26, critical t(32)=2.04. To achieve a difference between the HR-ASD and LR (n=32) groups, the present sample had a power of (1-β)=.29, critical t(41)=2.02. Finally, to achieve a difference between the HR-non ASD and LR groups, the present sample had a power of (1-β)=.43, critical t(53)=2.01.

Table 5: SCAS-P mean scores and group differences for each group

SCAS subscale (SD)	HR-ASD	HR-non ASD	LR	MANOVA
	***N*=15**	***N*=23**	***N*=36**	
Total	26.20 (20.86)[a]	17.91 (8.55)	12.22 (7.27)[b]	$F(2, 68)=9.87$, $p<.001$, $\eta^2=.225$
Separation Anxiety	6.27 (4.20)[a]	4.87 (2.82)[a]	2.94 (2.14)[b]	$F(2, 68)=9.23$, $p<.001$, $\eta^2=.213$
OCD	2.27 (3.41)[a]	1.00 (1.51)	0.67 (0.99)[b]	$F(2, 68)=7.15$, $p=.002$, $\eta^2=.174$
Social Phobia	5.07 (5.65)	4.13 (2.67)	2.64 (2.98)	$F(2, 68)=3.31$, $p=.043$, $\eta^2=.089$
Physical Injury/Fears	4.53 (2.85)	3.43 (2.25)	2.97 (2.08)	$F(2, 68)=2.36$, $p=.102$, $\eta^2=.065$
Panic/Agoraphobia	2.93 (4.62)[a]	.074 (.097)[b]	.053 (.094)[b]	$F(2, 68)=7.15$, $p=.002$, $\eta^2=.174$
Generalised Anxiety	5.13 (3.66)[a]	3.74 (2.05)	2.47 (1.40)[b]	$F(2, 68)=9.96$, $p<.001$, $\eta^2=.225$

Groups denoted with different subscript letters (a, b, c) differed significantly with Bonferonni correction applied (p<.05). HR/LR indicates high-risk or low-risk group; ASD autism spectrum disorder; SD standard deviation; OCD Obsessive Compulsive Disorder

Table 6: SCAS-C mean scores and group differences for each group

SCAS subscale	HR-ASD	HR-non ASD	LR	MANOVA
	***N*=11**	***N*=23**	***N*=32**	
Total	23.82 (10.59)	23.26 (10.19)	23.75 (11.44)	$F(2, 60)=.019$, $p=.982$, $\eta^2=.001$
Separation Anxiety	6.45 (4.06)	5.87 (3.38)	5.09 (3.15)	$F(2, 60)=1.15$, $p=.322$, $\eta^2=.037$
OCD	1.82 (1.66)	2.48 (2.23)	2.41 (2.70)	$F(2, 60)=.615$, $p=.544$, $\eta^2=.020$
Social Phobia	3.64 (2.94)	4.09 (2.64)	4.56 (2.86)	$F(2, 60)=.503$, $p=.607$, $\eta^2=.016$
Physical Injury/Fears	4.82 (3.25)	3.87 (3.07)	3.94 (3.18)	$F(2, 60)=.291$, $p=.749$, $\eta^2=.010$
Panic/Agora phobia	2.64 (2.62)	2.52 (2.13)	2.56 (3.51)	$F(2, 60)=.021$, $p=.979$, $\eta^2=.001$
Generalised Anxiety	4.45 (2.51)	4.43 (1.73)	5.19 (2.48)	$F(2, 60)=.611$, $p=.546$, $\eta^2=.020$

Groups denoted with different subscript letters (a, b, c) differed significantly with Bonferonni correction applied (p<.05). HR/LR indicates high-risk or low-risk group; ASD autism spectrum disorder; SD standard deviation; OCD Obsessive Compulsive Disorder

3.3.2 Psychometric properties and inter-rater agreement on SCAS-P and SCAS-C

Internal consistency of the SCAS-P and SCAS-C was evaluated within the whole sample and individually for the HR and LR groups. Within the entire sample, the SCAS-P had excellent internal consistency, α=.92. When examined separately within each group, SCAS-P had excellent internal consistency in the HR group, α=.92, and good consistency in the LR group, α=.77. SCAS-C also had excellent internal

consistency within the entire sample, α=.80. It had excellent internal consistency within the LR group, α=.83, and good consistency within the HR group, α=.77.

Table 7 presents the intra-class correlations between SCAS-P and SCAS-C scores for each subscale and the total score, within the HR and LR groups separately. In the HR group, agreement on total scores did not reach statistical significance but there was significant agreement on the subscales of separation anxiety, *ICC(*3,2)=.47, *F*(32, 32)=1.88, *p*=.04, and physical injury fears, *ICC(*3,2)=.59, *F*(32, 32)=2.41, *p*=.01. In the LR group, there was significant agreement on total anxiety scores, *ICC(*3,2)=.44, *F*(31, 31)=2.86, *p*=.002, and the physical injury fears subscale, *ICC(*3,2)=.46, *F*(31, 31)=1.94, *p*=.04.

Table 7: Intra-class correlations for SCAS-P and SCAS-C scores in HR and LR groups

Subscale	HR (95% CIs)	LR (95% CIs)
Separation anxiety	.468 (-.064-.737)*	.210 (-.352-.573)
OCD	.117 (-.751-.560)	-.060 (-.731-.409)
Panic/Agoraphobia	.379 (-.224-.689)	.195 (-.365-.563)
Physical Injury Fears	.590 (.164-.798)**	.462 (-.049-.731)*
Generalised Anxiety	.129 (-.814-.576)	.068 (-.286-.404)
Social Phobia	.160 (-.706-.585)	.339 (-.214-.658)
Total Anxiety	.362 (-.304-.687)	.442 (-.208-.750)**

* Indicates *p*<.05; ** *p*<.0; HR/LR high-risk or low-risk group; ASD autism spectrum disorder; CI Confidence Interval; OCD Obsessive Compulsive Disorder.

3.3.3 Association between anxiety, ASD symptoms, cognitive functioning and language

Table 8 summarises the Pearson correlation coefficients for associations between SCAS-P, SCAS-C total scores and measures of ASD severity, cognitive functioning and language. Within the HR group, there were significant associations between SCAS-P total score and each of the SCQ domains, including social interaction (p=.005), communication (p=.004) and RRB (p<.001). There were no significant associations between SCAS-P and cognitive functioning or language ability.

Post hoc power analyses were conducted for each correlation to determine the power that the present sample had to achieve a medium effect size (r=.30). The present HR sample had a power of (1-β)=.47 to achieve a medium sized effect for the correlation between SCAS-P and SCQ. For the WASI, the present sample had a power of (1-β)=.45 to achieve a medium sized effect. Finally, for the CELF, the present sample had a power of (1-β)=.39 to reach a medium sized effect.

On the other hand, SCAS-C total score was significantly associated with the CELF Repeating sentences scale score, (p=.037). There were no other significant associations between SCAS-C score and measures of ASD and cognitive functioning.

Again, post hoc power analyses were conducted for each correlation to determine how much power the present sample had to achieve a medium sized effect, as described above. For the correlation between SCAS-C and SCQ, the present sample had a power of (1-β)=.39 to achieve a medium sized effect. For the WASI, the present

sample had a power to (1-β)=.41. Finally, for the CELF, the sample had a power of (1-β)=.36 for a medium sized effect.

Table 8: Pearson correlation coefficients for the associations between SCAS-P, SCAS-C and measures of ASD symptoms, adaptive functioning and language ability

Measure	SCAS-P	SCAS-C
SCQ	*N*=38	*N*=32
Total	.545**	.110
Social	.446**	.083
Communication	.462**	.175
RRB	.667***	.062
WASI-II	*N*=37	*N*=34
FSIQ	-.108	-.161
VCI	-.015	-.191
PRI	-.239	-.063
CELF	*N*=32	*N*=29
Concepts/directions	.099	-.226
Repeating sentences	.198	-.397* (*N*=28)

Group sizes are smaller for some variables due to missing data. * indicates $p<.05$, ** $p<.01$, *** $p<.001$; HR/LR high-risk or low-risk group; ASD autism spectrum disorder; SCAS-P Spence Children's Anxiety Scale-Parent version; SCAS-C Spence Children's Anxiety Scale-Child version; OCD Obsessive Compulsive Disorder; CELF Clinical Evaluation of Language Essentials; SCQ Social Communication Questionnaire; RRB Restricted and Repetitive Behaviour.

To examine the independent contributions of sex, social ability, communication difficulties and RRBs on total anxiety symptoms, a linear regression was run with SCAS-P total as the dependant variable and SCQ social, SCQ communication and SCQ

RRB and sex as independent variables. The overall model significantly predicted anxiety symptoms, $F(4, 31)=9.86$, $p<.001$, $r^{2=}.56$. The SCQ RRB score was significantly associated with SCAS-P total score, $\beta=1.07$, $t(35)=4.10$, $p<.001$. On the contrary, neither the SCQ social ($\beta=-.353$, $t(35)=-1.32$, $p=.20$) nor communication ($\beta=-.13$, $t(35)=-.44$, $p=.66$) predicted anxiety symptoms. Finally, sex ($\beta=.23$, $t(35)=1.84$, $p=.08$) had a trend level association with SCAS-P score. A post hoc power analysis was conducted to determine the power that the present sample had to achieve a medium effect size of $R^2=.30$, which corresponds to $f^2=.39$ (Cohen, 1973). The present sample ($n=38$) had power of $(1-\beta)=.83$, critical $F(2, 33)=2.66$ to achieve a medium effect size in this regression analysis.

Finally, to determine whether the association between ASD severity and anxiety was driven by the HR-ASD group, follow-up Pearson correlations were run between each SCQ domain and SCAS-P separately within the HR-ASD and HR-non ASD groups. Within the HR-ASD group, there were significant associations between SCAS-P total score and SCQ Communication ($r(14)=.56$, $p=.04$) and RRB ($r(14)=.80$, $p=.001$), as well as the SCQ total score ($r(14)=.65$, $p=.01$). Within the HR-non ASD group, there were no significant associations between any of the SCQ subscales and SCAS-P. Figure 1 shows the association between SCQ total score and SCAS-P total score separately for the HR-ASD and HR-non ASD groups.

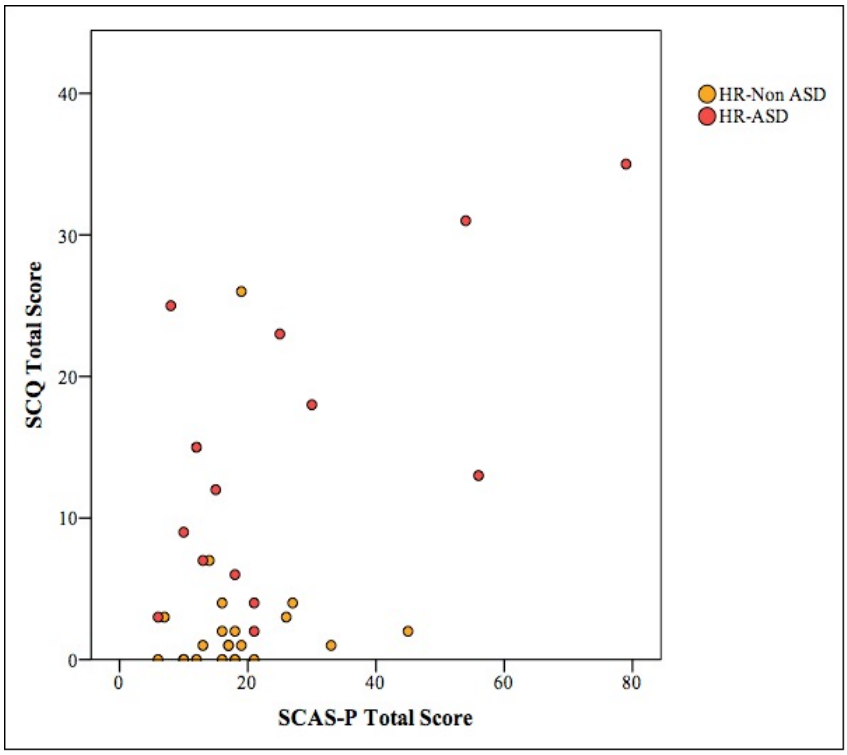

Figure 1. Scatter plot showing association between SCAS-P and SCQ total scores in the HR group

3.4 Discussion

In this chapter, the prevalence of anxiety symptoms among high-risk children with ASD (HR-ASD), those without ASD (HR-non ASD) and LR controls was examined. This is the first high-risk for ASD study that reports on anxiety symptoms during middle childhood. Using a parent-report questionnaire revealed that there was significantly elevated anxiety among the HR-ASD group, and slightly elevated anxiety in the HR-non ASD group on one subscale. On the contrary, using a self-report questionnaire did not yield any significant group differences. There was low to moderate agreement in self- and parent- report in both the HR and LR groups. Finally, parent-reported anxiety symptoms were associated with the core symptoms of ASD

(particularly RRBs) in the HR group, but this was driven primarily by the HR-ASD group.

3.4.1 Prevalence of co-occurring anxiety

Anxiety symptoms obtained from parent-report revealed heightened prevalence of anxiety symptoms in the HR group. In particular, the HR-ASD group scored higher on total anxiety and most subscales compared to the LR group. The HR-non ASD group, on the other hand, had higher separation anxiety scores than the LR group and lower panic/agoraphobia scores than the HR-ASD group, but did not differ significantly from either group on any other domain or the total score. A follow-up analysis was run (see Appendix 2) to test whether HR-non ASD children who manifested increased ASD severity (HR-Atyp group), due to scoring above cut-off on one of the clinical measures (ADOS and/or ADI-R), had increased anxiety compared to those who exhibited typical development. There was no evidence of increased anxiety severity among this group, contradicting previous research by Hallett, Ronald, et al. (2013), who found increased anxiety among twins of children with ASD who had themselves had features of BAP. These findings must be taken with some caution due to the modest size of the HR-Atyp group (n=7), as it is possible that there was not enough statistical power to detect a difference within this group. Furthermore, both Miller et al. (2015) and Schwichtenberg et al. (2013) report elevated anxiety among HR-non ASD siblings aged ~5 and ~3 years, respectively, but these studies had a much larger sample size than the one in this study. It is also important to take note of the fact that the HR-non ASD group had non-significant, elevated anxiety scores across

subscales compared to the LR group with strong effect sizes, some of which may reach significance with a larger sample size.

The finding of heightened separation anxiety among the HR group is consistent with previous literature of school-aged children with ASD (Gillott, Furniss, & Walter, 2001). This thesis extends prior findings to suggest that separation anxiety is also elevated among siblings that do not meet diagnostic criteria for ASD. Contrary to previous findings, social phobia was not elevated in the HR-ASD group (Bellini, 2004; Hallett, Lecavalier, et al., 2013). Among non-ASD children, prevalence of social anxiety increases with age and is more highly evident during adolescence (Costello, Mustillo, Erkanli, Keeler, & Angold, 2003), therefore it is possible that our sample was still too young for symptoms of social anxiety to be fully manifest.

Contrary to findings from parent-report, self-reported anxiety symptoms did not differ across groups. In general, the LR and HR-non ASD groups reported more anxious symptoms than parents did. The HR-ASD group reported slightly lower scores than parents did. Generally, this finding is consistent with reports that children with ASD tend to under-report symptoms while non-ASD children report more severe anxiety than parents do (Gillott, Furniss, & Walter, 2001; Mazefsky, Kao, & Oswald, 2011). However, this finding must be treated with caution, as the HR-ASD group mean total anxiety score was closer to parent report than in the other two groups. It is possible that parents of the HR-non ASD and LR participants underestimated anxiety levels in their children.

Parents reported higher anxiety symptoms for girls than boys across groups. However, there was a significant group by sex interaction, where this sex difference

was found to be significant only in the HR-ASD group. On self-report, there was an overall sex difference on OCD symptoms, with girls reporting more symptoms than boys, but there were no other sex differences or a group by sex interaction. There is a broad literature reporting increased anxiety symptoms among females in non-ASD populations (for review see McLean & Anderson, 2009) and findings from this chapter are in line with previous findings. However, it is unusual that sex differences were not significant in the HR-non ASD and LR groups. It is possible that this is due to the modest sample size in this study. While the HR-ASD group did have the smallest sample size, the sex differences in this group may have been strong enough to be statistically significant.

Sex differences in anxiety symptoms have not been studied as extensively among individuals with ASD and findings are currently equivocal. While some studies report no sex differences in anxiety symptoms (e.g. Lai, Lombardo, Pasco, Ruigrok, Wheelwright, Sadek, Chakrabarti, et al., 2011), others report increased internalising difficulties among adolescent girls with ASD (Solomon, Miller, Taylor, Hinshaw, & Carter, 2012) and more anxious behaviours and sleep problems among female toddlers with ASD (Hartley & Sikora, 2009). Differences across studies are likely due to differential participant characteristics and the use of self- and caregiver reported symptoms. Further investigation into sex differences in co-occurring anxiety symptoms in individuals with ASD are highly relevant to better understand ASD manifestation among females and could assist in clinical practice.

3.4.2 Inter-rater agreement and psychometric properties

Both the SCAS-P and SCAS-C had very good internal consistency in both the HR and LR groups. For the self-report questionnaire, this suggests that the children in both groups were able to understand the questions and responded consistently throughout (Mazefsky, Kao, & Oswald, 2011). On the contrary, agreement in self- and parent-reported anxiety symptoms was low to moderate across subscales and the total score for both the HR and LR groups. This is unsurprising, as numerous studies report poor agreement between child and parent accounts of anxiety symptoms (Klein, 1991), but the Spence Children's Anxiety Scale (Spence, 1998) used in this study is reported to have higher rates of agreement than other anxiety scales (Nauta et al., 2004). Inter-rater agreement for the physical injury fears subscale was significant in both groups. There was also significant agreement for separation anxiety scores in the HR group and the total anxiety score in the LR group. These findings coincide with previous research that reports improved inter-rater agreement for more overt, behavioural symptoms of anxiety (March, Parker, Sullivan, Stallings, & Conners, 1997; Zainal et al., 2014). The items that constitute physical injury fears (e.g. 'my child is scared of dogs') and separation anxiety (e.g. 'my child would be afraid of being on his/her own at home') are perhaps more readily observable than items that relate more to internal cognitions or emotions (e.g. 'my heart suddenly starts to beat too quickly for no reason').

Hallett, Ronald, et al. (2013) report higher agreement in parent- and self-reported anxiety symptoms than observed in this study. However, the participants were adolescents aged 10-15 years. It is possible that by this age, anxiety difficulties among young people with ASD become more pronounced and cause more severe impairment,

increasing parental awareness. It is equally possible that adolescents with ASD have more capability in reporting on their own symptoms than children do.

3.4.3 Association between anxiety, ASD symptoms and cognitive functioning

Among the HR group, parent-reported anxiety symptoms were significantly associated with ASD severity. When the different domains of ASD symptomatology (social, communication and RRBs) were taken together, this association was only significant for RRBs. Furthermore, a follow-up analysis revealed that the association between ASD symptoms and anxiety was specific to the HR-ASD group, but not the HR-non ASD participants. There was no association between parent-reported anxiety and cognitive functioning or language ability. Self-reported anxiety, on the other hand, was associated with expressive language ability, but not IQ or ASD severity.

As outlined in Chapter 1, the association between parent-reported anxiety and RRBs is consistent with numerous studies reporting similar associations (e.g. Hallett, Lecavalier, et al., 2013; Sukhodolsky et al., 2008). There are several accounts that try to explain the association between anxiety and RRBs in children with ASD. The first possibility is that RRBs serve to manage arousal and are, thereby, performed in an attempt to reduce the feeling of anxiety (Leekam, Prior, & Uljarevic, 2011). Uljarević and Evans (2016) examined the association between fearfulness and RRBs among children with ASD, children with Down Syndrome, and two groups of TD controls – one group that was matched on chronological age and the other group that was matched with the clinical groups on mental age. There was a significant association between fearfulness and RRBs in all groups except for the TD controls matched on chronological age. The authors suggest that RRBs serve an adaptive purpose to help

manage distress during early childhood but that, as TD children develop self-regulatory skills, the use of RRBs declines (Evans, Lewis, & Iobst, 2004). Among children with ASD, where atypicalities in executive functioning abilities are readily reported (Hill, 2004), children persist in using RRBs, instead of self-regulatory cognitive mechanisms, to manage distress. Given the developmentally inappropriate nature of this strategy, it may increase anxiety symptoms in the long term (Uljarević & Evans, 2016).

There has also been suggestion of interplay between sensory over-responsivity, which is also associated with anxiety among individuals with ASD (Green & Ben-Sasson, 2010; Green, Ben-Sasson, Soto, & Carter, 2012), and RRBs in their association to anxiety among individuals with ASD. For example, Lidstone et al. (2014) report that sensory sensitivity mediates the association between anxiety and RRBs among individuals with ASD. Similarly, using structural equation modelling, Wigham, Rodgers, South, McConachie, and Freeston (2014) suggest that the association between RRBs and sensory sensitivity is mediated by anxiety and intolerance of uncertainty. These findings suggest that individuals with ASD exhibit heightened sensory responsiveness, which is associated with increased distress, anxiety and resistance to change. RRBs may, in part, act to reduce the impact of sensory over-arousal, thereby reducing distress and anxiety.

In spite of the strong evidence supporting these theories regarding the association between anxiety and RRBs, several limitations must be considered. For example, White, Oswald, Ollendick, and Scahill (2009) note that anxiety is associated with more severe challenging behaviours among children with ASD. It is, therefore, possible that parents report higher symptoms of both ASD and anxiety when their

child's behaviour is more difficult to manage. This is especially a possibility given that the association between ASD symptoms and anxiety was only evident in parent-reported measures of both constructs within this sample. Self-reported anxiety was not associated with ASD severity at all, suggesting that it is not necessarily only children with more severe ASD that under-report anxiety symptoms.

There was also no association between parent- or self- reported anxiety and IQ, contradicting previous findings (Hallett, Lecavalier, et al., 2013; Sukhodolsky et al., 2008). However, it is important to note that in these studies, the sample is divided into groups that have normative IQ (IQ>70) and intellectual disability (IQ<70). Within the present sample, only two children fell into the range of intellectual disability and all others had normative IQ. Likewise, self-reported anxiety was associated with expressive language ability in the HR group, such that children with better language reported fewer symptoms.

3.4.4 Strengths, limitations and implications for future work

This chapter examined the prevalence of anxiety, obtained through self- and parent-report, in children at high familial risk for ASD, aged 6-8 years. Parent-report revealed that the HR group had heightened anxiety compared to LR controls and that this was particularly true for children who themselves met diagnostic criteria for ASD. On the contrary, there were no group differences on self-report. There was also moderate to poor inter-rater agreement on the self- and parent- report in both the HR and LR groups. However, the scale used had good psychometric properties in both groups. Finally, within the HR group, anxiety symptoms were associated with ASD severity, particularly RRBs.

This presents important implications for both research and clinical practice. In spite of the high prevalence of anxiety among first-degree relatives of individuals with ASD (Lainhart, 2009), the manifestation of these symptoms, particularly among younger age groups, remains under-explored. The findings from this chapter suggest that, among HR children, ASD severity and particularly RRBs are associated with anxiety. Furthermore, this association appears to be unique to children who themselves meet diagnostic criteria for ASD. CBT techniques have been modified to incorporate focus on the core features of ASD in treatment (Ung, Selles, Small, & Storch, 2015). The findings from this chapter suggest that such modifications may be useful even among young children with ASD. Furthermore, additional work is necessary to understanding the role of RRBs in the development and maintenance of anxiety in individuals with ASD.

One of the limitations of this study was the small sample size, particularly of the HR-ASD group. This was particularly highlighted when examining the post hoc power analyses, which revealed that the present sample had weak to moderate power in detecting significant effects, with power being below 50% for most analyses. Given the modest size of the HR group, it also was difficult to assess anxiety symptoms among the group of children that manifested aspects of BAP. While there was no evidence of elevated anxiety among the HR-Atypical group, this could be largely due to the small sample size. Furthermore, the unique associations between ASD symptoms and anxiety in the HR-ASD group also need to be taken with some caution given the small sample size and multiple comparisons that were run. These associations need to be examined more with a larger sample and more parsimonious statistical methods. A further limitation is that of shared method variance, where variance can be attributed to the

measures used rather than the actual constructs being assessed (Podsakoff, MacKenzie, Lee, & Podsakoff, 2003). This is particularly relevant, as the measures used to assess anxiety and ASD symptomatology were largely parent-report questionnaires, which could account for the strong association between these symptoms. It is important to note that a majority of the research investigating the association between ASD severity and co-occurring anxiety symptoms utilises a similar approach (e.g. Sukhodolsky et al., 2008). This highlights the need for more objective measures of both ASD symptomatology and anxiety that go beyond parent- and self-report questionnaires.

Furthermore, the analyses in this chapter relied primarily on parent-reported symptoms of both anxiety and ASD severity. This has important limitations for several reasons. As noted above, parents who themselves have higher psychopathology rate their children's anxiety symptoms more highly (Becker, Jensen-Doss, Kendall, Birmaher, & Ginsburg, 2016). Given the higher prevalence of both ASD symptoms and anxiety, among other conditions, in family members of children with ASD, it is possible that parents of children with ASD may have also had elevated symptoms of these conditions, thus reporting more severe anxiety and ASD severity in their children. Unfortunately, this study did not include measures of parental psychopathology or functioning, limiting the ability to formally examine this in analyses.

The findings from this chapter highlight the need to investigate the associations between the neurocognitive factors that are associated with anxiety in non-ASD populations and parent-/self-report of anxiety in children with ASD. Such investigation may help elucidate whether the mechanisms that correlate with anxiety are also present among individuals with ASD and elevated anxiety symptoms. This will be addressed

further using a threat bias task in Chapter 5. The longitudinal associations between anxiety and ASD need to be examined to help disentangle some of the associations between core ASD symptoms and anxiety severity, which will be done in Chapter 6.

Chapter 4
Review of threat bias paradigms used to assess cognitive mechanisms associated with anxiety in young children

4.1 Introduction

Anxiety disorders are highly prevalent and can cause significant impairment among children and adolescents (Langley, Bergman, McCracken, & Piacentini, 2004). Up to 20% of children are reported to experience symptoms of an anxiety disorder (Beesdo, Knappe, & Pine, 2009). While the prevalence and severity of paediatric anxiety is more commonly reported in older age groups, there is evidence to suggest that it is present in early childhood, by the age of ~3 years, at the same rates as in older children (Egger & Angold, 2006; Franz et al., 2013). Despite this, there is presently a scarcity in research investigating the symptom presentation and neurocognitive correlates of anxiety in early childhood (Hirshfeld-Becker, Micco, Mazursky, Bruett, & Henin, 2011). This is highly relevant as treatment of anxiety disorders is most frequently aimed at restructuring the cognitive underpinnings of the disorder (Hofmann & Smits, 2008; James, James, Cowdrey, Soler, & Choke, 2013) and research during early childhood is necessary to inform clinical practice for early interventions.

The investigation of the neurocognitive correlates of anxiety in early childhood is relevant both for this thesis and research on co-occurring anxiety in ASD more broadly. There is a vast body of research aimed at examining the prevalence of co-occurring anxiety within ASD and how anxiety symptoms relate to the core features of ASD (Hallett, Lecavalier, et al., 2013; Simonoff et al., 2008; White, Oswald, Ollendick,

& Scahill, 2009). However, there remains a dearth in research examining the neurocognitive correlates of anxiety among individuals with ASD. Such investigation is necessary to help characterise the aetiology and manifestation of anxiety in ASD. It is important to understand whether the cognitive architecture of anxiety among individuals with ASD is similar to that observed in non-ASD populations. Likewise, given that research into the neurocognitive correlates of anxiety in ASD remains novel, evidence from non-ASD populations can serve as a foundation for identifying key mechanisms and measures. However, given the heterogeneity of ASD symptoms and diverse functioning among individuals with the condition (Happe, Ronald, & Plomin, 2006; Jeste & Geschwind, 2014; Ring, Woodbury-Smith, Watson, Wheelwright, & Baron-Cohen, 2008), specific modifications may be required to existing techniques to make them more suitable for the ASD population. For example, intellectual disability and reduced communicative capacity are prevalent in ASD (Boucher, 2003; Matson & Shoemaker, 2009), therefore it is necessary to identify measures that would allow individuals with these difficulties to participate. Furthermore, the age of the participants in this study is relatively young (6-8 years). Therefore, it is necessary to go beyond general paediatric research and focus specifically on investigating younger age groups to identify appropriate measures for young children and those who may be non-verbal or have intellectual disability.

4.1.1 Cognitive mechanisms associated with anxiety

As discussed in Chapter 1, prominent cognitive theories posit that anxiety is characterised by maladaptive cognitive schemas, which predispose an individual to biasedly process or construe information, favouring aspects of the environment that are

deemed threatening or dangerous (Beck & Clark, 1997; Beck, Emery, & Greenberg, 1985; Eysenck, 1992). Such cognitive biases are considered an important aspect of the aetiology and maintenance of anxiety disorders (Bar-Haim, Lamy, Pergamin, Bakermans-Kranenburg, & van IJzendoorn, 2007). It is important to also note that, due to evolutionary factors, most healthy individuals are primed to respond more intensely to threatening stimuli because it signals the presence of danger in their environment (LoBue & DeLoache, 2008). However, this bias is *enhanced* among those who have heightened anxiety and persists in the absence of objective threat or danger (Beck, Emery, & Greenberg, 1985). The association between cognitive biases to threat and anxiety symptoms has been demonstrated experimentally in a myriad of research (Clark & Beck, 2010). The most prominent mechanisms that have been associated with anxiety are *attentional bias* and *interpretative bias* of threat (Bar-Haim, Lamy, Pergamin, Bakermans-Kranenburg, & van IJzendoorn, 2007; Castillo & Leandro, 2010; Hadwin, Garner, & Perez-Olivas, 2006).

Attentional bias is most frequently measured using tasks that compare reaction times (RTs) to threatening and non-threatening stimuli. Multiple paradigms have been used to assess how threat bias manifests in different components of attention. The dot-probe and emotional Stroop tasks, which measure hypervigilance to and interference caused by threatening stimuli, respectively, are the most widely used measures of threat bias. In the dot-probe paradigm, a threatening stimulus and a non-threatening stimulus are presented simultaneously and, after their offset, a probe is presented in the same spatial location as one of the stimuli (MacLeod & Mathews, 1988; MacLeod, Mathews, & Tata, 1986). Individuals with heightened anxiety are reported to be faster in detecting the probe when it had previously been paired with a threatening stimulus than a non-

threatening one, suggesting that they are displaying hypervigilance for threat (Bar-Haim, Lamy, Pergamin, Bakermans-Kranenburg, & van IJzendoorn, 2007; MacLeod & Mathews, 1988; MacLeod, Mathews, & Tata, 1986). There has also been suggestion that hypervigilance can be observed when threatening stimuli are presented for short durations (e.g. <500ms), while attentional avoidance occurs when stimuli are presented for longer durations (e.g. >1000ms) and individuals with heightened anxiety take longer to respond to a probe that has been paired with the threatening stimulus (Koster, Verschuere, Crombez, & Van Damme, 2005; Mogg, Bradley, De Bono, & Painter, 1997; Mogg, Bradley, & Hallowell, 1994; Mogg, Bradley, Miles, & Dixon, 2004). However, this finding has been somewhat inconsistent and attentional orienting depends on multiple factors, including the type of stimuli used and its visual properties (Koster, Verschuere, Crombez, & Van Damme, 2005).

The emotional Stroop task (MacLeod, Mathews, & Tata, 1986) is a modified version of Stroop's original paradigm, where participants are asked to read names of colours that have been presented in colour-congruent (e.g. the word "blue" printed in blue ink) or colour-incongruent (e.g. the word "blue" printed in red ink) stimuli (Stroop, 1935). In the emotional version, threatening and non-threatening stimuli are paired with different colours and participants are asked to name the colours (MacLeod, Mathews, & Tata, 1986). Participants with heightened anxiety are reported to be slower to name a colour that has been paired with a threatening stimulus than a non-threatening one (Bar-Haim, Lamy, Pergamin, Bakermans-Kranenburg, & van IJzendoorn, 2007). While these two tasks are the most widely used, numerous other paradigms have been used to suggest that anxiety is associated with other aspects of attention as well, such as delayed disengagement from threat (Fox, Henderson, Rubin,

Calkins, & Schmidt, 2001; Yiend & Mathews, 2001). Attentional disengagement from threatening stimuli will be discussed in greater detail in Chapter 5.

Interpretation bias refers to the tendency of individuals with elevated anxiety to assign threatening meaning to ambiguous stimuli or scenarios (Castillo & Leandro, 2010; Hadwin & Field, 2010; Hadwin, Garner, & Perez-Olivas, 2006). Paradigms used to evaluate interpretative biases require participants to evaluate emotionally ambiguous stimuli. Studies using such paradigms report that individuals with heightened anxiety evaluate ambiguous stimuli as more threatening than non-anxious participants do (Castillo & Leandro, 2010; Hadwin, Garner, & Perez-Olivas, 2006).

4.1.2 Threat bias in childhood anxiety

Both attentional and interpretative bias have been studied among children and, while there is less consistency than in adult research, evidence suggests that an association between threat bias and anxiety can be detected in childhood (Roy et al., 2008; Waters, Mogg, Bradley, & Pine, 2008). However, Hadwin and Field (2010) suggest that a limitation of using threat bias paradigms in child populations is that they have been directly acquired from adult research and may not capture crucial developmental aspects of threat cognition. Research with children also often includes participants with wide age ranges, from middle childhood to late adolescence (e.g. Roy et al., 2008), but data is not analysed separately for different age groups, making it difficult to discern age-related differences in cognitive bias. In addition to this, two recent studies suggest that RT-based paradigms may not be best suited to detecting bias to threat in young children (Brown et al., 2014; Dudeney, Sharpe, & Hunt, 2015). Dudeney, Sharpe, and Hunt (2015) suggest that the dot probe and emotional Stroop

tasks are widely used in child research, but that the latter has had more success in detecting an association between threat perception and anxiety. However, across tasks, the association between anxiety and threat bias is more readily observable among older age groups. Brown et al. (2014) evaluated the psychometric properties of several RT tasks (including the emotional dot probe, visual search and Stroop tasks) in children aged 8-10 years. Among this age group, the tasks exhibited poor reliability, weak association to anxiety symptoms and there was low convergence on performance across tasks.

It is important to note that there are several limitations present in these two studies, which make it difficult to fully characterise the association between threat bias and anxiety in younger age groups. For example, Dudeney, Sharpe, and Hunt (2015) did not include several prominent studies of threat bias in childhood, which largely include RT-based paradigms, such as the dot probe task, with school-aged children (e.g. Waters & Kershaw, 2015). Furthermore, when Brown et al. (2014) examined the association between anxiety and threat bias, stringent criteria were put in place to account for multiple testing that resulted from including a variety of measures (α=.05/32). Therefore, it is unclear whether the criteria of these two studies made it more challenging to detect an association between RT-based tasks measuring threat bias and anxiety symptoms. Nevertheless, these studies do raise concern about the use of RT-based paradigms among school-aged children. Additionally, when applying these paradigms to even younger, pre-school aged children, several additional limitations may need to be considered.

4.1.3 Challenges to evaluating anxiety and cognitive bias in early childhood

There is growing evidence that anxiety emerges early in childhood and that its prevalence among pre-school aged children is comparable to rates reported in older children (Egger & Angold, 2006; Franz et al., 2013). Yet, despite this, much of the research on paediatric anxiety focuses on children who are school-aged (~8 years) and older (Hirshfeld-Becker, Micco, Mazursky, Bruett, & Henin, 2011). This is largely due to the belief that school-aged children have developed sufficient verbal and reading capacity to complete the necessary measures (Jensen, Fabiano, Lopez-Williams, & Chacko, 2006). Furthermore, many parent-report measures of anxiety symptoms have been validated among children aged ~8 years and older (e.g. Nauta et al., 2004). There are fewer measures available for use with pre-school aged children, although the ones available have been well-validated (e.g. Spence, 1998).

Some of the problems identified in applying prominent threat bias tasks in child research may be even more pronounced among younger, pre-school aged children. For example, the use of RT-based paradigms may be more challenging because young children tend to exhibit higher variation in RT performance than older children do, making it difficult to detect significant differences across groups (Lange-Küttner, 2012). Developmental theories posit that threat bias may not be observable in early childhood, as the attentional and emotional skills required to maintain such biases may not yet be fully developed (Field & Lester, 2010). Field and Lester (2010) propose that, early in development, all children exhibit heightened attentiveness to threat-relevant information. Over time, typically developing children learn to inhibit responses to threatening stimuli, while heightened attentiveness to threat persists among children

who develop anxiety. Therefore, it may not be possible to detect differences in threat perception among anxious and non-anxious children early in development. Furthermore, the cognitive mechanisms elicited by threat bias paradigms, such as the ability to inhibit responding to certain stimuli, may not be fully developed in pre-school aged children (Iida, Miyazaki, & Uchida, 2010). Consequently, it may be difficult to demonstrate differences in an aspect of cognition among anxious and non-anxious children if neither group has fully developed this cognitive ability. Finally, the use of interpretation bias paradigms may also be challenging to use among very young children, as performance on such tasks relies on story comprehension and verbal responding, which are not fully developed in early childhood (Tompkins, Guo, & Justice, 2013).

4.1.4 Threat bias and temperament in early development

While the use of threat bias paradigms in paediatric research has been called into question, studies examining threat processing in early childhood suggest that typically developing children as young as ~3 years exhibit enhanced attending to evolutionarily threatening stimuli, such as snakes (LoBue & DeLoache, 2008). Furthermore, research investigating the cognitive mechanisms associated with temperamental traits in early development suggests that it is possible to detect differential responding to threat among children with distinct temperamental profiles (Cole, Zapp, Fettig, & Perez-Edgar, 2016; LoBue & Perez-Edgar, 2014; Nakagawa & Sukigara, 2012; Perez-Edgar, McDermott, et al., 2010; Perez-Edgar et al., 2011). For example, pre-school aged children that have temperamental profiles associated with the later development of anxiety (e.g. Negative Affect, Behavioural Inhibition) exhibit both

increased hypervigilance to threatening stimuli on an RT-based paradigm (LoBue & Perez-Edgar, 2014), as well as prolonged disengagement from threat on an eye-tracking task (Nakagawa & Sukigara, 2012). Attentional bias to threat, measured using the dot probe task, in early childhood (~5 years) has also been found to moderate the association between early temperamental risk and the later development of anxiety problems. Children with more difficult temperament, who also exhibit greater threat bias, are more likely to develop anxiety difficulties later in childhood (Cole, Zapp, Fettig, & Perez-Edgar, 2016; Perez-Edgar, Bar-Haim, et al., 2010; Perez-Edgar et al., 2011).

These findings suggest that pre-school children can reliably perform threat bias tasks and that their performance maps on to both parent-report and laboratory observation of temperament. The next important step is to determine whether an association between threat bias and anxiety can be detected using the measures outlined, and to identify the paradigm(s) best capable of achieving this.

4.1.5 Aims of the present review

The aim of this chapter is to facilitate evidence-based investigation of the cognitive correlates of anxiety in early development. The specific objective is to conduct a mixed-methods literature review on the association between threat bias and anxiety among young and pre-school aged children to address the following questions:

1) Is it possible to detect an association between threat bias and anxiety among children younger than school-age?

2) If it is possible to detect an association between threat bias and anxiety, which paradigms are the most effective in measuring this association?
3) Do specific modifications need to be made to existing paradigms to make them more suitable for younger age groups?

After identifying the paradigms that are used to measure threat bias, specific aspects of the methodology will be evaluated to determine whether these measures are suitable for testing children with ASD. In particular, modifications that threat bias paradigms may require among children with ASD will be discussed.

4.2 Method

4.2.1 Mixed-Method Review

The present review used a mixed-method approach, whereby a systematic search strategy, with specific inclusion and exclusion criteria, was employed to identify relevant records to include in the review. However, no specific criteria were used to evaluate the quality of the records included. Instead, a narrative approach was employed to describe the methods used in each study and to report on the main findings. This mixed-methods approach was selected as the aim of this chapter was to describe and reflect on the various methods used to assess threat bias among young children. Thus, a more inclusive approach, which facilitated a discussion of the strengths and limitations of various methods was deemed more appropriate. Furthermore, while specific criteria have been established to evaluate the quality of research papers describing healthcare interventions (e.g. Downs & Black, 1998), many of the criteria described are not directly relevant when assessing experimental research.

There are several key limitations in research examining threat bias, which were taken into consideration when evaluating the quality of records included in this review. These limitations include inconsistency in the specific details of the experimental tasks, variation in the analytic approach used to measure the association between threat bias and anxiety, and differential participant characteristics across studies (Cisler, Bacon, & Williams, 2009). The present review used inclusion/exclusion criteria and data extraction methods that were aimed at addressing some of these limitations. There were several criteria that were considered particularly relevant in assessing the quality of research records and informing the inclusion criteria and data extraction strategies. The criteria included (1) use of a validated measure of anxiety symptoms, (2) inclusion of a detailed description of participant characteristics and (3) inclusion of a comprehensive description of the experimental procedure and analyses performed.

4.2.1 Search Strategy

Systematic searches were conducted in electronic bibliographic databases, including Web of Science (all databases) and Ovid (Psych*INFO,* Psych Articles, Embase, Medline). Searches were restricted to English-language papers, published in peer reviewed journals. The following search terms were used: (threat bias* OR attention bias* OR interpretation bias* OR cognitive bias* OR emotional stimuli*) AND anxiety AND (children* OR early childhood* OR young children* OR preschool*). The terms were entered as free text and all results were evaluated by hand, based on the inclusion and exclusion criteria (outlined below). Reference lists of the articles selected for inclusion were examined to identify any further articles that met inclusion criteria. Finally, an author search was conducted in the above databases for

authors of the articles selected for review. Year of publication was not restricted and searches were conducted between December 2015 and February 2016.

4.2.2 Inclusion and Exclusion criteria

To be included in this review the articles must: a) have tested typically developing child participants, aged 8 years and younger, b) examined cognitive bias to threat, c) included a validated measure of anxiety symptoms, and d) examined the association between cognitive bias to threat and anxiety. Exclusion criteria included: a) having participants aged over 8 years, b) studies where anxiety-related constructs (e.g. temperament, spider fear) were measured instead of anxiety, c) case studies or observational studies.

4.2.3 Data Extraction

Data extraction was performed by the first author (BM) and 20% of the selected studies were reviewed by the second reviewer to ensure that they met inclusion/exclusion criteria and that selection was not biased. Data extraction was performed to examine the methodology used to assess cognitive bias and specific task design features (e.g. stimulus type, stimulus presentation duration, participation required from children), participant characteristics (age, sex, IQ), anxiety symptomatology (clinical anxiety vs. sub-clinical symptoms, anxiety type, state vs. trait anxiety), and findings (association between cognitive bias and anxiety). The present review refrained from performing statistical analyses on effect sizes, as the aim was to evaluate specific aspects of the methodology used rather than the magnitude of findings.

4.3 Results

4.3.1 Search results

Figure 2 illustrates the results of the search strategy. The search terms yielded 501 records, 253 of which were duplicates. A further 3 records were identified by searching the references of the articles included. The 251 records were screened for eligibility and 229 papers were excluded. The 22 remaining records were evaluated using the inclusion/exclusion criteria and 4 were excluded, resulting in 18 records eligible for data extraction.

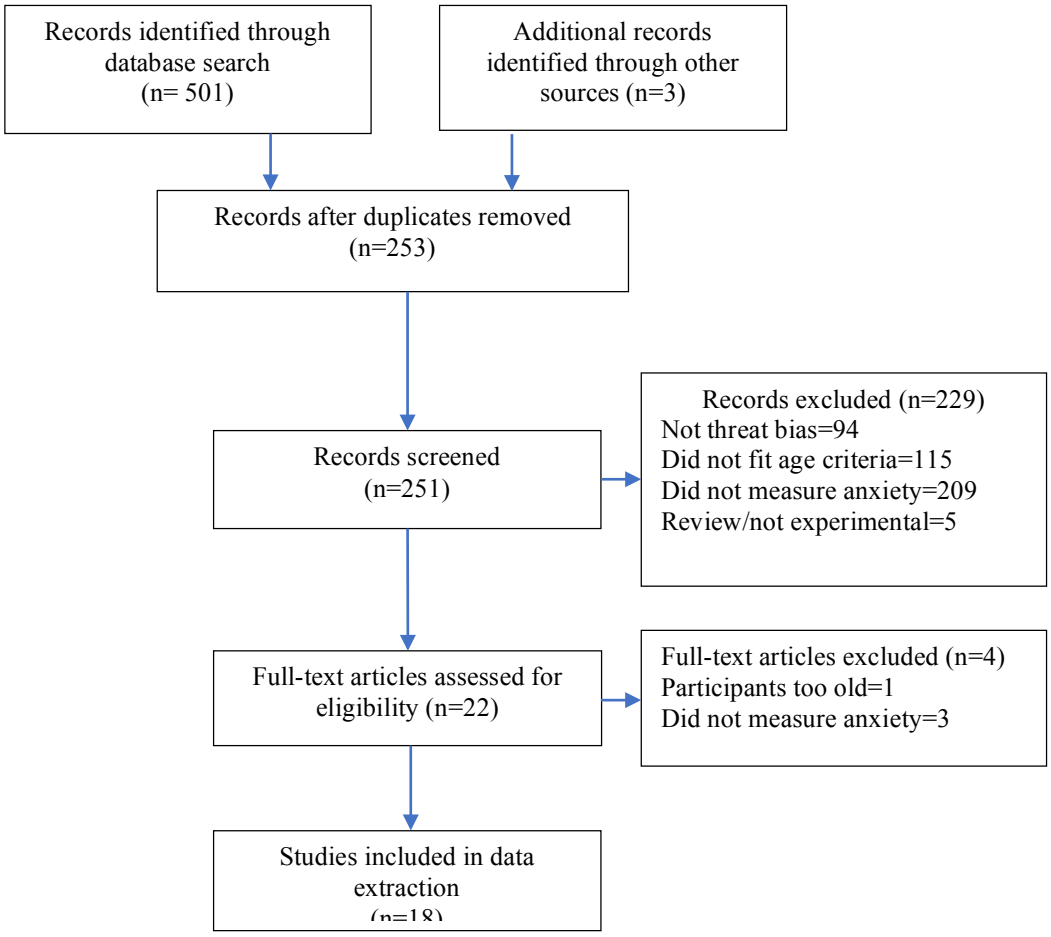

Figure 2. Flow-chart summarising stages of the systematic search

4.3.2 Data extraction

Of the 18 studies that met inclusion criteria, the information of interest was the type of tasks used and their features (e.g. stimuli, duration of stimulus presentation), age group of participants, measures used to assess anxiety symptoms, and reported association between anxiety symptoms and threat bias. The findings are broken down into studies using attentional bias, interpretation bias and working memory tasks and those using measures of neural activity and physiological arousal. A summary of the studies reviewed is presented in Table 9.

4.3.3 Measure used and task characteristics

Five studies measured attentional allocation to threatening, compared to neutral stimuli. Four of these studies used the dot probe paradigm to examine hypervigilance to (Briggs-Gowan et al., 2015; Mian, Carter, Pine, Wakschlag, & Briggs-Gowan, 2015; Susa, Pitică, Benga, & Miclea, 2012) and one examined avoidance (Brown et al., 2013) of threat in children with elevated anxiety and controls. In all studies, human facial expressions were used as stimuli and threat was induced through negative (angry or disgusted) facial expressions and compared to happy and neutral ones. The length of stimulus presentation was consistent across studies, being shown for 500ms in all paradigms measuring hypervigilance and only differed in the study examining avoidance, where stimuli were presented for 1000ms. All studies using the dot probe task reported a significant association between attentional allocation to threatening stimuli and symptoms of anxiety. Two studies (Brown et al., 2013; Mian, Carter, Pine, Wakschlag, & Briggs-Gowan, 2015) examined both categorical and dimensional associations between threat bias and anxiety, while one used a dimensional approach

only (Briggs-Gowan et al., 2015; Susa, Pitică, Benga, & Miclea, 2012). All studies reported a significant association between threat bias and anxiety, demonstrating that children with elevated anxiety exhibited greater threat bias compared to children with low anxiety. Furthermore, using a dimensional approach, anxiety symptom severity was significantly associated with threat bias scores.

One study (Dodd et al., 2015) examined attentional allocation to angry and neutral face pairs using an eye-tracking paradigm. *Initial hypervigilance* for angry, compared to neutral, faces was assessed by comparing how often participants made first fixations to faces showing each emotion type upon stimulus presentation. *Attentional maintenance* was also assessed by comparing how long participants viewed each stimulus type after first fixation. Stimuli were presented for 1250ms and of particular interest for hypervigilance were fixation patterns at 500ms, to be consistent with data reported from studies using the dot probe paradigm. There were no significant group differences in hypervigilance or attentional maintenance for angry and neutral faces. However, children with high anxiety spent less time looking at faces overall compared to non-anxious children.

Four studies examined the association between interpretation bias of ambiguous stimuli and anxiety symptoms (Berry & Cooper, 2012; Dodd, Hudson, Morris, & Wise, 2012; Eley et al., 2008; Ooi, Dodd, & Walsh, 2015). All studies used the ambiguous stories task, where participants were presented with scenarios that had ambiguous emotional valance and were asked to complete the sequence of events in the stories. The number of threatening interpretations were recorded and compared across anxious and non-anxious participants. Dodd, Hudson, Morris, and Wise (2012) and Ooi, Dodd,

and Walsh (2015) used scenarios that related to physical threat, social threat and separation anxiety, while Berry and Cooper (2012) presented social scenarios only. Participants were instructed to provide responses in different ways across studies. Two studies (Dodd, Hudson, Morris, & Wise, 2012; Ooi, Dodd, & Walsh, 2015) used story stems, while one study (Eley et al., 2008) provided participants with 4 response options that varied in valance (threatening and non-threatening) and one study (Berry & Cooper, 2012) encouraged participants to make as many attributions as possible. Additionally, Ooi, Dodd, and Walsh (2015) allowed participants to use toys and props to facilitate responses. While all studies used ambiguous stories, some incorporated additional tasks. Berry and Cooper (2012) also measured *reappraisal* by asking participants to make alternative interpretations of the ambiguous stories. Eley et al. (2008) used both ambiguous stories and a homophone task, in which homophones (words with the same pronunciation but different meaning) that could be interpreted as threatening, neutral or positive were presented, and asked participants to construct sentences with each word.

Interpretation bias paradigms produced varied findings when the association between threat interpretation and anxiety was evaluated. Two studies (Berry & Cooper, 2012; Ooi, Dodd, & Walsh, 2015) found no significant association between child anxiety symptoms and threatening interpretations. However, Ooi, Dodd, and Walsh (2015) asked parents to describe how they would explain the stories to their children and child threat interpretation was significantly associated with the threat content in the parents' explanations. Dodd, Hudson, Morris, and Wise (2012) report that highly anxious children made more threatening interpretations than non-anxious children did. Anxiety symptoms were measured longitudinally and threat interpretation at baseline

was also associated with anxiety symptoms 1 year later, but not 2 or 5 years later. Finally, Eley et al. (2008) reported a significant association between threatening interpretations and anxiety, but this association became non-significant when symptoms of depression were controlled for.

Four studies examined the neural correlates of threat processing and anxiety (DeCicco, O'Toole, & Dennis, 2014; DeCicco, Solomon, & Dennis, 2012; O'Toole, DeCicco, Berthod, & Dennis, 2013; Solomon, DeCicco, & Dennis, 2012). Three of these studies examined the *Late Positive Potential (LLP)*, an Event Related Potential (ERP) component on the visual-cortical areas that is modulated by emotional content of visual stimuli, while viewing threatening and non-threatening images. DeCicco, Solomon, and Dennis (2012) also presented threatening stories alongside the images. These two studies also examined *reappraisal*, where participants were provided with positive information alongside the threatening images, to see if this would result in a change in the pattern of LLP response. The stimuli used in all three studies were images of threatening, pleasant and neutral scenes taken from the International Affective Picture System database (IAPS; Lang, Bradley, & Cuthbert, 2008) and were presented for 2000ms. DeCicco, Solomon, and Dennis (2012) report an association between anxiety symptoms and heightened LLP activity during the viewing of threatening images. DeCicco, O'Toole, and Dennis (2014) report that greater reduction in LLP activity during reappraisal was associated with lower anxiety symptoms.

O'Toole, DeCicco, Berthod, and Dennis (2013) used a flanker task, where faces depicting threatening (angry) and non-threatening (happy and neutral) expressions were presented as distractor stimuli for 200ms. EEG activity was recorded alongside the

flanker task, with specific focus on the N170 ERP, which represents neural processing of faces. Anxiety symptoms were measured at baseline and at a follow-up 2 years later. N170 activity was not significantly associated with anxiety symptoms at baseline, but increased N170 activity to angry compared to happy faces was associated with increased anxiety symptoms 2 years later.

Three studies examined the impact of threatening stimuli on working memory functioning in children with elevated anxiety (Cheie & Visu-Petra, 2012; Cheie, Visu-Petra, & Miclea, 2012; Visu-Petra, Ţincaş, Cheie, & Benga, 2010). The paradigms used range from simple immediate and delayed recall tasks (Cheie & Visu-Petra, 2012), visual search (Cheie, Visu-Petra, & Miclea, 2012) and odd one out tasks (Visu-Petra, Ţincaş, Cheie, & Benga, 2010). Cheie, Visu-Petra, and Miclea (2012) used threatening and neutral words and faces as stimuli in the immediate/delayed recall memory task. They report that, among anxious children, immediate recall was poorer for threatening words, while delayed recall was poorer for neutral words. On the contrary, anxious children had superior recollection of angry faces and poorer memory for happy faces. Visu-Petra, Ţincaş, Cheie, and Benga (2010) report that, on an odd one out task, highly anxious children had worse performance than controls when a happy face was the odd one out, but performed equally to controls when an angry face was the odd one out. Furthermore, throughout the task, highly anxious children had slower reaction times than low anxious children, except when the target was an angry face. Finally, Cheie, Visu-Petra, and Miclea (2012) used a visual search-memory detection task to examine working memory in the ability to detect a probe presented alongside different images and to recognise a probe previously presented, in the presence of angry, neutral and happy faces. Overall, highly anxious children took longer to complete the task,

particularly when executive functioning demands were higher, but there was no impact of stimulus type.

One study (Fulcher, Mathews, & Hammerl, 2008) examined evaluative learning, where faces showing neutral expressions were morphed to exhibit either happy or angry expressions. Participants were later presented with the original neutral faces and asked to rate how much they liked them. Eye-tracking was used in conjunction with the learning task to examine whether there were differences between anxious and non-anxious children in attention to faces that morphed into happy or angry expressions. Anxiety symptoms were significantly associated with the magnitude of children's evaluative learning. All children rated the faces that had been morphed into angry expressions as less likeable, but this was particularly enhanced among those who had heightened anxiety.

Finally, one study by Waters, Neumann, Henry, Craske, and Ornitz (2008) measured startle response and skin conductance in response to threatening stimuli. Children completed an emotion labelling task, where angry, neutral and happy facial expressions were presented, while arousal measures were taken concurrently with the task. Furthermore, if an expression was labelled incorrectly, the answer that was given was recorded. Within the anxious group, there was a significant association between startle response and accuracy in labelling neutral expressions. Higher anxiety scores were associated with reduced accuracy in labelling neutral faces. Furthermore, children who showed greater startle responses were more likely to mislabel neutral faces as sad.

4.3.4 Age differences on task performance

Participant age across studies ranged between 2 (Ooi, Dodd, & Walsh, 2015) and 8 years (DeCicco, O'Toole, & Dennis, 2014; Eley et al., 2008). Studies with the youngest participants, where children were aged 2-5 years (Ooi, Dodd, & Walsh, 2015) and 3-4 years (Dodd et al., 2015) did not detect a significant association between responsiveness to threat and anxiety symptoms. In studies where the age range was wider and included children aged 4 years and older (e.g. Briggs-Gowan et al., 2015; Cheie & Visu-Petra, 2012), a significant association between threat and anxiety did emerge. Two studies examined the association between age and task performance. DeCicco, Solomon, and Dennis (2012) reported that the neural correlates of reappraisal of threatening stimuli were observed in older children only, suggesting that this ability may not be fully developed in younger age groups. On the other hand, Fulcher, Mathews, and Hammerl (2008) compared children aged 7-8, 10-12 and 14-15 on their ability to perform the task (i.e. whether the evaluative learning effect could be observed) and did not find significant differences, although the association between the magnitude of evaluative learning and anxiety was not measured in children older than 8 years.

4.3.5 Anxiety measures

One study tested children with clinically diagnosed anxiety (Waters, Neumann, Henry, Craske, & Ornitz, 2008), while the rest used dimensional measures of anxiety symptoms. Parent-report questionnaires were used in all studies and two studies also employed a newly developed observational measure of anxiety symptoms, the Anxiety Dimensional Observation Scale (Anx-DOS; Mian, Carter, Pine, Wakschlag, & Briggs-

Gowan, 2015), alongside parent report. Both studies using the Anx-DOS report an association between observed anxiety symptoms, particularly fearfulness, and threat bias. However, Briggs-Gowan et al. (2015) report no association between task performance and parent-reported anxiety symptoms.

Table 9: Summary of study designs and participant characteristics of records included in review

Authors (Year)	**Cognitive Mechanism**	**Task Type**	**Stimuli**	**Anxiety Measure**	**Sample Size**	**Age Range**	**Target Group**	**Primary findings and Effect Sizes**
Briggs-Gowan et al. (2015)	Hypervigilance	Dot-Probe	Angry, happy and neutral faces	PAPA, Anx-DOS	218	48-84 months	Children of mothers who reported partner violence within the last year and scored above 80% on disruptive behaviour	Children with greater bias towards threat had higher anxiety than those with bias away from threat or no bias.
Mian, Carter, Pine, Wakschlag & Briggs-Gowan (2015)	Hypervigilance	Dot-Probe	Angry, happy and neutral faces	PAPA, Anx-DOS	252	37-87 months	Children who experienced domestic violence	Increased scores on fearfulness scale of Anx-DOS associated with greater bias towards angry faces (β=.19).
Brown, McAdams, Lester, Goodman, Clark & Eley (2013)	Avoidance	Dot-Probe	Negative, positive and neutral faces	DAWBA	247 twin pairs	8 years	Twin pairs for whom one twin had parental report of elevated anxiety symptoms at 7 years	Anxious children showed more avoidance of threatening faces than non-anxious children (d=.37)
Susa, Pitica, Benga & Miclea (2012)	Hypervigilance	Dot-Probe	Angry, happy and neutral faces	Spence Preschool Anxiety Scale	56	n/a (M=6 years)	Typically developing	There was a significant association between threat bias and anxiety (b=.02).

Authors (Year)	Cognitive Mechanism	Task Type	Stimuli	Anxiety Measure	Sample Size	Age Range	Target Group	Primary findings and Effect Sizes
Dodd, Hudson, Williams, Morris, Lazarus & Byrow (2015)	Hypervigilance and maintained attention	Passive viewing with eye-tracking	Angry and neutral child faces	ADIS-P	83	3-4 years	Children with elevated levels of BI (+1SD above group mean)	No group differences in initial fixation (d=.15) or sustained attention (η^2=.02) to angry faces.
Berry & Cooper (2001)	Interpretation bias and reappraisal	Ambiguous stories	Stories relating to self and others	RCMAS	60	6-7 years	Typically developing	No difference between high and low anx. in *self-referent* (d=.45) or *other referent* (d=.34) interpretations
Dodd, Hudson, Morris & Wise (2011)	Interpretation bias	Ambiguous stories	Stories relating to physical and social threat and separation anxiety	ADIS-P, Spence Preschool Anxiety Scale	131	3-4 years	Children with elevated levels of behavioural inhibition (BI)	Highly anxious children made more negative interpretations than non-anxious children (d=.51)
Eley et al. (2008)	Interpretation bias	Ambiguous stories, homophone task	Ambiguous scenarios and homophones	SCARED, Anxiety-related behaviour measure	299	8 years	High parent reported anxiety at age 7 years	Significant association between threatening interpretations in ambiguous scenarios and anxiety (r^2=.003)

Authors (Year)	Cognitive Mechanism	Task Type	Stimuli	Anxiety Measure	Sample Size	Age Range	Target Group	Primary findings and Effect Sizes
Ooi, Dodd & Walsh (2015)	Interpretation bias	Ambiguous stories task	Stories relating to physical, social threat and separation anxiety	Revised Preschool Anxiety Scale	50	2y, 7m – 5y, 8m	Typically developing	No significant association between anxiety and interpretation bias (r^2=.0001)
DeCicco, O'Toole & Dennis (2014)	Late Positive Potential ERP	Passive viewing and directed reappraisal	Negative, positive and neutral images	RCMAS-II	44	87-113 months	Typically developing	Larger LLP difference scores during reappraisal of negative images associated with reduced anxiety (r^2=.18)
DeCicco, Solomon & Dennis (2012)	Late Positive Potential ERP	Passive viewing and directed reappraisal	Negative, positive and neutral images	CBCL	34	5-7 years	Typically developing	LLP during negative image viewing associated with greater anxiety scores (r^2=.14)
Solomon, DeCicco & Dennis (2012)	LLP ERP	Passive viewing and directed reappraisal	Negative and neutral images	CBCL	59	60-84 months	Typically developing	No significant association between LLP and anxiety but LLP to unpleasant images associated with more fearfulness (r^2=.14)

Authors (Year)	Cognitive Mechanism	Task Type	Stimuli	Anxiety Measure	Sample Size	Age Range	Target Group	Primary findings and Effect Sizes
O'Toole, DeCicco, Berthod & Dennis (2013)	N170 ERP	Attention network task	Angry, neutral and happy faces	CBCL	51	5-7 at baseline, 7-9 at FU	Typically developing	Higher N170 amplitudes to angry faces associated with anxiety 2 years later (r^2=.53)
Cheie & Visu-Petra (2012)	Working memory	Immediate and delayed recall	Negative, positive and neutral faces; negative, positive and neutral words	Spence Preschool Anxiety Scale	76	45-85 months	Typically developing	Anxious children had worse memory for negative words in immediate recall (η_p^2=.06) and for neutral words in delayed recall (η_p^2=.06). They were also less able to recognise happy faces (η_p^2=.09)
Cheie, Visu-Petra & Miclea (2012)	Memory	Working memory detection task	Angry, happy and neutral faces	Spence Preschool Anxiety Scale	65	n/a (M=5.19 years)	Typically developing	No association between anxiety and memory of threatening faces.

Authors (Year)	Cognitive Mechanism	Task Type	Stimuli	Anxiety Measure	Sample Size	Age Range	Target Group	Primary findings and Effect Sizes
Visu-Petra, Tincas, Cheie & Benga (2010)	Memory	Odd one out task	Angry, happy and neutral faces	Spence Preschool Anxiety Scale	60	59-88 months	Typically developing	Highly anxious children more accurate in detecting probes following angry faces than low anxiety children.
Fulcher, Matthews & Hammerl (2008)	Evaluative Learning	Evaluative learning task	Ideographs of morphed faces	MASC	44	7-8 years	Typically developing	Anxiety significantly associated with evaluative learning of threatening faces (r^2=.13)
Waters, Neumann, Henry, Craske & Ornitz (2008)	Physiological arousal, emotion recognition	Emotion labelling task	Angry and neutral faces	ADIS-C; SCAS-P	25	4-8 years	Children with clinical anxiety	Higher anxiety associated with reduced accuracy in labelling neutral faces (r^2=.36)

The following acronyms were used for anxiety measure names: ADIS-P is Anxiety Disorders Interview Schedule – Parent Version, ADIS-C Anxiety Disorders Interview Schedule – Child Version, SCARED Screen for Child Anxiety Related Disorders, MASC Multidimensional Anxiety Scale for Children, PAPA Preschool Age Psychiatric Assessment, Anx-DOS Anxiety Dimensional Observation Scale, CBCL Child Behavior Checklist, SCAS-P Spence Children Anxiety Scale – Parent Version; RCMAS-II Revised Children's Manifest Anxiety Scale – 2nd Edition; DAWBA Development and Wellbeing Assessment.

4.4 Discussion

4.4.1 Summary

The present study employed a mixed-method approach to reviewed literature examining the association between cognitive bias to threat and anxiety in early childhood. While emerging research into the prevalence of anxiety in young children suggests that symptoms of the condition are present among children as young as the age of 3 years (Egger & Angold, 2006; Franz et al., 2013), there is presently a dearth in research examining the neurocognitive correlates of anxiety in early development. The scarcity in research was mirrored by the findings of this review, which identified only 18 studies that have examined an association between threat bias and anxiety among pre-school aged and young school-aged children. The studies reviewed in this chapter utilised a variety of paradigms, including those measuring attentional allocation (hypervigilance and avoidance) to threat, interpretation of ambiguous stimuli, and the impact of threatening stimuli on working memory, neural activity and physiological arousal. Contrary to prior suggestion (Brown et al., 2014; Dudeney, Sharpe, & Hunt, 2015), RT-based paradigms (i.e. the dot probe task) most reliably detected an association between threat processing and anxiety symptoms in young children. The findings of this review suggest that it is possible to demonstrate threat bias among highly anxious, pre-school children as young as 4 years. However, several further issues need to be considered when applying these paradigms to children with ASD.

4.4.2 Paradigms to measure association between threat bias and anxiety in early childhood

Multiple paradigms were used to examine the association between threat bias and anxiety in the studies reviewed. A majority of studies used the dot probe paradigm, which is among the most widely used measures of attentional bias to threat in child and adult research (Bar-Haim, Lamy, Pergamin, Bakermans-Kranenburg, & van IJzendoorn, 2007; Dudeney, Sharpe, & Hunt, 2015). All studies using the dot probe task detected an association between threat bias and anxiety; highly anxious children were faster to detect a probe paired with a threatening stimulus when stimuli were presented for short durations (500ms) and slower to detect a probe paired with threatening stimuli when they were presented for longer periods (1000ms) (Briggs-Gowan et al., 2015; Brown et al., 2013; Mian, Carter, Pine, Wakschlag, & Briggs-Gowan, 2015; Susa, Pitică, Benga, & Miclea, 2012). The time course of attentional bias found in these studies corresponds to what has been reported in older participants, where hypervigilance is observed at shorter presentations of stimuli while avoidance is observed after longer presentations when conscious processing has taken place (Gamble & Rapee, 2009; Koster, Verschuere, Crombez, & Van Damme, 2005). However, it is important to note that two of the studies using the dot probe did not find a significant association with parent-rated anxiety symptoms, only an observational measure of anxiety (Briggs-Gowan et al., 2015; Mian, Carter, Pine, Wakschlag, & Briggs-Gowan, 2015). This highlights the challenges of obtaining accurate informant reports of anxiety in very young children and emphasises the need for additional measures or multiple informants. It is also important to note that the effect sizes of the

findings in these studies were small to moderate. This suggests that, while a significant association can be detected among young children, it is relatively weak and may only increase in strength with age.

The findings from this review contradict prior research, which suggested that RT-based paradigms such, as the dot probe task, are not sensitive enough to detect an association between threat bias and anxiety in children (Brown et al., 2014; Dudeney, Sharpe, & Hunt, 2015). Dudeney, Sharpe, and Hunt (2015) conducted a meta-analysis on a much larger number of studies using the dot probe paradigm, and there was greater inconsistency in specific aspects of the methodology (e.g. the length of stimulus presentation). While the number studies reviewed in this chapter was much smaller, the methodology across studies was highly consistent. This could have contributed to the difference in findings in this review and the meta-analysis. Furthermore, the meta-analysis suggests that, unlike the dot probe paradigm, the emotional Stroop task is better suited in detecting threat bias among highly anxious children, particularly when linguistic stimuli were used. Surprisingly, none of the studies identified in this chapter used the Stroop task. However, depending on their reading ability, younger children may struggle to interpret the threat-value of linguistic stimuli, suggesting that this paradigm may not be appropriate for pre-school aged children.

On the contrary, research using interpretation bias tasks did not consistently detect an association between anxiety symptoms and a tendency to interpret ambiguous stimuli as threatening. An important limitation of interpretation bias tasks is their reliance on linguistic threat stimuli and verbal responses. Language ability and story comprehension are not fully developed in early childhood (Tompkins, Guo, & Justice,

2013), suggesting that the use of ambiguous stories or homophones is not a useful method of inducing threat among very young, pre-school aged children. Dodd, Hudson, Morris, and Wise (2012) used the ambiguous stories paradigm with children age 3-4 years and allowed them to use dolls and props to facilitate their responses, rather than solely relying on verbal responses. This study does report a significant association between interpretation bias and parent-reported anxiety symptoms both at baseline and one year later. Therefore, interpretation bias tasks may need to be modified to offer alternative methods of responding that supplement verbal answers.

Eley et al. (2008) also found increased interpretative bias among the highly anxious participants, but this effect became non-significant when depression symptoms were controlled for. These findings are not surprising, as studies examining the neurocognitive correlates of depression also report that children and adolescents with the condition tend to interpret ambiguous stimuli in a negative way (e.g. Orchard, Pass, & Reynolds, 2016). The pictorial stimuli used in RT-based paradigms tend to effectively represent danger (e.g. an angry face clearly signals threat), while the ambiguous stories used in many paradigms are more broadly negative and often represent unpleasant social situations, rather than specific danger (Eley et al., 2008). Thus, it is possible that interpretation bias paradigms capture the cognitive mechanisms of internalising difficulties more broadly and may not be specific to anxiety disorders.

Studies using working memory and learning paradigms also report equivocal results (Cheie & Visu-Petra, 2012; Cheie, Visu-Petra, & Miclea, 2012; Fulcher, Mathews, & Hammerl, 2008). A finding that is reported across these studies is that anxious children have worse performance on memory tasks than non-anxious controls

overall, except when the stimuli depict threat-relevant information (pictorial or linguistic). This suggests that anxious children do not necessarily have superior memory for threatening information compared to non-anxious children, but that they are better able to remember threatening, compared with non-threatening, material. However, Cheie, Visu-Petra, and Miclea (2012) did find that anxious children (aged 45-85 months) had better immediate recall for angry faces. Furthermore, Fulcher, Mathews, and Hammerl (2008) suggests that anxious children more readily learn to dislike neutral faces that had previously displayed a threatening expression. Given the diverse aspects of memory tested across these tasks, it is difficult to make firm conclusions about the utility of memory-based paradigms in assessing threat bias. It is important to note that research on memory biases in anxious adults has also produced equivocal results (Mitte, 2008; Williams et al., 2007).

Finally, measures of the neural and physiological correlates of anxiety suggest that young children do exhibit both heightened neural reactivity (DeCicco, Solomon, & Dennis, 2012; O'Toole, DeCicco, Berthod, & Dennis, 2013; Solomon, DeCicco, & Dennis, 2012) and physiological arousal (Waters, Neumann, Henry, Craske, & Ornitz, 2008) to threatening stimuli. However, the findings using these measures are also somewhat mixed in their methodology and the parameters utilised. Firstly, an association between the LLP ERP response to threatening scenes can be detected at the age of 5-7 years (DeCicco, Solomon, & Dennis, 2012; Solomon, DeCicco, & Dennis, 2012). On the other hand, N170 response to threatening faces at the age of 5-7 years is not associated with concurrent anxious symptoms but does predict anxiety two years later. While the N170 ERP component is specific to the processing of faces, the LLP

more generally relates to emotion regulation ability (Dennis & Hajcak, 2009). Therefore, it is possible that reduced emotion regulation is indicative of current anxiety, while biased processing of angry faces involves a developing, cumulative process and contributes to the development of anxiety later in childhood. These findings from EEG paradigms complement temperament research to suggest that heightened responsiveness to threat serves as a risk factor for the development of anxiety symptoms later in development (Cole, Zapp, Fettig, & Perez-Edgar, 2016; Perez-Edgar et al., 2011). Furthermore, as noted by DeCicco, Solomon, and Dennis (2012), children aged 7 years and younger do not exhibit the neural correlates of reappraisal that are observed in older populations, suggesting that this ability is still developing at this age.

Field and Lester (2010) suggest that, in early development, all children manifest a bias towards threatening stimuli and that typically developing children learn to inhibit responding to threat while those with anxiety do not. Thereby suggesting that, in young age groups, it is not possible to detect an association between threat bias and anxiety. However, the findings both from this review and temperament research (LoBue & Perez-Edgar, 2014; Nakagawa & Sukigara, 2012) suggest that it is possible to detect differential responding to threatening stimuli based on a child's emotional state. Children with heightened anxiety show a bias towards threatening stimuli on multiple paradigms, but RT-based tasks have thus far yielded the most consistent results in early development.

4.4.3 Methodological considerations for applying threat bias paradigms to test children with ASD

Because the prevalence of anxiety in children with ASD is very high (e.g. Simonoff et al., 2008) and there is evidence that symptoms are observed early in childhood (Davis et al., 2010), it is important to apply the methods used in non-ASD populations to examine the neurocognitive correlates of anxiety in ASD. However, multiple modifications may be required to adapt existing threat bias paradigms to be suitable for young children with ASD, as well as those that have reduced communicative skills and cognitive functioning.

4.4.3.1 Measuring Reaction Time (RT) in Children with ASD

Prior research has raised concerns about using RT-based paradigms, such as the dot probe task, to measure the association between threat bias and anxiety in children. However, the findings from this review suggest that the dot probe task is the most widely used and consistent paradigm to measure attentional bias in young children with heightened anxiety. Numerous studies report that children with ASD, who have average cognitive ability, can perform equally well on measures of RT as age and IQ-matched typically developing controls (Ferraro, 2016; Ozonoff & Strayer, 1997). However, using a meta-analysis, Landry and Parker (2013) suggest that individuals with ASD do exhibit slower reaction times than typically developing controls on tasks involving orienting of attention. This is particularly true for tasks that involve exogenous cueing with brief stimulus onset asynchrony (SOA), the time between the onset of one stimulus and another stimulus (Landry & Parker, 2013). This presents

difficulty in implementing tasks such as the dot probe, which are exogenous cueing tasks by design with short SOAs, where emotional stimuli are presented for very brief durations (e.g. 500ms). Furthermore, atypicalities in motor functioning and the higher prevalence of Attention Deficit Hyperactivity Disorder (ADHD) in children with ASD make measuring RTs in this population more challenging (Karalunas, Geurts, Konrad, Bender, & Nigg, 2014; Rinehart, Bradshaw, Brereton, & Tonge, 2001). For example, even if participants with ASD can perform an RT task, having a co-occurring condition like ADHD may make it more difficult for them to sustain attention to the task and focus on the stimuli.

Threat bias tasks, particularly those measuring RTs, are generally adaptations of basic perceptual paradigms, which have been adapted to include emotional stimuli (MacLeod, Mathews, & Tata, 1986). While attention orienting tasks, such as the dot probe may be more challenging for children with ASD, there are other cognitive tasks where individuals from this population excel. For example, children with ASD have faster RTs on visual search tasks compared to controls (Joseph, Keehn, Connolly, Wolfe, & Horowitz, 2009). Superior performance on visual search detection has also been observed among toddlers at increased familial risk for ASD (Gliga, Bedford, Charman, & Johnson, 2015). Therefore, tasks measuring other aspects of cognition can also be adapted to include emotional stimuli and, therefore, assess threat bias (e.g. Cheie, Visu-Petra, & Miclea, 2012). Similarly, children with ASD are also reported to have difficulty in flexibly shifting attention and take longer to disengage attention from a stimulus (Landry & Bryson, 2004). This effect has also been observed among high-risk infants who go on to develop ASD at 36 months of age (Elsabbagh et al., 2013). It

would be useful to assess attentional disengagement from threat among children with ASD to determine whether this general cognitive style impacts on threat processing and co-occurring anxiety symptoms.

Another alternative is the use of visual inspection tasks, where participants are asked to state which of two parallel lines is longer and their response times are measured (Garaas & Pomplun, 2008). Individuals with ASD, even those who have reduced cognitive functioning, perform equally well on inspection time tasks as typically developing controls do (Scheuffgen, Happé, Anderson, & Frith, 2000; Wallace, Anderson, & Happé, 2009). Inspection time tasks can also be modified to include emotionally-relevant stimuli and the use of such tasks may minimise the impact of cognitive ability on task performance. Finally, Brown et al. (2014) detail a novel "missile probe" task, which has a similar design to the dot probe task but calibrates the duration of probe presentation online to ensure a 75% accuracy rate, reducing data loss. This paradigm allows for comparison of differential error rates across conditions as well as comparison of RTs. Therefore, even if participants struggle with RT performance, sufficient data can still be collected for analysis.

4.4.3.2 Measuring threat bias in individuals with ASD and reduced cognitive functioning

Co-occurring anxiety symptoms are less frequently reported among individuals with ASD and intellectual disability (IQ<70), than among those with average cognitive functioning (e.g. Hallett, Lecavalier, et al., 2013; Sukhodolsky et al., 2008). However, there is currently limited information about the manifestation of anxiety symptoms among individuals with ASD and reduced cognitive functioning. It is particularly

unclear whether the lower prevalence of co-occurring anxiety in this population is due to difficulty in ascertaining information about internalising symptoms from individuals with reduced cognitive and/or verbal ability (Wood & Gadow, 2010). For this reason, it is necessary to validate informant reports of anxiety by examining whether they associate to threat bias. Furthermore, if individuals with ASD and intellectual disability do manifest threat bias in the absence of reported anxiety symptoms, this would suggest that they may have higher levels of anxiety than caregivers can perceive. Threat bias has also been studied among individuals with Williams Syndrome (WS), a neurodevelopmental condition where there is also a high prevalence of anxiety. Individuals with WS who have average IQ do exhibit hypervigilance for threatening stimuli, but threat bias is not observed among those with intellectual disability (McGrath et al., 2016). Comparisons across individuals with ASD who have intellectual disability and those who have average IQ are also necessary to discern cognitive related differences in the correlates of anxiety symptoms. Such investigation may help identify differential treatment strategies for those with intellectual disability.

However, the paradigms that are suitable for use among individuals with intellectual disability, particularly those with reduced communicative ability, are limited. For example, it may not be possible to assess interpretative bias among participants with reduced verbal ability or those who cannot communicate verbally at all. Some of the paradigms identified in this review may provide a useful foundation for testing individuals with ASD and reduced cognitive functioning. For example, passive-viewing tasks that are supplemented with measures of attention (eye-tracking) or neural activity (EEG) may help identify neurocognitive responding to threatening

stimuli in participants that have reduced ability to perform RT or interpretative bias tasks. Care must be taken to ensure that participants view stimuli that are presented during the task. However, such paradigms have been used in very young children and infants at risk for ASD and rewarding stimuli, such as animations, can be used instead of a fixation cross to draw attention to the centre of the screen during prior to stimulus presentation (e.g. Elsabbagh et al., 2011).

4.4.3.3 Ethical considerations

In most studies using threat bias tasks, participants are warned that they will view threatening images and must consent prior to starting a task. Individuals with ASD often have difficulty identifying and communicating their own emotional states (e.g. Silani et al., 2008). This implies that even if children with ASD can refuse/provide consent, they may struggle to verbalise concerns and predict the impact that viewing threatening stimuli may have on them. Additional caution is needed to ensure that individuals with ASD do not experience distress during tasks that involve the presentation of threatening stimuli.

4.4.4 Conclusion

The present systematic review identified experimental paradigms that have been used to evaluate the association between threat bias and anxiety in early development. The experimental measures used among very young children were generally the same as the paradigms used in older children and adults (Bar-Haim, Lamy, Pergamin, Bakermans-Kranenburg, & van IJzendoorn, 2007; Dudeney, Sharpe, & Hunt, 2015). Overall, the dot probe task, an RT-based paradigm, yielded the most consistent findings

of an association between threat bias and anxiety among children as young as 37-87 months (~3 to 7 years).

The findings from this review contradict prior research, which suggests that it may not be possible to observe threat bias in early development (Field & Lester, 2010) and that RT-based paradigms are unsuitable for young children (Brown et al., 2014; Dudeney, Sharpe, & Hunt, 2015). Threat bias modification training is showing increasing promise in reducing anxiety symptoms in children and supplementing Cognitive Behaviour Therapy as a treatment method (Shechner et al., 2014). The finding of increased threat bias among very young children suggests that such methods may be suitable for early interventions. This is highly relevant, as anxiety symptoms are reported to emerge in very early childhood, as early as ~3 years (Egger & Angold, 2006; Franz et al., 2013). Furthermore, several studies in this review suggest that threat bias in early development is associated with anxiety in later childhood (O'Toole, DeCicco, Berthod, & Dennis, 2013). These findings highlight the need for longitudinal studies to replicate such findings and further investigate the role of early threat bias on the progression of anxiety symptoms.

As discussed, the measures identified in this review are generally appropriate for use when testing children with ASD. However, certain modifications may be required to ensure that tasks are suitable for children with ASD and reduced cognitive or verbal ability. Alternative tasks that do not require measures of RT to orient to an exogenous stimulus, such as inspection time or missile probe tasks may also be used. Finally, eye-tracking and EEG measures may be beneficial to measure threat bias in individuals with ASD and reduced verbal ability.

Chapter 5
Anxiety and attentional bias to threat in children at increased familial risk for Autism Spectrum Disorder

5.1 Introduction

As outlined in Chapter 3, elevated rates of anxiety have been observed among individuals with ASD and their siblings (Mazefsky, Folstein, & Lainhart, 2008; Salazar et al., 2015; Simonoff et al., 2008; White, Oswald, Ollendick, & Scahill, 2009). However, there is a scarcity of research examining the shared underlying neurocognitive mechanisms of the two conditions. Wood and Gadow (2010) suggest that such investigation is highly relevant, as it is presently unclear whether the co-occurrence of ASD and anxiety represents a true comorbidity, the manifestation of two separate conditions in the same individual, or if it results from an overlap in symptom presentation and difficulties with self- and caregiver-report. One way to better understand the manifestation of anxiety within ASD is to examine whether the neurocognitive mechanisms that are associated with anxiety in non-ASD populations, such as increased attentional allocation to threat, are also present and relate to anxiety symptoms in children with ASD and their siblings.

Such investigation would help elucidate whether the prominent theories of anxiety, which describe a cognitive architecture characterised hypersensitivity to threat and danger (Beck, Emery, & Greenberg, 1985), also characterise anxiety among individuals with ASD. Given that most treatments for anxiety, such as Cognitive Behavioural Therapy (CBT) are aimed at restructuring maladaptive cognitions (Hofmann & Smits, 2008; James, James, Cowdrey, Soler, & Choke, 2013), it is

important to identify specific cognitions to target when administering treatment to individuals with ASD.

Furthermore, it is also important to examine how the neurocognitive correlates associated with anxiety map on to both parent- and self-reported anxiety symptoms among children with ASD. As outlined in Chapter 3, discrepancy in the severity of anxiety reported by children with ASD and their parents has been reported in multiple studies (e.g. Mazefsky, Kao, & Oswald, 2011). Therefore, examining whose report most strongly associates with an unbiased, experimental measure, would be highly beneficial for both research and clinical practice. Finally, the high-risk design used in this study enables the examination of differential cognitive mechanisms among siblings who develop ASD and those who do not.

5.1.1 Attentional bias to threat and anxiety

As discussed in Chapter 4, cognitive theories of anxiety disorders posit that highly anxious individuals may be particularly sensitive to threat-relevant information in the environment (Bar-Haim, Lamy, Pergamin, Bakermans-Kranenburg, & van IJzendoorn, 2007). Biased processing of threat is thought to contribute to both the development and maintenance of anxiety disorders (Beck & Clark, 1997; Beck, Emery, & Greenberg, 1985; Eysenck, 1992). This cognitive style has been demonstrated experimentally using a number of tasks that compare reaction times (RTs) to threatening and non-threatening stimuli (for review see Bar-Haim, Lamy, Pergamin, Bakermans-Kranenburg, & van IJzendoorn, 2007).

Given that most anxiety disorders first manifest in childhood (Beesdo, Knappe, & Pine, 2009), assessing threat bias among school-aged children at-risk for ASD may

be particularly relevant in describing the early processes associated with the development of anxiety in this population. The association between threat bias and anxiety has been reported in both adults and children, but a recent meta-analysis (Dudeney, Sharpe, & Hunt, 2015) suggests that the association between threat bias and anxiety becomes more readily observable among older children. Furthermore, Brown et al. (2014) suggested that RT-based paradigms have poor psychometric properties among school-aged children. Nevertheless, as the systematic review in Chapter 4 suggests, RT-based paradigms, such as the dot probe task, have thus far exhibited the most success in measuring the association between threat processing and anxiety among children as young as preschool-age (e.g. Mian, Carter, Pine, Wakschlag, & Briggs-Gowan, 2015).

5.1.1.1 Distinct components of attention measured in threat bias tasks

As noted in Chapter 4, the dot-probe paradigm is one of the most widely used measures of threat bias among both adults and children (MacLeod & Mathews, 1988; MacLeod, Mathews, & Tata, 1986). Studies using this task report that individuals with heightened anxiety are faster to detect a probe that has previously been paired with a threatening (compared to a neutral) stimulus (Bar-Haim, Lamy, Pergamin, Bakermans-Kranenburg, & van IJzendoorn, 2007). Faster orienting towards threatening stimuli has been suggested to reflect a state of hypervigilance that is present among highly anxious individuals (MacLeod & Mathews, 1988).

However, the dot-probe paradigm has received criticism for not differentiating between different components of attention. Fox, Henderson, Rubin, Calkins, and Schmidt (2001) argue that faster RTs to threatening stimuli may be a consequence of

delayed disengagement from, rather than faster orienting to, threatening stimuli. This argument posits that two equally possible interpretations exist; either individuals with anxiety are faster to detect a probe paired with a threatening stimulus because they orient towards it more quickly, or they are slower to detect a probe paired with a neutral stimulus because they have difficulty disengaging attention from the location where the threatening stimulus was previously presented. Studies using paradigms that disentangle different facets of attention corroborate the postulation that anxiety is specifically associated with delayed disengagement from threatening stimuli, but not faster orienting towards it (Salemink, van den Hout, & Kindt, 2007; Yiend & Mathews, 2001). Bar-Haim, Morag, and Glickman (2011) further suggest that training anxious children to flexibly disengage attention from threatening stimuli is successful in reducing anxiety symptoms.

This may be particularly relevant for individuals with ASD, who exhibit difficulty in flexibly shifting attention (Landry & Bryson, 2004). Perhaps this general cognitive style prevalent among individuals with ASD also contributes to cognitive processing in anxiety, resulting in more difficulty in shifting attention away from threat.

5.1.1.2 Association between threat bias and anxiety among individuals with ASD

While threat bias has been studied very extensively among individuals with anxiety disorders, there is a dearth in research investigating this among ASD populations and studies to date have yielded equivocal results. Two studies examined attentional bias to angry faces and found that young people with ASD and elevated anxiety did not exhibit enhanced engagement to or delayed disengagement from threat,

compared to participants with ASD who did not have heightened anxiety or TD controls (Hollocks, Ozsivadjian, Matthews, Howlin, & Simonoff, 2013; May, Cornish, & Rinehart, 2015). On the other hand, using an eye-tracking paradigm, White, Maddox, and Panneton (2015) found that prolonged fixation to threatening faces, depicting expressions of disgust and anger, was associated with fear of negative social evaluation (a construct linked to social phobia) in adolescents with ASD.

In contrast to these studies, Isomura, Ogawa, Shibasaki, and Masataka (2015) found that children with ASD, who did not have clinical-level anxiety symptoms, exhibited prolonged disengagement from threatening (snakes) compared with non-threatening (flowers) stimuli. While it is not unusual to find a general bias to threat in children and adults (Lobue and Deloache 2008), participants with ASD had longer disengagement from the threatening stimuli than TD controls. It is important to note that, although participants in this study did not have clinical diagnoses of anxiety, subclinical symptoms or traits were not measured. Given that delayed disengagement is frequently observed among individuals with ASD and anxiety symptoms were not measured, it is unclear whether the attentional bias to threat reported in this study is a consequence of ASD symptoms, anxiety, or an interplay of both.

5.1.2 Social and non-social threat stimuli

One of the limitations of previous studies examining threat bias in ASD is the use of human facial expressions as stimuli. There is a broad literature suggesting atypical face processing and reduced emotion recognition ability among individuals with ASD (e.g. Harms, Martin, & Wallace, 2010). A recent meta-analysis suggests that individuals with ASD exhibit reduced performance on tasks that measure emotion

recognition, particularly for negative emotions such as anger and fear (Uljarević & Hamilton, 2013). Multiple studies also report both reduced accuracy in emotion labelling and attenuated neural activity when viewing emotional faces among first-degree relatives of individuals with ASD (Oerlemans et al., 2014; Spencer et al., 2011; Sucksmith, Allison, Baron-Cohen, Chakrabarti, & Hoekstra, 2013).

A recent systematic review (Pergamin-Hight, Naim, Bakermans-Kranenburg, van, & Bar-Haim, 2015) suggests that content specificity is an important factor in eliciting threat bias. Thus, individuals with a specific type of anxiety disorder exhibit a stronger bias towards stimuli that are disorder-congruent or personally-relevant (Pergamin-Hight, Naim, Bakermans-Kranenburg, van, & Bar-Haim, 2015). In the context of this evidence, the use of threatening facial expressions as stimuli may not be salient enough to detect an association between anxiety and attentional bias in ASD populations. On the contrary, individuals with ASD have exhibited heightened neural responses to unpleasant non-social stimuli, comparable to neural activity observed in TD controls (Silani et al., 2008), which is perhaps why bias to images of snakes compared to flowers was observed in children with ASD (Isomura, Ogawa, Shibasaki, & Masataka, 2015).

Given the evidence outlined, the use of non-social threatening stimuli may be better suited to detect threat bias among individuals with ASD. A similar approach has yielded promising findings among individuals with other neurodevelopmental conditions, who have elevated anxiety. In particular, individuals with Williams syndrome (WS), a genetic condition caused by microdeletion of genes on chromosome 7, exhibit several features similar to individuals with ASD, such as heightened prevalence of anxiety and atypicalities in processing facial stimuli (Dykens, 2003). In a

seminal study, Meyer-Lindenberg et al. (2005) report that individuals with WS exhibit attenuated amygdala activation to threatening social stimuli, but heightened amygdala activation to non-social threat stimuli. Subsequently, Dodd and Porter (2011) demonstrated that individuals with WS exhibit heightened threat bias to non-social threat stimuli and that this is associated with anxiety severity. Thus, a similar approach of using non-social threat stimuli may yield a significant association between threat bias and anxiety among individuals with ASD.

An additional challenge exists in selecting appropriate non-threatening comparison stimuli. Children with ASD often exhibit fears and phobias of unusual or commonplace objects (Kerns et al., 2014; Mayes et al., 2013). As a consequence, the traditional use of neutral stimuli may not be as clearly non-threatening to children with ASD. Perhaps more clearly positively valenced stimuli may be more effective in detecting differences in attentional allocation to threatening and non-threatening information.

5.1.3 The present study

The present study sought to extend current understanding of anxiety in ASD by examining the association between self- and parent-reported anxiety and threat bias, in a cohort of children at high familial risk for ASD (HR), some of whom met diagnostic criteria for ASD (HR-ASD) and others who did not (HR-non ASD), compared to low-risk (LR) controls. Importantly, one aim is to address limitations in previous work by examining bias to non-social threatening stimuli, which may be more salient among children with ASD. Chapter 3 examined the prevalence of anxiety among children at high-risk for ASD using parent- and self-report questionnaires. The parent-report

measure suggested that anxiety was elevated in the HR group, especially among the HR-ASD children. There was also evidence of heightened anxiety, albeit to a lesser degree, in the HR-non ASD group, particularly within the domain of separation anxiety. On the contrary, there were no significant group differences on the self-report measure.

Therefore, the aims of this chapter are two-fold. Primarily, this chapter will examine whether high-risk siblings manifest heightened threat bias for non-social threat stimuli and whether this is associated with anxiety symptoms. A further aim is to investigate possible differential associations between parent- and self-reported anxiety with threat bias. This approach will help clarify whether threat bias, a feature widely observed among anxious individuals, is also present among children with ASD and those at-risk for ASD. Furthermore, it may help clarify discrepancies in self and caregiver reported symptoms of anxiety.

Given the present literature, this study aims to address the following hypotheses:

1. Children at HR for ASD will show evidence of attentional threat bias. In light of the literature suggesting that anxiety may be associated with prolonged disengagement from threat (Fox, Russo, Bowles, & Dutton, 2001) and reports that children with ASD have difficulty in flexibly shifting attention (Landry & Bryson, 2004), it can be predicted that threat bias will be observed through delayed disengagement from, rather than faster orienting to, threatening stimuli.
2. The parent-reported anxiety measure suggests that anxiety is most highly elevated in the HR-ASD group and, to a lesser extent, the HR-non ASD group. Therefore, it can be predicted that threat bias will also be highest among

children in the HR-ASD group, followed by those who are HR-non ASD, and lowest in LR controls.

3. Since children with ASD report heightened fear of atypical or commonplace objects (Kerns et al., 2014; Mayes et al., 2013), threat bias will be more readily observed when comparing threatening with positive, rather than threatening with neutral, stimuli within the HR sample.
4. Finally, there will be an association between parent-reported anxiety symptom severity and attentional threat bias, regardless of ASD severity. On the contrary, given suggestions that individuals with ASD tend to under-report anxiety symptoms and that self-report measures have reduced sensitivity in this population (Mazefsky, Kao, & Oswald, 2011), an association between self-reported anxiety and threat bias is not expected to emerge.

5.2 Method

5.2.1 Emotional Spatial Cueing task

A modified version of the spatial cueing task (Posner, Snyder, & Davidson, 1980) was used to measure attentional bias. The task was adapted to include emotional stimuli and has been previously used to measure both attentional engagement to and delayed disengagement from threatening stimuli in anxiety (e.g. Yiend & Mathews, 2001). Prior to administering the task to the cohort in this study, the task was piloted with a group of typically developing children (aged 4-8 years) and healthy adults. The results from the pilot phase are presented in Appendix 3.

5.2.1.1 Stimuli

Sixty digitised colour photographs were selected from the International Affective Picture System database (IAPS; Lang, Bradley, & Cuthbert, 2008) and were chosen because they had been used (or had similar content to those used) in previous studies of emotional picture processing in TD children (Hajcak & Dennis, 2009; McManis, Bradley, Berg, Cuthbert, & Lang, 2001). Of these, 20 were classified as threatening, 20 as neutral and 20 as positive[4] based on ratings of affective valance and emotional arousal previously made by adult participants. A subset of these images was also rated by children aged 7-11 years (Lang, Bradley, & Cuthbert, 2008). Threatening images included pictures of animals (e.g. snakes, spiders) and unpleasant scenes (e.g. injections) but none relied on human facial expressions to induce threat. Positive and neutral images were matched as closely as possible in content, colour, orientation, level of detail and brightness, through visual inspection.

Threatening images (M=3.36, SD=0.64) were rated by the IAPS sample (Lang, Bradley, & Cuthbert, 2008) as less pleasant than neutral (M=5.04, SD=0.33) or positive (M=7.44, SD=0.50) ones and both threatening (M=6.07, SD=0.70) and positive (M=5.44, SD=0.80) images were rated as more emotionally arousing than neutral images (M=2.78, SD=0.50). Each picture subtended 4 by 3 inches and was presented either to the left or to the right of the fixation cross (4 inches between the centre of the fixation cross and the centre of the image) on a grey background. The task was

---

[4] The following IAPS images were used: ***Threatening*** (1050, 1120, 1201, 1300, 1525, 1930, 1932, 3210, 6190, 9312, 6370, 9373, 9440, 9480, 9590, 9592, 9622, 9902, 9909, 9940), ***Neutral*** (2038, 2396, 2579, 5390, 5520, 5530, 5740, 7004, 7006, 7025, 7035, 7050, 7060, 7100, 7140, 7150, 7175, 7217, 7233, 7595) and ***Positive*** (1710, 1750, 1920, 1999, 2650, 5450, 5460, 5470, 5480, 5621, 5910, 7250, 7270, 7330, 7430, 8200, 8260, 8420, 8490, 8510).

presented on a 15-inch colour monitor and was programmed using E-Prime version 2.0 (Psychology Software Tools Inc., 2012).

5.2.1.2 Procedure

Participants were given 30 practice trials with neutral stimuli, followed by 240 experimental trials in 4 blocks of 60 trials each. All 60 images (20 threatening, 20 neutral, and 20 positive) were presented within each block with equal presentations on the right and left of the fixation cross. Each image was presented once in every block, with both the order and assignment to congruent or incongruent trial randomised within each block.

Each trial began with a fixation cross at the centre of two empty rectangles (4 by 3 inches) for a jittered duration of 875-1275ms. In order to minimise eye movements, participants were instructed to keep their eyes on the fixation cross throughout the task. Subsequently, an image (threatening, neutral or positive) appeared in either the right or the left rectangle for 500ms. The image was then removed and replaced by a target (a star) at the centre of one of the rectangles and remained on screen until the end of the trial. In 70% of trials, the target appeared in the location of the image cue (congruent) and in 30% of trials the target was in the opposite location (incongruent). The sequence of events in a congruent and an incongruent trial are presented in Figure 3.

The uneven distribution of congruent and incongruent trials was done in order to facilitate covert orienting of attention in response to cueing. When a greater portion of trials are congruent, participants are more likely to covertly shift attention to the cued location because it is an accurate predictor of the target location most of the time,

resulting in faster RTs on congruent trials and slower RTs on incongruent trials (Posner, Snyder, & Davidson, 1980). Since enhanced attending is expected towards congruent trials, the slower RTs on incongruent trials are indicative of attentional disengagement.

Participants were asked to locate the target by pressing one of two buttons to indicate right or left. A new trial began once participants had made a response or after 3000ms. The reaction time (RT) to detect the target was measured as the time, in milliseconds (ms), from target onset to button press. Feedback was given after each trial, indicating whether the response was correct, incorrect or if participants were too slow to respond. Mean RTs for each stimulus type (threatening, neutral and positive) in both congruent and incongruent trials were used in analyses.

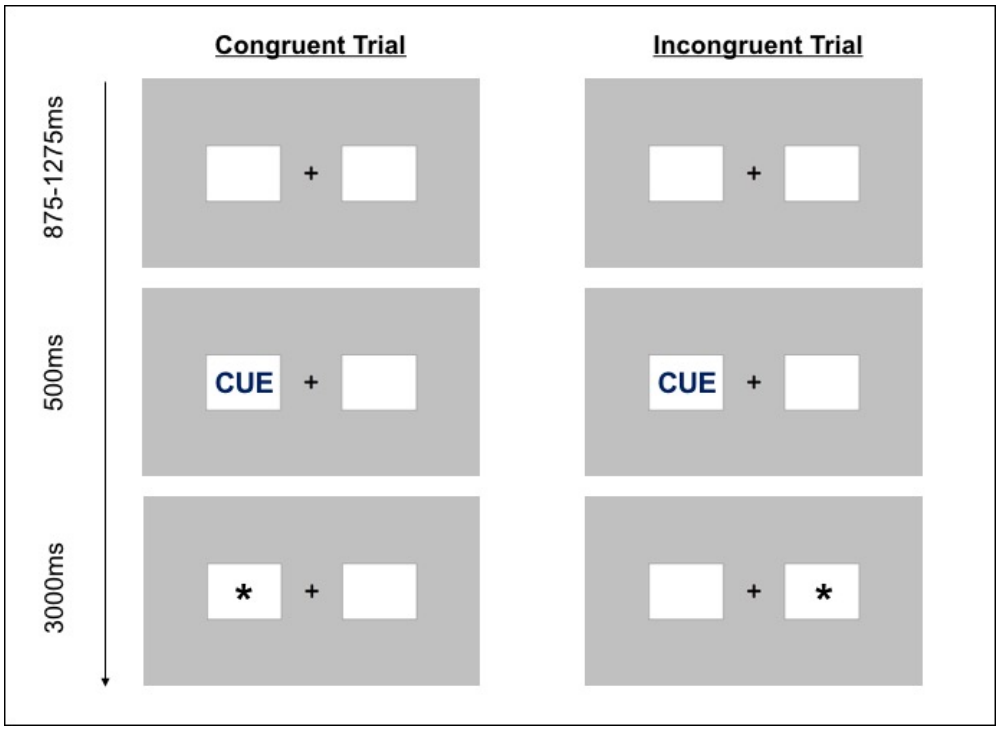

Figure 3. Sequence of events in a congruent trial (left) and an incongruent (right) trial of the Emotional Spatial Cueing task

5.2.2 Measures of anxiety, ASD severity and cognitive functioning

5.2.2.1 Anxiety symptoms

As described in Chapter 2, anxiety symptoms were measured using the Spence Children's Anxiety Scale – Parent and Child Report versions (SCAS-P/C; Nauta et al., 2004; Spence, 1998). On the SCAS-P, there were group differences on multiple subscales and the total score. Consequently, only the total score was used in the analyses in this chapter as it is most relevant to capture all the facets of anxiety. While individuals with anxiety disorders do exhibit stronger biases towards disorder-congruent stimuli (Pergamin-Hight, Naim, Bakermans-Kranenburg, van, & Bar-Haim, 2015), general threat bias is observed across different types of anxiety disorders (Roy et al., 2008), so there was no strong reason to examine the association between threat bias and a particular subtype of anxiety. Contrary to parent-reported symptoms, there were no significant group differences on the SCAS-C total score or any of the subscales. Thus, the use of the total score was deemed justifiable, as there was no apparent need to examine any particular self-reported subscale. While the SCAS-C allows for the conversion of the total score to age and sex normed t-scores, the SCAS-P does not. Therefore, to ensure comparability across the two measures, only the raw scores were used for each measure.

5.2.2.2 Measure of ASD severity

The Social Responsiveness Scale-Second Edition (SRS-2; Constantino, 2012) was used to measure ASD severity. In Chapter 3, the association between anxiety and ASD symptoms was measured using the SCQ (Rutter, Bailey, & Lord, 2003) because it

enabled examination of different core features of ASD (Social, Communication and RRB). However, unlike the SCQ, the SRS allows for conversion of raw scores to age and sex normed t-scores. Given that there were sex differences in anxiety in the HR-ASD group (see Chapter 3) and that sex was incorporated into the analyses in this chapter, having ASD severity ratings which take sex into account was deemed favourable. The SCQ and SRS t-scores were highly correlated in this sample ($r(68)=.63$, $p<.001$).

5.2.2.3 Measure of cognitive functioning

The Wechsler Abbreviated Scales of Intelligence – Second Edition (WASI-II; Wechsler, 2011) was used to measure of cognitive ability. The WASI-II was included in this chapter due to the vastly reported association between cognitive ability and performance on RT tasks (for review see Sheppard & Vernon, 2008). Furthermore, in Chapter 2, the HR-non ASD group were reported to have significantly reduced WASI-II scores compared to the HR-ASD and LR groups. Although, it is important to note that participants in this group did not exhibit evidence of intellectual disability (IQ<70). Nevertheless, WASI-II was included to ensure that group differences on the threat bias task were not attributable to cognitive ability. A common statistical approach used when such group differences emerge is to co-vary for cognitive ability in analyses. However, Miller and Chapman (2001) suggest that such an approach is not appropriate when testing individuals who have been pre-assigned to groups (as is the case in this study). They suggest that these differences may be substantive and related to group status in a meaningful way. Therefore, this chapter will report all analyses without covariates, but Appendix 4 will present the analyses with WASI-II FSIQ included as a

covariate to ensure that the pattern of results does not change when IQ is taken into account.

5.2.3 Statistical analyses

5.2.3.1 Group differences in threat bias

To examine group differences in threat bias, performance on each of the 6 trial types (threat congruent, positive congruent, neutral congruent, threat incongruent, positive incongruent and neutral incongruent) were compared across the three groups (HR-ASD, HR-non ASD and LR) using MANOVA. Additionally, 6 indices of attentional engagement and disengagement were computed. Attentional engagement indices were computed by calculating the difference in mean RTs for non-threatening and threatening *congruent* trials. Three engagement indices were computed, including threat compared with neutral ("threat-neutral engage"), threat compared with positive ("threat-positive engage") and positive compared with neutral ("positive-neutral engage"). Attentional disengagement was computed by calculating difference in mean RTs for threatening and non-threatening *incongruent* trials. Again, three disengagement indices were computed comparing threatening with neutral ("threat-neutral disengage"), threatening with positive ("threat-positive disengage") and positive with neutral ("positive-neutral disengage").

Group differences in these 6 indices were compared between the 3 groups (HR-ASD, HR-non ASD, LR) using a MANOVA. Where significant group differences emerged, planned comparisons were carried out between each pair of groups, with Bonferonni correction applied for multiple testing. Furthermore, if group differences were detected on a particular bias index, follow-up tests were conducted to ensure that

the bias score significantly differed from 0. To do this, one-sample t-tests were run on the selected bias score within each group, with Bonferonni correction applied for multiple testing (α=.05/3=.02).

Given that significant group differences emerged in FSIQ and there were sex differences in anxiety symptoms (see Chapters 2 and 3), these analyses were repeated and co-varied for FSIQ and sex, to ensure that these factors did not alter the pattern of findings. This is presented in Appendix 4.

5.2.3.2 Association between threat bias and anxiety

The association between threat bias and anxiety was examined in two steps. First-order Pearson correlations were run between each of the threat engagement and disengagement indices (threat-neutral engage, threat-positive engage, threat-neutral disengage and threat-positive disengage), SCAS-P total score, SCAS-C total score, SRS t-score, and WASI FSIQ, with Bonferonni adjusted *p*-values used to account for multiple analyses (α=.05/8=.01).

Because a significant association emerged between the threat-positive engage index and SCAS-P (see results), a follow-up linear regression was performed to assess the contribution of this attentional index to anxiety severity, co-varying for ASD severity and sex. As FSIQ was not significantly associated with SCAS-P total score or the threat-positive engagement index, it was not included the regression analysis. Furthermore, as SCAS-C total score was not associated with any threat bias index (see results), a follow-up regression analysis was not performed. Cohen's *d*, η^2 and r^2 were used to indicate the effect size (Cohen 1973). Post hoc power analyses were carried out

using G*Power (Faul, Erdfelder, Buchner, & Lang, 2009; Faul, Erdfelder, Lang, & Buchner, 2007).

5.3 Results

5.3.1 Preparation of RT data

RTs on trials with incorrect responses or ones where the participant did not make a response were removed from further analysis. This resulted in removal of 4.41% of RT data from the HR-ASD group, 1.17% from the HR-non ASD group, and 3.48% from the LR group. Additionally, trials with RTs below 100ms, which are indicative of automatic responding (Whelan, 2008), and trials with RTs that were 3SD above the participant's group mean were removed. This resulted in removal of a further 1.53% of RT data from the HR-ASD group, 2.87% from the HR-non ASD group, and 2.60% from the LR group. One participant from the HR-ASD group and 2 from the LR group had fewer than 50% valid trials in multiple conditions after removal of incorrect data and outliers, and were removed from further analyses. Additionally, 1 LR participant had unusually long RTs (+3SD compared to group RT) on multiple conditions and was also removed from further analyses. Two HR children were unable to complete the task due to having limited language and not being able to follow task instructions. A further 4 HR and 5 LR participants did not complete the task due to time constraints on the day of testing. As in prior analyses, the children who lost diagnosis from the 36-month to 7-year visits were excluded from analyses. This resulted in 35 HR (11 HR-ASD and 21 HR-non ASD) and 29 LR having useable RT data for analysis.

5.3.2 Group differences in threat bias

Table 10 provides a summary of the scores on each trial type for the HR-ASD, HR-non ASD and LR groups. The HR-non ASD group had slower RTs than the LR group in the Threat Incongruent (p=.03, d=.75) and Neutral Incongruent (p=.04, d=.73) conditions. The HR-ASD group had slower RTs than the LR group on Positive Congruent trials (p=.03, d=.08). The HR-ASD group also showed trend-level, longer RTs on Threat Incongruent (p=.07, d=.74) and Neutral Congruent (p=.07, d=.88) trials. Finally, the HR-non ASD group had trend-level, longer RTs than the LR group on the Threatening Congruent trials (p=.07, d=.67).

Table 10: Scores on the threat bias task in the HR-ASD, HR-non ASD and LR groups

Cond.	HR-ASD	HR-non ASD	LR	MANOVA
TC	677.45 (91.26)	676.75 (89.77)	624.38 (59.32)	$F(2, 57)= 3.53, p=.04, \eta^2=.11$
TI	738.55 (102.86)	733.00 (80.85)[a]	659.17 (112.24)[b]	$F(2, 57)= 4.66, p=.01, \eta^2=.14$
NC	674.18 (76.19)	649.60 (88.77)	613.62 (61.44)	$F(2, 57)= 3.11, p=.05, \eta^2=.09$
NI	704.64 (94.14)	728.05 (114.65)[a]	650.76 (95.70)[b]	$F(2, 57)= 3.60, p=.03, \eta^2=.11$
PC	695.36 (88.19)[a]	644.20 (89.04)	621.62 (66.93)[b]	$F(2, 57)= 3.50, p=.04, \eta^2=.11$
PI	710.27 (95.07)	717.50 (105.24)	659.90 (86.19)	$F(2, 57)= 2.56, p=.09, \eta^2=.08$

TC abbreviates Threat Congruent; TI Threat Incongruent; NC Neutral Congruent; NI Neutral Incongruent; PC Positive Congruent; PI Positive Incongruent; ASD Autism Spectrum Disorder; HR High Risk; LR Low Risk.

Figures 4 and 5 provide a summary of the engagement and disengagement index scores for each group. The MANOVA comparing the 6 attentional engagement and disengagement indices between the three groups revealed only one significant difference, in the threat-positive engagement index, $F(2, 58)=6.54$, $p=.003$, $\eta^2=.18$. Follow-up planned pairwise contrasts for the threat-positive engagement index revealed that the HR-non ASD group took significantly longer to engage with threatening stimuli (compared to positive stimuli) than both the HR-ASD ($p=.003$, $d=1.25$) and the LR ($p=.04$, $d=.82$) groups.

A post hoc power analysis was conducted to examine how much power the present sample that completed the task ($n=64$) had to achieve a medium effect size of $\eta^2=.06$, which corresponds to a power of $f=.25$, as described in Chapter 3. Overall, the current sample had a power of $(1-\beta)=.40$, critical $F(2, 61)=3.15$, to achieve a medium effect. Further post hoc analyses were carried out to determine how much power each group (HR-ASD, HR-non ASD, LR) had to achieve a medium sized difference ($d=.50$) with one of the other groups. To achieve a difference between the HR-ASD ($n=11$) and HR-non ASD ($n=21$), the present sample had a power of $d=.26$, critical $t(30)=.20$. To achieve a difference between the HR-ASD and LR ($n=29$) groups, the present sample had a power of $d=.28$, critical $t(38)=.20$. Finally, to detect a difference between the HR-non ASD and LR groups, the present sample had a power of $d=.40$, critical $t(48)=.20$.

Follow-up, one-sample t-tests were run on the threat-positive engagement index within each group to confirm that this bias score was significantly different from 0. Threat-positive engagement was significantly different from 0 in the HR-non ASD

group ($t(20)=-5.32$, $p<.001$), but not in the HR-ASD ($t(10)=1.22$, $p=.13$) or the LR ($t(20)=-.35$, $p=.73$) groups.

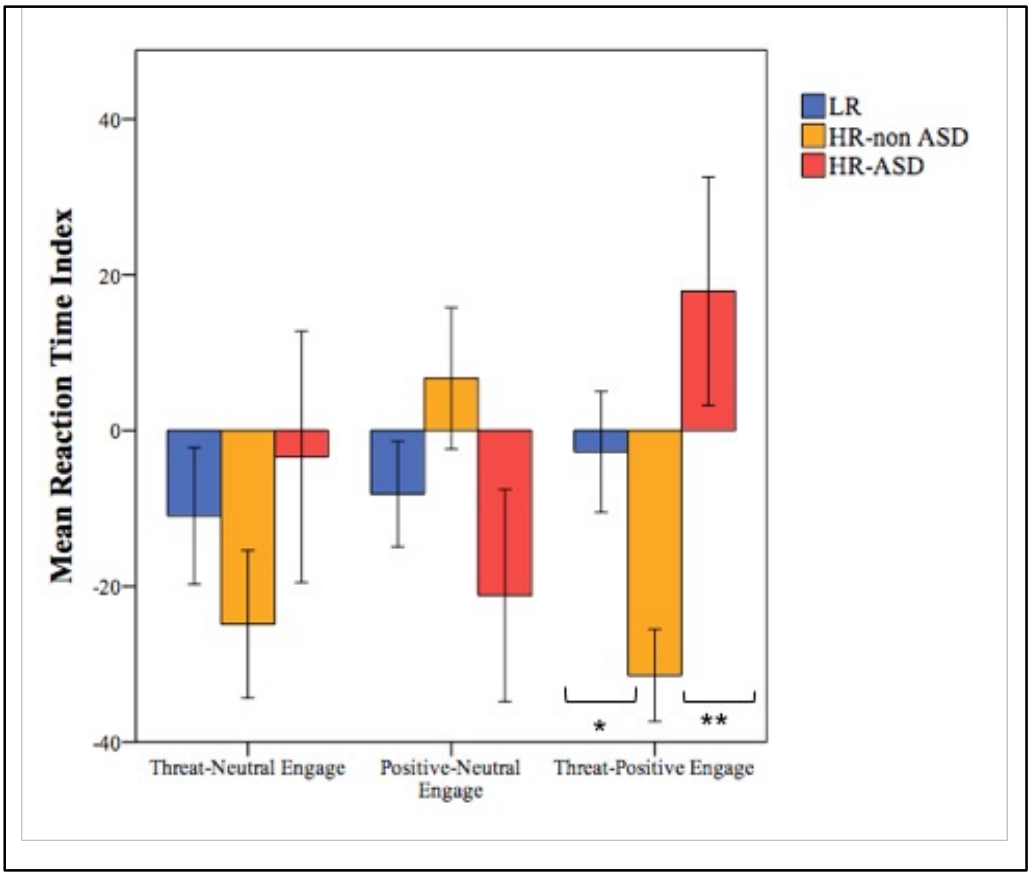

Figure 4. Threat engagement indices in the HR-ASD, HR-non ASD and LR groups. Significant differences are denoted with asterisks (*p<.05, **p<.01). Error bars represent +/- SE of the mean.

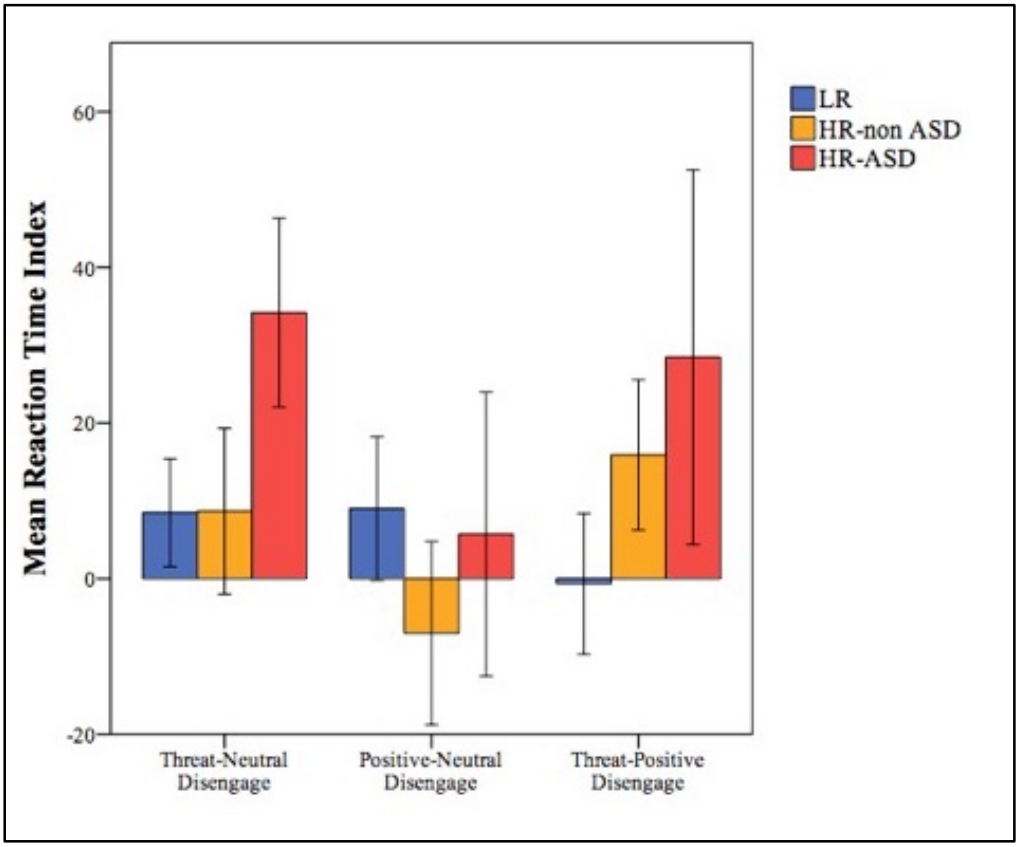

Figure 5. Threat disengagement indices in the HR-ASD, HR-non ASD and LR groups. Significant differences are denoted with asterisks (*p<.05, **p<.01). Error bars represent +/- SE of the mean.

5.3.3 Association between threat bias and anxiety symptoms

There was a significant association between SCAS-P total score and the threat-positive engagement index, $r(60)=.35$, $p=.01$, $r^2=.12$, but not any of the other attention indices (see Table 11). There was also a significant association between SCAS-P total score and SRS t-score, $r(60)=.60$, $p=.01$, $r^2=.36$. Since FSIQ was not associated with SCAS-P total score or any of the threat bias indices, it was removed from further analyses.

Post-hoc power analysis was conducted to determine the power that the current sample who had completed SCAS-P questionnaires (n=60) had in detecting a medium sized effect, r=.30. The analysis revealed that the present sample had power of (1-β)=.67, critical t(58)=2.00 in detecting a significant association between SCAS-P total score and any of the threat bias indices.

There was a trend-level association between SCAS-C total score and the threat-positive engagement index, r(57)=.23, p=.09, r^2=.01. On the other hand, there were no significant associations between SCAS-C total score and SRS t-scores or FSIQ. Associations between SCAS-P, SCAS-C and the threat-positive engagement index are presented in Figures 6 and 7.

Post-hoc power analysis was conducted to determine the power that the current sample who had completed SCAS-C questionnaires (n=57) had in detecting a medium sized effect. The analysis revealed that the present sample had power of (1-β)=.65, critical t(55)=65 in detecting a significant association between SCAS-C total score and any of the threat bias indices.

Table 11: First-order Pearson correlation coefficients showing the association between threat bias indices, SCAS-P, SCAS-C, SRS-2 t-score and WASI-II FSQI

	SCAS-P	SCAS-C	SRS-2	WASI-II FSIQ
Threat-Neutral engage	.19	.20	.00	.00
Threat-Positive engage	.35*	.23	.21	-.07
Threat-Neutral Disengage	.16	-.18	.27	-.10
Threat-Positive Disengage	.10	-.21	.22	-.24
SCAS-P	1			
SCAS-C	.29*	1		
SRS t-score	.60*	.15	1	
WASI-II FSIQ	-.16	.02	-.29	1

Associations denoted with an asterisk (*) were significant, with Bonferonni correction applied (p=.05/7=.007). SCAS-P abbreviates the Spence Children's Anxiety Scale-Parent Report; SRS-2 Social Responsiveness Scale; WASI-II FSIQ Wechsler Abbreviated Scales of Intelligence, 2nd Edition Full Scale IQ.

Further analyses were conducted to examine the association between anxiety and threat bias, taking into account the contributions of ASD severity and sex. Linear regression was run with SCAS-P total score as the dependent variable, and the threat-positive engagement index as the independent variable, co-varying for SRS t-score and sex. The overall model accounted for a significant proportion of variance in anxiety

symptoms, $F(3, 49)=20.61$, $p<.001$, $r^2=.56$. Both the threat-positive engagement index ($\beta=.25$, $t(49)=2.59$, $p=.01$) and SRS t-score ($\beta=.61$, $t(49)=6.19$, $p<.001$) were significantly associated with SCAS-P total score. Sex ($\beta=.17$, $t(49)=1.76$, $p=.08$) had a trend-level association with SCAS-P total score. A post hoc power analysis revealed that the regression analysis with the present sample size ($n=53$) had a power of ($1-\beta$)=.61, critical $F(3, 49)=2.79$, to detect significant effects with a medium effect size ($f^2=.15$).

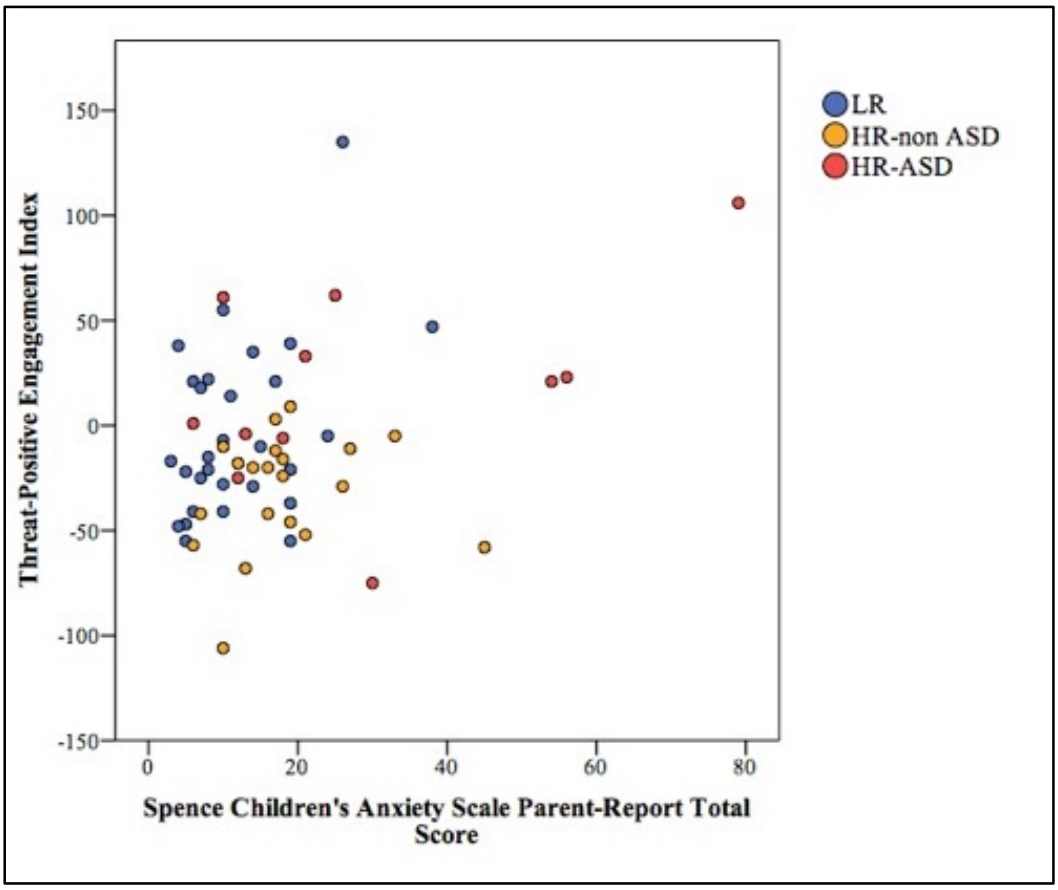

Figure 6. Association between the threat-positive engagement index and SCAS-P total score, with data points marked by group (HR-ASD, HR-non ASD and LR)

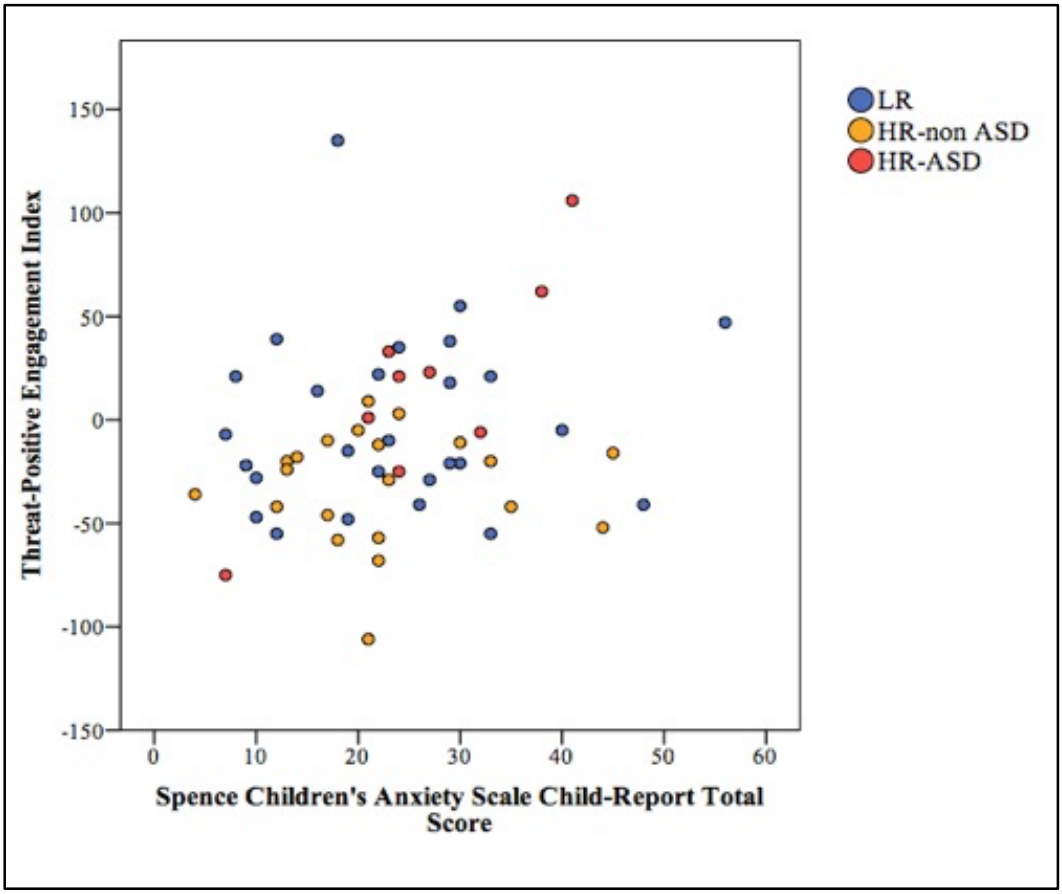

Figure 7. Association between the threat-positive engagement index and SCAS-c total score, with data points marked by group (HR-ASD, HR-non ASD and LR)

5.4 Discussion

The present study is the first to examine the association between attentional bias to threat, anxiety and ASD symptoms within the context of a high-risk for ASD sibling design. Attentional bias was enhanced in the HR-non ASD group, who exhibited longer latencies to detect threatening (compared with positive) stimuli than both the HR-ASD and LR groups. Engagement with threatening stimuli was significantly associated with parent-reported anxiety symptoms, even after taking ASD severity and sex into account. On the contrary, while the HR-ASD group had elevated anxiety, they did not show evidence of threat bias. These findings suggest that the cognitive mechanisms

associated with anxiety in non-ASD populations also relate to anxiety in "non-affected" siblings of children with ASD, but may not be present in those that have ASD.

5.4.1 Attentional bias to threat in children at high-risk for ASD

The emotional spatial cueing task allowed exploration of multiple attentional systems (both attentional orienting and disengagement). It was predicted that the HR-ASD group would exhibit delayed disengagement from threatening stimuli and that this would be associated with anxiety severity. When the analysis was run using the raw scores, there were group differences across conditions. The HR group were generally slower to respond than the LR group across trials, this reached significance for the HR-non ASD group, and was at trend-level for the HR-ASD group. This finding generally seems to reflect overall slower RTs in the HR group compared to LR, which is consistent with prior literature suggesting that children with ASD are slower to make responses on RT-based paradigms (Landry & Parker, 2013).

When the threat bias indices were compared across groups, several unexpected findings emerged. Firstly, despite having heightened anxiety, the HR-ASD group did not manifest delayed disengagement from or enhanced orienting towards threatening stimuli. On the other hand, the HR-non ASD group had significantly longer latencies when engaging with threatening, compared with positive, stimuli than both the HR-ASD and LR groups. Findings remained unchanged when sex and IQ were co-varied (see Appendix 4).

While the direction of bias observed in the HR-non ASD group is unexpected, numerous studies report prolonged latencies to engage with threatening stimuli and suggest this to be indicative of bias away from threat (Koster, Crombez, Verschuere,

Van Damme, & Wiersema, 2006). Typically, such an attentional pattern is observed when stimuli are presented for long durations and there is sufficient time for conscious processing to occur (Koster, Verschuere, Crombez, & Van Damme, 2005; Mogg, Bradley, De Bono, & Painter, 1997), but the time course of attentional processing in anxious children is less conclusive than in adults (Waters, Kokkoris, Mogg, Bradley, & Pine, 2010). However, multiple studies with both anxious adults and children report attentional avoidance when stimuli are presented for 500ms, as they were in the experimental task used in this study (Koster, Crombez, Verschuere, Van Damme, & Wiersema, 2006; Waters & Kershaw, 2015; Waters, Mogg, & Bradley, 2012). Bar-Haim, Lamy, Pergamin, Bakermans-Kranenburg, and van IJzendoorn (2007) suggest that individuals typically begin to process images consciously at approximately 500ms and inconsistencies in previous studies could be largely due to methodological differences, such as use of colour vs. grey scale images and differential onset of target stimulus (Koster, Crombez, Verschuere, Van Damme, & Wiersema, 2006).

It is also important to note that attentional bias in the HR-non ASD group was observed when comparing threatening images with positive, rather than neutral, images. Given the evidence of atypical fear processing in individuals with ASD, it is possible that the neutral images may have presented a certain level of ambiguity and more highly positive images were needed to offset the impact of the threatening stimuli. Research on fear conditioning in ASD suggests that individuals with the condition may have difficulty extinguishing previously learned fear associations (Top et al., 2016). This suggests that they have difficulty distinguishing between threat and safety cues and inhibiting fear responses when they are no longer relevant (Top et al., 2016; Waters & Kershaw, 2015). Furthermore, children with ASD are reported to have

atypical fears and phobias, frequently of commonplace objects (Kerns et al., 2014; Mayes et al., 2013). There is presently a scarcity of studies that explores fear processing in siblings of children with ASD. The threatening stimuli used in this study generally presented evolutionarily-relevant threats (e.g. snakes, spiders) or scenes depicting physical threat (e.g. injections, car crashes). Preschool children, as young as 3 years, exhibit enhanced attentional bias for evolutionary threat (LoBue & DeLoache, 2008). These findings suggest that such threat stimuli are equally salient among unaffected siblings of children with ASD. However, future studies assessing threat bias in children with ASD or their siblings would benefit from asking participants to rate the valence of the images.

5.4.2 Threat bias, anxiety symptoms and ASD severity

A further aim of the present study was to examine the association between anxiety, threat bias and ASD severity. Both parent- and self-report measures of anxiety were used in analyses, to compare how attention bias mapped on to each informants' account of anxiety symptoms. In addition to observing increased bias away from threat in the HR-non ASD group, parent-reported anxiety was significantly associated with both this particular index of threat bias and with ASD severity. On the other hand, self-reported anxiety was not associated with threat bias or ASD severity.

The association between heightened anxiety and ASD severity is unsurprising, as multiple studies report such an association among individuals with ASD (Hallett, Lecavalier, et al., 2013; Sukhodolsky et al., 2008) and anxiety was most highly elevated in the HR-ASD group. The association between anxiety and the threat-positive engagement index remained significant even when taking into account ASD severity

and sex. This implies that the increased threat bias observed in the HR-non ASD group is not merely a by-product of having symptoms of ASD, but is uniquely associated with anxiety. While the difference was not significant, the HR-non ASD did have higher mean scores on the anxiety measure than LR controls at trend-level, which may have reached significance with a larger sample size. They were also reported to have significantly higher separation anxiety. Thus, the heightened threat bias in the HR-non ASD group suggests that anxiety functions similarly among unaffected siblings of children with ASD as it does in non-ASD populations. Furthermore, longitudinal studies in non-ASD populations suggest that increased attentional bias to threat in childhood is a risk factor for the development of anxiety related difficulties in adolescence (Cole, Zapp, Fettig, & Perez-Edgar, 2016; Perez-Edgar, McDermott, et al., 2010). Therefore, the elevated threat bias observed in the HR-non ASD group could also indicate risk for the development of more severe anxiety in later development.

The HR-ASD group, on the other hand, had markedly higher parent-reported anxiety levels compared to LR controls across multiple domains but did not exhibit attentional bias to threat. While it is possible that the modest size of the HR-ASD group (n=11) meant that there was insufficient power to detect a significant effect, the HR-non ASD group did have significantly higher threat bias than HR-ASD group, with a large effect size, d=1.25 (Cohen, 1973). Multiple studies have reported elevated rates of anxiety in individuals with ASD, but found no evidence of an association between anxiety symptoms and bias to socially threatening stimuli (Hollocks, Ozsivadjian, Matthews, Howlin, & Simonoff, 2013; May, Cornish, & Rinehart, 2015). In this study, we failed to observe an association between anxiety and bias to non-social threat. Given these findings, it is possible that anxiety among ASD populations is not

characterised by biased attentional allocation to threat, but that different mechanisms are involved. For example, increased anxiety within ASD may be more attributable to worries about uncertainty (Wigham, Rodgers, South, McConachie, & Freeston, 2014), fear of unwanted change and reduced ability to cope with distress, rather than biased attentional allocation to threat (Hollocks, Ozsivadjian, Matthews, Howlin, & Simonoff, 2013; May, Cornish, & Rinehart, 2015). Thus, it is possible that the stressors associated with anxiety in ASD cannot easily be portrayed using visual stimuli. On the contrary, Sharma, Woolfson, and Hunter (2014) report that specific aspects of interpretation bias of ambiguous scenarios, particularly greater expectancy of negative outcomes and lower perceived emotional coping potential, are associated with elevated anxiety in children with ASD. Perhaps a paradigm measuring interpretation bias, which relies on vignettes rather than visual stimuli, is better suited to capture the complex stressors that are prevalent among individuals with ASD. Although, as noted in Chapter 4, this method may not be suitable for individuals with ASD who have reduced communicative ability.

5.4.3 Comparison between parent- and self-report anxiety symptoms and threat bias

Accurately assessing anxiety symptoms among individuals with ASD is highly challenging (Wood & Gadow, 2010). One of the most prominent factors is the discrepancy observed in self- and caregiver- report of anxiety symptoms and reduced sensitivity of current measures in ASD-populations (Mazefsky, Kao, & Oswald, 2011). There is presently a dearth in research examining how neurocognitive correlates of anxiety map on to self- and parent-reported measures of anxiety among individuals with ASD and those at-risk for ASD. The findings from this study suggest that threat

bias was significantly associated with parent-reported anxiety but not self-report (although this did reach trend-level significance). However, while parents reported children in the HR-ASD group as having the most severe anxiety, it was the HR-non ASD group that demonstrated the highest threat bias. It is possible that parents overestimated anxiety levels of children in the HR-ASD group. On the other hand, these findings also suggest that parents are able to report on anxiety levels in unaffected high-risk siblings with a degree of accuracy. In order to better understand how neurocognitive correlates of anxiety map onto either respondent's reported symptoms, it is necessary to better characterise the mechanisms that underlie anxiety among children with ASD.

Multiple studies suggest that children as young as pre-school age are able to accurately report on their own anxiety symptoms (Spence, 1998). Furthermore, Bitsika, Sharpley, Andronicos, and Agnew (2015) suggest that self-reported anxiety is more strongly associated with salivary cortisol than parent-report among adolescent boys with ASD. However, the findings of the current study contradict prior research to suggest that, among school-aged children, parent-report is more strongly associated with threat bias than self-report is. Perhaps, by adolescence, both children with ASD and those who are typically developing are better able to reflect on their internal states than parents are.

5.4.4 Strengths, limitations and implications for future research

The present study is the first to explore symptoms of anxiety and attentional bias to threat in children with increased familial risk for ASD. The findings have implications for both research and clinical practice. These findings suggest that in

unaffected siblings, the cognitive correlates of anxiety are similar to those found in non-ASD populations. However, the HR-ASD group did not exhibit heightened bias to threat, despite having elevated anxiety by parent report. In line with previous research, this finding could suggest that the cognitive correlates of anxiety in children with ASD are different from those observed in anxious individuals without ASD. Further investigation is required to understand the neurocognitive mechanisms that underlie anxiety in ASD. This could have important implications for clinical practice, as existing therapies for anxiety may need to be modified to suit the specific needs of children with ASD, particularly as threat bias modification therapy is showing increasingly promising results in treating anxiety in children (Shechner et al., 2014).

One limitation of the present study was the small sample size, particularly within the HR-ASD group. Post hoc power analyses revealed that the present sample had weak to moderate power in detecting a group difference in threat bias. However, there was improved power in detecting a significant association between threat bias and anxiety, with the power index being over 60% for both the SCAS-P and SCAS-C measures. Nevertheless, it was not possible to examine associations between threat bias and anxiety independently for each group. It was also not possible to explore these associations in relation to clinically diagnosed anxiety, only a dimensional measure of anxiety symptoms. Future research should examine whether the association between threat bias and anxiety is present in children who are at high-risk for ASD and meet diagnostic criteria for anxiety disorders.

A further limitation is that the highly varied nature of the IAPS images meant that it was difficult to control the visual properties (e.g. luminance, spatial frequency and colour) of the stimuli used in the emotional spatial cueing task. However, to control

for a possible mismatch in the visual properties of the stimuli, each image was presented once in every block, with both the order and assignment to trial type (congruent/incongruent) randomised to ensure that no one image was presented in a particular location or trial type, thus reducing the potential for particular images biasing participants' attention.

Finally, there is a need for longitudinal studies to explore the development and trajectories of anxiety in ASD and non-ASD siblings. In Chapter 6, I will examine the association between dysregulated temperament in infancy and school-age anxiety symptoms in the present cohort.

Chapter 6
Dysregulated temperament in infancy and toddlerhood among children at high familial risk for ASD and its association with anxiety symptoms in middle childhood

6.1 Introduction

Identifying early predictors and developmental pathways of psychiatric conditions is of great importance for both research and clinical practice. Many psychiatric conditions have neurodevelopmental and polygenic underpinnings, but are also influenced by environmental and experiential factors (Cramer et al., 2011). It is widely accepted that harmful environmental factors can increase the risk of developing psychopathology (Rutter, 2005). Similarly, however, interventions that target specific risk factors, before the onset of the disorder, can significantly reduce the risk of developing psychopathology (Webster-Stratton & Taylor, 2001). Such targeted interventions are most effective when administered in early development, while there is greater potential to harness neuroplasticity and make lasting changes (Cramer et al., 2011; Webster-Stratton & Taylor, 2001). Thus, identification of the predictors of psychopathology through the use of prospective longitudinal methodology is of high significance in identifying the relevant factors to focus on in early intervention.

In recent years, prospective longitudinal studies of infants at-risk for ASD have identified several early risk-markers of the condition (e.g. Jones, Gliga, Bedford, Charman, & Johnson, 2014). Recently developed targeted interventions administered to high-risk infants have shown promise in reducing some of the autism-risk behaviours (e.g. Green et al., 2015). Given the high prevalence of co-occurring psychopathology among individuals with ASD (e.g. Simonoff et al., 2008), research into identifying the

early markers for these conditions among high-risk infants is also highly relevant. The aim of this chapter, therefore, is to investigate potential risk factors in infancy and toddlerhood, which place children at high-risk for ASD at increased risk for developing co-occurring anxiety as well. Such research may help elucidate the shared aetiology of the two conditions and increase our understanding of the mechanisms leading to such high co-occurrence.

One of the earliest identified risk factors of anxiety is the manifestation dysregulated temperament (e.g. Costello, Egger, & Angold, 2005). In particular, heightened levels of Negative Affect, the propensity to experience high levels of distress, in early childhood, are associated with increased anxiety later in childhood and adolescence (e.g. Fox & Pine, 2012). Atypicalities in temperament, including increased Negative Affect, are also observed among infants at high-risk for ASD, particularly among those who themselves meet diagnostic criteria (e.g. Clifford et al., 2013). In spite of the overlap in the early temperamental characteristics observed among infants at-risk for ASD and for anxiety, the association between early Negative Affect and the emergence of anxiety symptoms among children with ASD has not yet been examined.

6.1.1 The construct of temperament

Temperament research has an extensive history, which has culminated in the development of multiple operational definitions and methods of measurement (for review see Rothbart, 2011). Thomas and Chess (1977) first defined temperament as an individual's behavioural style, which is essentially the "how" of behaviour and is distinct from skill (the "how well") or motivation (the "why"). This model defined temperament across nine dimensions, which measured aspects of emotional reactivity and attention and constituted three higher-order classifications that described children

as “difficult”, “easy” and “slow to warm up”. The New York Longitudinal Study (NYLS; Thomas & Chess, 1984) assessed the associations between these aspects of temperament in early childhood through to adulthood and reported stability across time, suggesting that temperamental characteristics emerge early in life and persist throughout development. Since the formation of Thomas and Chess’s model, numerous taxonomies of temperament have emerged and have received empirical support (for review see Rettew & McKee, 2005). Unfortunately, however, there is still insufficient consensus regarding the taxonomy of temperament (De Pauw & Mervielde, 2010). This thesis focuses primarily on the taxonomic model outlined by Rothbart and colleagues, whose age-specific measures of infant and early childhood temperament have been well validated and used widely in developmental research.

Rothbart and Deryberry (1981) advanced the definition by Thomas and Chess (1977) to describe temperament as individual differences in reactivity and self-regulation, which have a *constitutional* basis. Within this model, reactivity refers to an individual’s response to changes in the environment, while self-regulation describes the processes that modulate reactivity. Furthermore, the description of these individual differences as constitutional implies that the processes involved in the development of temperament include an interplay between heritable, biologically based traits, maturation and experience (Rothbart & Deryberry, 1981). Whittle, Allen, Lubman, and Yucel (2006) suggest that the affective and regulatory mechanisms of temperament have neurobiological bases, being modulated by activity in the amygdala and regions involved in cognitive control. In turn, these neurobiological mechanisms have underlying genetic bases. Therefore, temperamental traits have been suggested as a

potential endophenotypes in conditions that have a complex genetic heritability, such as ASD (Garon et al., 2016).

In Rothbart's model, temperament is measured across multiple dimensions that cluster around three general higher-order factors – *Negative Affect, Surgency* and *Effortful Control* (Putnam, Rothbart, & Gartstein, 2008). Negative Affect and Surgency constitute the reactive components of temperament, while Effortful Control is the regulatory element (Rothbart & Bates, 1998). Negative affect refers to a child's inclination to experience distress and displeasure in response to variations in the environment, including fearfulness, sadness, anger and frustration (Rothbart & Bates, 1998). On the contrary, Surgency is akin to extraversion and depicts levels of positive affectivity and approach (Rothbart & Bates, 1998). Finally, Effortful Control describes the attentional and behavioural mechanisms used to modulate reactivity (Rothbart & Posner, 2006).

Rothbart, Ahadi, Hershey, and Fisher (2001) suggest that temperament emerges in infancy and develops over time but that these traits persist until middle childhood. For example, by 2-3 months, infants exhibit behaviours associated with Surgency and approach, such as smiling and laughter (Rothbart, 2007). Negative Affectivity also emerges during the first year of life, with the manifestation of anger and frustration by 2-3 months and fearfulness by 7-10 months (Rothbart & Bates, 1998). Fearfulness and distress to novelty tend to peak between 9 and 18 months, but then decline by ~24 months in most children (Warren & Sroufe, 2004). Effortful control begins to emerge by 12 months but does not become fully stable until ~36 months (Kochanska, Murray, & Harlan, 2000; Rothbart, Ellis, Rosario Rueda, & Posner, 2003). There is evidence supporting the continuity of temperament, as both parent-report and laboratory

observation of temperamental dimensions in infancy and toddlerhood are significantly associated with temperament at age 7 years (Rothbart, Ahadi, & Evans, 2000).

6.1.2 The association between temperament and psychopathology

Dysregulated temperament in infancy and early childhood has been associated with a number of adverse outcomes later in life (Rothbart, 2004). However, the mechanisms through which temperament contributes to psychopathology are not fully clear. Four models have been proposed to explain the association between early difficulties in temperament and later psychopathology (Nigg, 2006; Rettew & McKee, 2005): (a) the *spectrum* model proposes a dimensional approach, suggesting that psychopathology is an extreme manifestation of temperament with shared aetiological underpinnings, (b) the *vulnerability/risk* model suggests that temperament and psychopathology are aetiologically distinct, but that specific dimensions of temperament increase one's risk for developing a particular condition, (c) the *psychoplastic effect* model proposes that temperament influences the course of a disorder once it occurs; and (d) *scar effects*, where the pathological processes associated with a disorder also alter a person's temperamental traits.

As outlined by two comprehensive reviews (Nigg, 2006; Rettew & McKee, 2005), evidence from studies examining the association between temperamental dimensions and psychopathology support the vulnerability/ risk model. While temperament readily accounts for a significant proportion of the variation in psychopathology, the correlation coefficients of these associations are often small to moderate in magnitude (Eisenberg & Morris, 2002; Eisenberg et al., 2009; Nigg, 2006; Rettew & McKee, 2005), suggesting that they are not extremes of the same dimension. This could be due to measurement error, but occurs even when measures of

temperament and psychopathology are obtained concurrently from the same respondent and statistically corrected for attenuation (Nigg, 2006; Rettew & McKee, 2005). Behavioural genetics studies have thus far produced equivocal results (Rettew & McKee, 2005). While there is evidence for the heritability of temperamental style (Emde et al., 1992; Whittle, Allen, Lubman, & Yucel, 2006), psychiatric disorders and their associated temperamental dimensions share little genetic overlap (Gjone & Stevenson, 1997). Nigg (2006) suggests that the association between temperament and psychopathology occurs through a diathesis-stress (Ingram & Luxton, 2005) or a gene by environment (Moffitt, 2005; Rutter, 2005) model, where difficult temperament alone does not predispose a child to developing disorder, but interacts with environmental risks to increase the likelihood of psychopathology.

6.1.3 Temperamental dimensions and the development of anxiety disorders

Pioneering work by Watson, Clark, and Carey (1988) emphasised the role of mood factors in the aetiology of internalising disorders. In particular, their model suggests that both anxiety and depression are characterised by heightened levels of Negative Affect, but that they can be distinguished by levels of positive affectivity, which is reduced only among individuals with depression. Watson and Clark (1984) suggest that individuals with high levels of Negative Affect are likely to continually experience discomfort, even in contexts that do not pose high threat. This account is consistent with the cognitive theories of anxiety, which propose that highly anxious individuals perceive threat in the environment, even in the absence of any objective danger (Beck, Emery, & Greenberg, 1985). As outlined in Chapter 1, developmental theories of anxiety disorders also stress the role of Negative Affect as a risk factor for the condition. A review by Lonigan, Vasey, Phillips, and Hazen (2004) proposes that

the development of anxiety disorders in children involves an interplay between Negative Affect and Effortful Control. This model suggests that anxiety is associated with high levels of Negative Affect, partly because increased negative emotionality is associated with biased attentional processing of threat-relevant information. Levels of Effortful Control can moderate the extent of these attentional biases and promote more adaptive coping strategies to stressors. Therefore, both high levels of Negative Affect and low levels of Effortful Control are necessary for the development of anxiety (Muris & Ollendick, 2005).

6.1.3.1 The taxonomy of Negative Affect and its association to anxiety disorders

The association between Negative Affect in infancy and toddlerhood and the development of anxiety disorders has received widespread empirical support. However, variability and lack of consensus in taxonomic the models of temperament complicate the narrative of these findings. While some studies directly examine the association between Negative Affect and anxiety (Cote et al., 2009), others focus on specific dimensions (e.g. fearfulness, shyness) that constitute Negative Affect (Dyson, Klein, Olino, Dougherty, & Durbin, 2011). Other taxonomic models have been developed to describe behaviours similar to those that constitute Negative Affect. For example, Kagan, Reznick, and Snidman (1987) described the temperamental trait of Behavioural Inhibition (BI), which is characterised by the propensity to experience distress and to withdraw from novel situations or people. There is substantial overlap in the constructs of BI and Negative Affect to the extent that some studies measure BI through parent-report of shyness and fearfulness (Dyson, Klein, Olino, Dougherty, & Durbin, 2011) using the scales developed by Rothbart and Deryberry (1981). Other models, describe

"withdrawal behaviours", which are characterised by high levels of distress to changes in the environment and shyness towards strangers (Rapee, 2002).

Regardless of the specific taxonomy used, there is consensus that a temperamental style characterised by high levels of distress to novelty, fearfulness and weariness of strangers, is associated with the development of anxiety disorders (Fox, Henderson, Marshall, Nichols, & Ghera, 2005; Fox & Pine, 2012; Muris & Ollendick, 2005; Schwartz, Snidman, & Kagan, 1999). Rapee (2002) argues that temperamental characteristics associated with withdrawal (fearfulness and shyness) are the most robust predictors of anxiety and that other risk factors (e.g. parental anxiety and maladaptive coping styles) are either mediated or moderated by temperament. Karevold, Roysamb, Ystrom, and Mathiesen (2009) also report that both childhood temperament (shyness and emotionality) and environmental factors (maternal distress, adversity and support) contribute to the development of anxiety in adolescence, but that most risk factors are partially mediated by temperament. This association can be detected very early in life, with some studies reporting that Negative Affect at the age of 3-5 months is associated with later anxiety (Cote et al., 2009; Kagan, Snidman, Zentner, & Peterson, 1999). Furthermore, there is evidence that Negative Affect and anxiety have similar neurocognitive correlates, such as heightened attentional bias to threatening stimuli (Cole, Zapp, Fettig, & Perez-Edgar, 2016; Gaffrey, Barch, & Luby, 2016; Nakagawa & Sukigara, 2012; Perez-Edgar et al., 2011).

While temperament is largely considered to be a stable trait (Rothbart, Ahadi, & Evans, 2000), numerous studies report a discontinuity in Negative Affect over time and not all children who are high on this trait go on to develop anxiety disorders (for review see Degnan & Fox, 2007). This is consistent with the observations of Warren and

Sroufe (2004), who suggest that fearfulness and distress to novelty tend to decline by ~24 months. The decline of negative emotionality can be attributed to a number of environmental and intrinsic resilience factors, including the development of attentional and inhibitory control processes (Degnan & Fox, 2007). Therefore, some researchers posit that children who exhibit continually high and stable levels of Negative Affect are the ones who are at the most heightened risk for anxiety (Costello, Egger, & Angold, 2005; Degnan & Fox, 2007).

6.1.3.2 The association between Effortful Control and anxiety

Fewer studies have examined the interplay between Negative Affect and Effortful Control in the development of anxiety disorders. Lonigan and Vasey (2009) report that Effortful Control moderates the association between Negative Affect and threat bias, such that children who have both elevated Negative Affect and reduced Effortful Control exhibit heightened attentional allocation to threatening stimuli. However, other studies (Cole, Zapp, Fettig, & Perez-Edgar, 2016) have not supported these findings and suggest that threat bias moderates the association between Negative Affect and social withdrawal (behaviours associated with Social Anxiety), such that children with high levels of Negative Affect and the propensity to attend to threatening stimuli are more likely to exhibit withdrawal behaviours. Effortful Control, on the other hand, has a direct, negative association with later social withdrawal.

Eisenberg et al. (2009) report that, while Negative Affect is directly associated with internalising difficulties, Effortful Control is associated with these problems to the degree that it predicts levels of maladjustment over time. Thus, children with high Effortful Control may be better able to cope with their internalising symptoms than those with reduced levels. Correspondingly, Nakagawa and Sukigara (2013) report that

among infants, those with higher levels of Effortful Control manifest less Negative Affect.

6.1.4 Temperament among infants at high-risk for ASD

Individuals with ASD are reported to have atypical temperamental profiles, including high levels of Negative Affect, withdrawal, and reduced attentional flexibility (De Pauw, Mervielde, Van Leeuwen, & De Clercq, 2011). Prospective longitudinal studies of infants at high-risk for ASD suggest that these temperamental characteristics manifest in the first year of life and may be particularly characteristic of the infants who later develop ASD (Bryson et al., 2007; Clifford et al., 2013; Del Rosario, Gillespie-Lynch, Johnson, Sigman, & Hutman, 2014; Zwaigenbaum et al., 2005). By the age of 12 months, high-risk infants exhibit heightened irritability and distress, and are more difficult to soothe than LR infants (Bryson et al., 2007; Zwaigenbaum et al., 2005).

Studies that have followed high-risk infants to the age when a research diagnosis of ASD could be made (24-36 months), suggest that heightened levels of Negative Affect and reduced Effortful Control are especially prevalent during the first two years of life among children who meet diagnostic criteria for ASD (Clifford et al., 2013; Garon et al., 2009). Clifford et al. (2013), who reported on the temperamental profiles of the cohort of children from this study, also examined temperament among high-risk siblings who had typical development and those who were considered 'atypical' (as described in Chapter 2). Both groups of children exhibited 'intermediate' levels of Negative Affect and Effortful Control, where they did not differ significantly from either the HR-ASD or LR groups. Del Rosario, Gillespie-Lynch, Johnson, Sigman, and Hutman (2014) report that, at 24-months, infants who meet criteria for

ASD have heightened Negative Affect, but that group differences become non-significant by the age of 36-months. Garon et al. (2016), on the other hand, report that infants who were diagnosed 'late' (at the age of 36 months) had higher levels of Negative Affect than those who received an early diagnosis (at 24 months). The authors propose that infants with the most severe ASD, who are diagnosed early, are more likely to be placid and disengaged, while those diagnosed later engage with the environment to a greater, albeit more negative, degree. Furthermore, within this study, the high-risk infants exhibited elevated Negative Affect at the age of 12 months, but this was not significantly associated with ASD severity at 36 months.

Finally, Garon et al. (2009) used discriminant function analysis to identify the combination of temperamental traits that best distinguished the high-risk children with ASD, high-risk children with typical development and low-risk controls. Two distinct dimensions emerged, which described behavioural approach and emotion regulation. Children who met diagnostic criteria for ASD at 36 months exhibited the highest levels of emotion dysregulation, including higher negative emotionality and social fear, lower attentional control and reduced approach behaviours. The approach dimension significantly discriminated the high-risk children who had ASD from those who did not and from low-risk controls. On the other hand, reduced emotion regulation distinguished the entire high-risk group from controls. This suggests that temperamental traits associated with reduced emotion regulation may be part of the BAP, rather than being unique to clinical-level ASD.

6.1.5 Heightened Negative Affect as an early marker of anxiety in children with ASD

Mundy, Henderson, Inge, and Coman (2007) suggest that early temperamental atypicalities in ASD may act as modifier processes, which contribute to the wide heterogeneity observed in this condition. According to this model, modifiers like temperament interact with the core features of ASD to contribute to the differences in development and behaviour observed among individuals with ASD. Correspondingly, Negative Affect has been associated with numerous aspects of functioning among children with ASD, including sensory hypersensitivity, withdrawal and problem behaviours (Brock et al., 2012; Chuang, Tseng, Lu, & Shieh, 2012; Kerekes et al., 2013). However, the contribution of early Negative Affect to the development of co-occurring anxiety among individuals with ASD has not yet been examined.

Tonnsen, Malone, Hatton, and Roberts (2013) examined the association between Negative Affect in infancy and anxiety at 71 months in children with Fragile X syndrome, who are also at increased risk for developing both ASD and anxiety. Negative Affect significantly predicted anxiety severity, but not ASD symptoms, within this cohort. In light of these findings, and the widely reported association between anxiety and Negative Affect in non-ASD populations, their association in children at high-risk for ASD warrants further investigation.

6.1.6 Aims and hypotheses

The aims of the present chapter are threefold. Firstly, while there are multiple reports of the temperamental characteristics of children at-risk for ASD, no study to date has examined temperament in high-risk children beyond the age of 36 months.

Therefore, it is presently unclear whether the temperamental profiles of high-risk infants, such as heightened Negative Affect and reduced Effortful Control, persist beyond toddlerhood. Furthermore, given that a proportion of the high-risk participants in this cohort has received a 'late' diagnosis of ASD and the outcome groups have changed since the 36-month visit, re-examination of the previous patterns is warranted. The second aim of this chapter is to examine the development of Negative Affect in high-risk and low-risk infants. Given reports that Negative Affect declines in typically-developing children by the age of ~24 months (Warren & Sroufe, 2004), it is possible that low-risk infants also exhibit a decline in this factor while high-risk infants maintain persistently high levels over time. This would also place high-risk infants at increased risk for developing anxiety (Degnan & Fox, 2007). A further aim is to investigate whether changes in Negative Affect are associated with the development of Effortful Control. Finally, the association between Negative Affect and Effortful Control in infancy and toddlerhood and anxiety symptoms at 7-years will be examined. I also aim to identify the earliest time that an association between atypical temperament and anxiety can be detected.

I propose the following hypotheses:

1. HR children will exhibit high levels of Negative Affect, and lower Effortful Control and Surgency at all ages, including the 36-month and 7-year visits. A pattern is expected to emerge where these characteristics are most pronounced in the HR-ASD group, but are also elevated in the HR-non ASD group, compared to LR controls.
2. As outlined above, Warren and Sroufe (2004) suggest that at ~24 months of age, most children show a decline in Negative Affect, while some children

continue to have persistently high levels, increasing their risk for developing anxiety. Thus, it can by hypothesised that the LR group will show a decline in Negative Affect at 24-months, while children in the HR group will have persistently high levels of this trait.

3. Reduced Effortful Control will be associated with higher Negative Affect across time.
4. Higher Negative Affect and reduced Effortful Control in infancy and toddlerhood will be associated with increased anxiety at 7-years, in the HR group, even when ASD severity and developmental level are taken into account. Given that both Negative Affect and Effortful Control begin to emerge within the first year of life (Rothbart & Deryberry, 1981; Rothbart, Ellis, Rosario Rueda, & Posner, 2003), an association between these factors and anxiety can be expected as early as 7-14 months. The association between temperament and anxiety will continue to be significant at 24- and 36-months.

6.2 Method

6.2.1. Temperament measures

Temperament was assessed at each visit (7 months, 14 months, 24 months, 36 months and 7 years) using age appropriate parent-report questionnaires, developed by Rothbart and Colleagues. The *Infant Behavior Questionnaire – Revised* (IBQ-R; Gartstein & Rothbart, 2003) was used at the **7-month and 14-month** visits (hereafter IBQ1 and IBQ2, respectively). The *Early Childhood Behavior Questionnaire* (ECBQ; Putnam, Gartstein, & Rothbart, 2006) was used at the **24-month** visit. The *Child Behavior Questionnaire* (CBQ) *very short-form* (Putnam & Rothbart, 2006) was administered at the **36-month** visit and the *Child Behavior Questionnaire standard-*

form (CBQ; Rothbart, Ahadi, Hershey, & Fisher, 2001) was used at the **7-year** visit (hereafter CBQ1 and CBQ2, respectively). Each questionnaire assesses temperament across multiple dimensions (summarised in Table 12). The three main factors of Negative Affect, Effortful Control and Surgency are computed by averaging scores on specific dimensions. The factor affiliation of each dimension was identified through factor analysis, which was performed separately for the IBQ-R, ECBQ and CBQ by the authors of each measure (Gartstein & Rothbart, 2003; Putnam, Gartstein, & Rothbart, 2006; Rothbart, Ahadi, Hershey, & Fisher, 2001). The CBQ very-short form (Putnam & Rothbart, 2006) does not contain dimension scores and the three factors are computed by averaging specific items, which were identified through factor analysis by the authors. Table 13 summarises the factor affiliation of the dimensions on each of the measures.

The *IBQ-R* (Gartstein & Rothbart, 2003) is suitable for assessing temperament in infants aged 3-12 months. The scale consists of 191 items, which ask parents to indicate how frequently their child has engaged in a range of behaviours over the last 7 days (e.g. 'cry or fuss before going to sleep for naps'). Responses are recorded on a 7-point Likert-scale, ranging from 'never' to 'always' and a 'not applicable' option is also given. Temperament is measured across 14 dimensions (summarised in Tables 12 and 13), which are computed by averaging pre-specified items. The scale has good to excellent psychometric properties (Gartstein & Rothbart, 2003) and internal consistency within our sample was good for IBQ 1 (α=.72) and acceptable for IBQ 2 (α=.51).

The *ECBQ* (Putnam, Gartstein, & Rothbart, 2006) is suitable for assessing temperament among children aged 18-36 months. The format of the ECBQ is similar to

the IBQ-R (Gartstein & Rothbart, 2003), but includes 201 age-appropriate items assessing the child's behaviour over the last two weeks (e.g. 'After getting a bump or scrape how often did your child forget about it in a few minutes?'). Temperament is measured across 18 dimensions, 8 of which are also present in the IBQ-R (Gartstein & Rothbart, 2003) and 10 new ones. The ECBQ is reported to have very good psychometric properties (Putnam, Gartstein, & Rothbart, 2006) and internal consistency within this sample was excellent (α=.97).

The *CBQ* (Putnam & Rothbart, 2006; Rothbart, Ahadi, Hershey, & Fisher, 2001) is suitable for assessing temperament in children aged between 36 months and 7 years. Parents are presented with various statements (e.g. my child gets quite frustrated when prevented from doing something he/she wants to do) and asked to indicate how true each is of their child's reactions or behaviours over the last 6 months. The CBQ Standard Form (Rothbart, Ahadi, Hershey, & Fisher, 2001) consists of 195 items and measures temperament across 15 dimensions that are extensions of the IBQ-R and ECBQ. The CBQ very-short form (Putnam & Rothbart, 2006) contains 36 items, which were selected from the standard version. Responses are recorded on a 7-point Likert scale (ranging from 'extremely untrue of your child' to 'extremely true of your child') and a 'not applicable' option is also provided. Both scales are reported to have very good psychometric properties (Putnam, Gartstein, & Rothbart, 2006; Rothbart, Ahadi, Hershey, & Fisher, 2001) and internal consistency within this sample was good for both CBQ 1 (α=.74) and for CBQ 2 (α=.82).

Table 12: Names and definitions of each dimensions measured on the IBQ-R, ECBQ and CBQ

Dimension	**Definition**
Activity level	Gross motor activity levels
Anger/frustration	Negative emotions resulting from interruption of tasks or goals.
Approach	Levels of excitement of anticipated pleasurable events.
Attentional focusing	Ability to focus and flexibly shift attention.
Attentional shifting	Ease of shifting attention from one activity to another.
Cuddliness	Desire for closeness and physical contact with others.
Discomfort	Negative emotions resulting from change in sensory input from environment.
Distress to limitations	Negative emotions/behaviour when unable to perform particular action.
Duration of orienting	Ability to focus and flexibly shift attention.
Falling reactivity/rate of recovery	Rate of recovery from arousal or distress; ease of falling asleep.
Fear	Negative emotions related to anticipated pain or distress.
High intensity pleasure	Level of pleasure/enjoyment resulting from situations where there is high stimulation or complexity or novelty.

Impulsivity	Speed of initiating a response.
Inhibitory control	Ability to suppress inappropriate responses when asked to do so and the ability to plan actions.
Low intensity pleasure	Level of enjoyment resulting from situations with low stimulus intensity or novelty or complexity.
Motor activation	Motor movements, repetitive motion, fidgeting.
Perceptual sensitivity	Level of sensitivity to low intensity stimuli from external environment.
Positive anticipation	Level of positive reactivity or excitement to anticipated situations.
Sadness	Amount of negative emotions, sadness, low energy.
Shyness	Wariness of novel situations or people.
Smiling and laughter	Positive affect in response to changes in the environment.
Sociability	Seeking and enjoying interaction with other people.
Soothability	Rate of recovery from distress or arousal.
Vocal reactivity	Vocalisation during daily activity.

Definitions of the dimensions were obtained from Putnam, Rothbart, and Gartstein (2008)

Table 13: Summary of factor affiliation on each version of the temperament scales (IBQ-R, ECBQ, CBQ)

	Temperament Scale								
	IBQ-R			ECBQ			CBQ		
Dimension	NA	EC	SU	NA	EC	SU	NA	EC	SU
Activity level			X			X			X
Anger/frustration				X			X		
Approach			X						
Attentional focusing					X			X	
Attentional shifting					X				
Cuddliness		X			X				
Discomfort				X			X		
Distress to limitations	X								
Duration of orienting		X							
Falling/ROR	X(R)								
Fear	X			X			X		
HIP			X			X			X
Impulsivity						X			X

Inhibitory control					X			X	
LIP		X			X			X	
Motor activation				X					
Perceptual sens.			X	X				X	
Positive anticipation						X			
Sadness	X			X			X		
Shyness				X					X
Smiling and laughter			X						
Sociability						X			
Soothability		X		X(R)			X(R)		
Vocal reactivity			X						

(R) indicates that the dimension was reverse coded when computing the factor score. IBQ-R denotes Infant Behavior Questionnaire – Revised. ECBQ Early Child Behavior Questionnaire. CBQ Child Behavior Questionnaire. NA Negative Affect. EC Effortful Control. SU Surgency.

6.2.1.1 Inconsistency in factor affiliation across temperament scales

While the IBQ-R, ECBQ and CBQ (Gartstein & Rothbart, 2003; Putnam, Gartstein, & Rothbart, 2006; Putnam & Rothbart, 2006; Rothbart, Ahadi, Hershey, & Fisher, 2001) have been widely used to measure temperament in children from infancy to middle childhood, several challenges exist to using these scales in longitudinal analyses. As is evident from Table 13, the dimensions that each factor is composed of vary across the three measures. In some cases, this is justified because the types of behaviours that children engage in change from infancy to the age of 7-years. Certain aspects of behaviour that are relevant and observable at a particular age, may not be evident at other ages (e.g. vocal reactivity is not relevant once a child develops speech). However, in most of these cases, new dimensions that have been added to the scales for older children and are direct extensions of dimensions on the versions used with younger children. For example, the dimensions of 'Attentional Focusing' and 'Soothability' on the ECBQ and CBQ are extensions of the 'Duration of Orienting' and 'Falling Reactivity/Rate of Recovery' dimensions on the IBQ-R, respectively (Putnam, Rothbart, & Gartstein, 2008). The dimensions and corresponding factor scores are significantly correlated across the three measures, suggesting continuity of these temperamental characteristics (Putnam, Rothbart, & Gartstein, 2008).

However, it is more problematic that three dimensions (Perceptual Sensitivity, Shyness and Soothability) change factor affiliation across measures. This is problematic for several reasons; if a distinct pattern of group differences is observed in the factor scores at different times, it will be difficult to discern whether these are true changes or if they result from a change in the composition of the factor scores. A further problem emerges when trying to examine associations between the factors

measured at different times, as having dimensions that load on to both factors may result in artefactual associations between them. To resolve this issue, various authors have used distinct approaches, for example Putnam, Rothbart, and Gartstein (2008) suggest that it is suitable to use both the factor and dimension scores in longitudinal analyses, given their significant associations across measures. Tonnsen, Malone, Hatton, and Roberts (2013), on the other hand, computed the Negative Affect scores using dimensions that consistently loaded onto that factor. For the analyses in this chapter, the three dimensions that exhibited inconsistent factor affiliation across measures were removed when computing factor scores. For the 36-month visit, items that corresponded to those dimensions were removed when calculating factor scores. Given that unexpected findings emerged at the 36-month visit (see results), it was important to ensure that this was not an artefact of the alteration of factor scores, so analyses were repeated with the original factor structure and are presented in Appendix 5. Furthermore, to ensure comparability of the measures across time, the means of the factor scores were standardised through z-transformation (Fischer & Milfont, 2015).

6.2.2 Measures of ASD severity, developmental level and anxiety

The Spence Children's Anxiety Scale – Parent report (SCAS-P; Nauta et al., 2004) administered at the 7-year visit was used to examine association between temperament and anxiety. Furthermore, as outlined in Chapter 2, measures of ASD symptomatology and developmental level were collected at every visit and will be used in the analyses in this chapter.

6.2.2.1 Measures of ASD severity

As the ADOS/AOSI were administered at every visit, these measures will be used to assess ASD severity. The AOSI (Bryson, Zwaigenbaum, McDermott,

Rombough, & Brian, 2008) was administered at the **7-month** and **14-month** visits while the ADOS (Lord et al., 2000; Lord et al., 2012) was used at the **24-month**, **36-month** and **7-year** visits. Different Modules of the ADOS were used at each visit and administered to children based on their developmental level and language ability. Therefore, the use of Calibrated Severity Scores, (CSS; Hus, Gotham, & Lord, 2014), which are described in more detail in Chapter 2, is preferable because they provide a measure of ASD severity that takes into account the module used and the child's age/developmental level. CS scores are not available for the AOSI, so raw scores must be used for this measure. Gammer et al. (2015) examined the association between AOSI and ADOS scores within the HR sample from this study and reported significant correlations with 14-month AOSI scores and ADOS raw scores at 24-months and 36-months. Using CS scores, we find a similar pattern, where there is a trend-level association between 14-month AOSI raw scores and ADOS CS scores at 24-months ($r(51)=.24$, $p=.09$) and a significant association with ADOS CS scores at 36-months ($r(55)=.31$, $p=.02$) and 7-years ($r(41)=.47$, $p=.002$). Therefore, it was deemed appropriate to use AOSI raw scores in conjunction with the ADOS CSS for these analyses.

6.2.2.2 Measures of developmental level

At the 7-month, 14-month, 24-month and 36-month visits, the Mullen Scales of Early Learning (MSEL; Mullen, 1995) were used to measure developmental level, while the Wechsler Abbreviated Scales of Intelligence-2nd Edition (WASI-II; Wechsler, 2011) was used at the 7-year visit. Standard Scores (SS) for both the MSEL and WASI-II will be used in these analyses, as the scales have equivalent means and standard deviations ($M=100$, $SD=15$).

6.2.3 Statistical analyses

6.2.3.1 Preparation of temperament data

While temperament data was collected from all children that participated in each visit, for this analysis only children who took part in the 7-year follow-up and those who were assigned to a diagnostic outcome group will be used in analyses. As in previous chapters, the three participants who lost diagnosis from the 36-month to the 7-year visits will be excluded from analyses. Dimension and factor scores will be computed for each temperament measure as outlined above. The 'not applicable' response was treated as missing data and items with this response were excluded when computing the mean dimension scores. Once dimension and factor scores were computed, the factor scores were z-transformed. The data were also screened for any outliers that were ±3SD from their group mean. However, no such cases were identified. There was missing data at most visits; 6.67% at 7-years, 2.67% at 36-months, 5.33% at 24-months, 2.66% at 14-months and no missing data at 7-months.

6.2.3.2 Demographic characteristics, ASD severity and developmental level

Demographic characteristics (age and sex ratio at each visit) were assessed using ANOVA and chi-squared tests, where appropriate. To examine group differences in ASD severity across time, AOSI total scores and ADOS CS scores were compared across the HR-ASD, HR-non ASD and LR groups using ANOVA. As the ADOS was not administered to the LR group at the 24-month visit, only the HR-ASD and HR-non ASD group scores were compared for this visit. Finally, to assess group differences in cognitive ability across time, Mullen SS and WASI-FSIQ were compared across the HR-ASD, HR-non ASD and LR groups using ANOVA.

6.2.3.3 Group differences in temperament across time

In order to assess group differences in the temperament factors across time, Multivariate ANOVA was used to compare the HR-ASD, HR-non ASD and LR groups on Negative Affect, Effortful Control and Surgency at each visit. Planned comparisons between each pair of groups were performed where significant differences emerged, with Bonferroni correction applied to account for multiple testing. Post hoc power analyses were carried out for each MANOVA to determine whether the sample size was sufficient at each time point to detect significant group differences.

6.2.3.4 Change in Negative Affect over time and its association with Effortful Control

To address whether Negative Affect levels change in the HR and LR groups across time, and whether change in Negative Affect is associated with levels of Effortful Control, a Generalized Estimating Equation (GEE; Liang & Zeger, 1986) was used. GEE can be used to test main effects, interactions and be applied to data that is categorical or continuous (Ballinger, 2004), making it a good option for this analysis which incorporated both data types. The scale response was set as linear and the correlation structure was set as autoregressive. This structure was selected because it is expected that the correlation coefficient for measures adjacent to each other in time will be stronger than for those further apart. A further advantage of GEE is that it performs analyses on all available pairs of data, so even if a participant has missing data at a particular time point, they can be included in the analysis.

Negative Affect z-scores (from every visit) were entered as the dependant variable. To assess for risk-group differences, group (HR, LR) was entered as a factor. To examine differences in Negative Affect over across time, visit (1-5) was also entered as a factor. Finally, to examine whether change across time differed in the HR

and LR groups, a group (HR, LR) by time (visits 1-5) interaction was entered. If a significant group by time interaction emerged, post hoc analyses were run to examine the difference in slopes for the HR and LR groups at each visit.

To assess the association between Negative Affect and Effortful Control, z-transformed Effortful Control scores were entered as a continuous predictor. Finally, cognitive ability (Mullen SS from visits 1-4 and WASI FSIQ from visit 5) were entered as covariates.

6.2.3.5 The association between Negative Affect, Effortful Control and anxiety

To examine the association between infant and toddler Negative Affect, Effortful Control and anxiety at 7-years, several steps were taken. Primarily, first-order Pearson correlation was conducted to examine the association between SCAS-P total score and Negative Affect and Effortful Control on IBQ1, IBQ2, ECBQ, and CBQ1 in the entire sample. This was done to determine whether there is a significant association between these aspects of temperament and anxiety and to identify the earliest time a significant association can be detected. First order Pearson correlation were also run between SCAS-P total score and Mullen scores from visits 1 to 4, to determine whether infant and toddler cognitive ability contributes to anxiety severity and if this needs to be co-varied for in further analyses.

If a significant association between temperament and anxiety was detected, follow-up regression analyses were run with temperament data from the earliest time point when the association was significant, to establish whether it would remain significant when taking into account group status and sex. This analysis included risk group instead of ASD severity for two main reasons. Firstly, the ADOS was not

administered to the LR group at 24 months so it would not be possible to enter these scores in a regression for the whole sample. Secondly, first-order Pearson correlation was run between SCAS-P total score and AOSI/ADOS scores for each visit (please see Appendix 5) and no significant associations were detected. In addition to this, correlation analysis was run between Negative Affect in visits 1-4 and ADOS total CS score at 7-years, where no significant associations emerged (see results). Thus, it was deemed that including ADOS and AOSI scores in the analysis would have been redundant. Sex was also included due to the significant sex differences in anxiety severity, which are presented in Chapter 3. As the earliest association between SCAS-P and Negative Affect was detected at 7-months (see results), data from this time point was used in the regression. Effortful Control and Mullen scores did not have a significant association with SCAS-P (see results), so they were removed from the regression. Thus, SCAS-P total score was entered as the dependant variable and IBQ1 Negative Affect was entered as the predictor, and risk group (HR, LR) and sex were entered as covariates. Post hoc power analyses were conducted for the correlation and regression analyses to determine whether the present sample size provided enough power to detect significant associations.

6.3 Results

6.3.1 Demographic characteristics, ASD severity and cognitive functioning

Table 14 summarises the demographic characteristics, AOSI/ADOS scores and MSEL standard scores for the HR-ASD, HR-non ASD and LR groups at visits 1-4. There were no significant group differences in age or sex ratio at any of the visits. There were also no significant group differences on AOSI or MSEL scores across the three groups at the 7-month visit. However, at the 14-month visit, the HR-ASD group

had higher AOSI scores than both the LR (p=.002, d=.92) and HR-non ASD (p=.01, d=.95) groups. The HR-ASD group also had lower MSEL scores than the LR group (p=.004, d=.96) at the 14-month visit. At this visit, the HR-ASD group also scored lower than the HR-non ASD group on the MSEL, but this only reached trend-level significance (p=.07, d=.79). At the 24-month visit, the HR-ASD group had higher ADOS CS scores than the HR-non ASD group (p=.01, d=.92). Furthermore, at the 24-month visit, both the HR-ASD (p=.001, d=1.05) and HR-non ASD (p=.02, d=.81) groups had lower MSEL scores than the LR group. At the 36-month visit, the HR-ASD group had higher ADOS CS (p=.03, d=.79) and lower MSEL (p=.01, d=.83) scores than the LR group.

Table 14: Demographic characteristics, ASD severity and cognitive functioning scores in the HR-ASD, HR-non ASD and LR groups at 7, 14, 24 and 36 months.

Time/measure (SD)	HR-ASD	HR-non ASD	LR	ANOVA/X^2
7-months	***N*=15**	***N*=24**	***N*=37**	
Age (months)	7.47 (1.36)	7.46 (1.22)	7.28 (1.19)	$F(2, 73)=.17$, $p=.84$, $\eta^2=.01$
Sex ratio (M:F)	7:8	5:19	15:22	$X^2(2)=3.48$, $p=.18$
AOSI total	8.80 (7.39)	9.25 (5.14)	6.70 (3.67)	$F(2, 73)=2.14$, $p=.13$, $\eta^2=.06$
MSEL SS	95.40 (18.39)	95.83 (9.88)	102.86 (10.76)	$F(2, 73)=3.23$, $p=.05$, $\eta^2=.08$
14-months	***N*=15**	***N*=23**	***N*=36**	
Age	13.80 (1.94)	13.74 (1.45)	13.72 (1.25)	$F(2, 71)=.02$, $p=.99$, $\eta^2<.001$

Sex ratio (M:F)	7:8	5:18	14:22	$X^2(2)=2.91$, $p=.23$
AOSI total	7.53[a] (5.45)	3.30[b] (3.20)	3.31[b] (3.57)	$F(2, 71)=6.99$, $p=.002$, $\eta^2=.16$
MSEL SS	93.33[a] (16.29)	104.48 (11.74)	108.46[b] (*N*=35) (15.28)	$F(2, 70)=5.74$, $p=.01$, $\eta^2=.14$
24-months	***N*=15**	***N*=24**	***N*=36**	
Age	24.20 (.94)	23.71 (1.40)	23.89 (.67)	$F(2, 72)=1.10$, $p=.34$, $\eta^2=.03$
Sex ratio (M:F)	7:8	5:18	14:22	$X^2(2)=2.91$, $p=.23$
ADOS CSS	4.80[a] (2.18)	2.79[b] (2.19)	N/A	$F(2, 37)=7.81$, $p=.01$, $\eta^2=.17$
MSEL SS	99.27[a] (21.40)	106.17[a] (15.81)	118.28[b] (*N*=32) (13.43)	$F(2, 68)=.8.18$, $p=.001$, $\eta^2=.19$
36-months	***N*=15**	***N*=24**	***N*=36**	
Age	37.13 (2.10)	37.21 (2.21)	37.86 (2.58)	$F(2, 72)=.78$, $p=.46$, $\eta^2=.02$
Sex ratio (M:F)	7:8	5:19	14:22	$X^2(2)=3.09$, $p=.21$
ADOS CSS	5.13[a] (2.70)	3.42 (2.54)	3.17[b] (2.25)	$F(2, 72)=3.61$, $p=.03$, $\eta^2=.09$
MSEL SS	98.60[a] (27.56)	109.83 (16.91)	117.17[b] (15.77)	$F(2, 72)=5.15$, $p=.01$, $\eta^2=.13$

Group sizes are smaller for some visits due to missing data. Groups denoted with different subscript letters (a, b, c) differed significantly with Bonferonni correction applied (p<.05). HR/LR indicates high-risk or low-risk group; ASD autism spectrum disorder; SD standard deviation; AOSI Autism Observation Schedule for Infants; ADOS Autism Diagnostic Observation Schedule; CSS Calibrated Severity Score; MSEL Mullen Scales of Early Learning; SS Scaled Score.

6.3.2 Group differences on temperament factor scores

Group means and comparisons on the temperament factor scores at each visit are summarised in Table 15. At the 7-month visit, the HR-non ASD group had lower Effortful Control than the LR group (p=.05, d=.63). The HR-non ASD group also had lower Effortful Control scores than the HR-ASD group, but this difference only reached trend-level significance (p=.07, d=.66). The HR-non ASD group also had significantly lower Surgency scores than the LR group (p=.02, d=.73) and lower scores on this factor than the HR-ASD group, which reached trend-level significance (p=.07, d=.77). At the 14-month visit, the HR-non ASD group had lower levels of Surgency than both the LR (p=.02, d=.74) and the HR-ASD (p=.02, d=.92) groups. At the 24-month visit, the HR-ASD group had higher levels of Negative Affect than both the LR (p=.04, d=.56) and HR-non ASD (p=.09, d=.52) groups. The HR-ASD group also had lower levels of Effortful Control than the LR group (p=.01, d=1.18). At the 36-month visit, the HR-ASD group had higher levels of Negative Affect than the LR group (p=.04, d=.71). Finally, at the 7-year visit, both the HR-ASD (p=.03, d=.80) and the HR-non ASD (p=.002, d=.94) groups had higher levels of Negative Affect than the LR group. The HR-ASD group also had lower levels of Effortful control than the LR (p<.001, d=1.40) and HR-non ASD (p=.03, d=.82) groups. The HR-non ASD group had somewhat lower Effortful Control scores than the LR group, but this only reached trend-level significance (p=.09, d=.62).

Post hoc power analyses were carried out to determine how much power the sample had at each time point to detect a significant group difference in any of the temperament factors with a medium sized effect (η^2=.06, f=.25). These analyses revealed that at the 7-month visit, the sample size (n=76) had a power of (1-β)=.47,

critical $F(2, 73)=3.12$ of detecting significant group differences in any of the temperament factors. At the 14- and 24-month visits, the sample size (n=73) had a power of $(1-\beta)=.45$, critical $F(2, 70)=3.13$ of detecting significant group differences. At the 36-month visit, the sample size (n=74) had power of $(1-\beta)=.45$, critical $F(2, 71)=3.13$ of detecting significant group differences. Finally, at the 7-year visit, the sample size (n=71) had a power of $(1-\beta)=.44$, critical $F(2, 68)=3.13$ of detecting significant group differences.

Further post hoc analyses were carried out to determine how much power each group (HR-ASD, HR-non ASD and LR) had to achieve a significant difference from one of the other groups on one of the temperament factors, with a medium sized effect (d=.50). At the **7-month visit**, to detect a difference between the HR-ASD (n=15) and HR-non ASD (n=24) groups, there was a power of $(1-\beta)=.44$, critical $t(37)=1.69$. Similarly, to detect a difference between the HR-ASD and LR (n=37) groups, there was a power of $(1-\beta)=.49$, critical $t(50)=1.68$. Finally to detect a difference between the HR-non ASD and LR groups, there was a power of $(1-\beta)=.60$, critical $t(59)=1.67$.

At the **14-month visit**, to detect a difference between the HR-ASD (n=14) and HR-non ASD (n=22) groups, there was a power of $(1-\beta)=.43$, critical $t(36)=1.69$. Similarly, to detect a difference between the HR-ASD and LR (n=36) groups, there was a power of $(1-\beta)=.48$, critical $t(48)=1.68$. Finally to detect a difference between the HR-non ASD and LR groups, there was a power of $(1-\beta)=.57$, critical $t(56)=1.67$.

At the **24-month visit**, to detect a difference between the HR-ASD (n=15) and HR-non ASD (n=23) groups, there was a power of $(1-\beta)=.43$, critical $t(36)=1.69$. Similarly, to detect a difference between the HR-ASD and LR (n=35) groups, there was

a power of (1-β)=.48, critical $t(48)=1.68$. Finally to detect a difference between the HR-non ASD and LR groups, there was a power of (1-β)=.57, critical $t(56)=1.67$.

At the **36-month visit**, to detect a difference between the HR-ASD (n=15) and HR-non ASD (n=23) groups, there was a power of (1-β)=.43, critical $t(36)=1.69$. Similarly, to detect a difference between the HR-ASD and LR (n=36) groups, there was a power of (1-β)=.48, critical $t(49)=1.68$. Finally to detect a difference between the HR-non ASD and LR groups, there was a power of (1-β)=.58, critical $t(57)=1.67$.

At the **7-year visit**, to detect a difference between the HR-ASD (n=13) and HR-non ASD (n=21) groups, there was a power of (1-β)=.40, critical $t(32)=1.69$. Similarly, to detect a difference between the HR-ASD and LR (n=37) groups, there was a power of (1-β)=.45, critical $t(48)=1.68$. Finally to detect a difference between the HR-non ASD and LR groups, there was a power of (1-β)=.56, critical $t(56)=1.67$.

Table 15: Summary of the temperamental factor scores at each visit for the HR-ASD, HR-non ASD and LR groups

Time/Factor	HR-ASD	HR-non ASD	LR	MANOVA
7-month IBQ-R	***N*=15**	***N*=24**	***N*=37**	
Negative Affect	.26 (1.149)	.18 (.88)	-.23 (.78)	$F(2, 73)=1.89$, $p=.16$, $\eta^2=.05$
Effortful Control	.29 (1.05)	-.44[a] (1.17)	.18[b] (.76)	$F(2, 73)=3.83$, $p=.03$, $\eta^2=.10$
Surgency	.25 (.93)	-.49[a] (1.00)	.22[b] (.94)	$F(2, 73)=4.63$, $p=.01$, $\eta^2=.11$
14-month IBQ-R	***N*=15**	***N*=23**	***N*=35**	

Negative Affect	.15	.14	-.16	$F(2, 70)=.84, p=.44, \eta^2=.02$
	(1.51)	(.99)	(.69)	
Effortful Control	-.25	-.19	.24	$F(2, 70)=1.96\ p=.15, \eta^2=.05$
	(1.06)	(1.06)	(.90)	
Surgency	.35[a]	-.53[b]	.20[a]	$F(2, 70)=5.44\ p=.01, \eta^2=.14$
	(.85)	(1.06)	(.90)	
24-month ECBQ	***N*=14**	***N*=22**	***N*=36**	
Negative Affect	.60[a]	-.12	-.16[b]	$F(2, 69)=3.43\ p=.04, \eta^2=.09$
	(1.83)	(.70)	(.53)	
Effortful Control	-.59[a]	-.17	.34[b]	$F(2, 69)=5.42\ p=.01, \eta^2=.14$
	(.72)	(1.18)	(.85)	
Surgency	.28	-.07	-.06	$F(2, 69)=.66\ p=.52, \eta^2=.02$
	(1.19)	(.92)	(.98)	
36-month CBQ	***N*=15**	***N*=23**	***N*=36**	
Negative Affect	.43[a]	.23	-.33[b]	$F(2, 71)=4.22\ p=.02, \eta^2=.11$
	(1.18)	(.80)	(.95)	
Effortful Control	.03	-.25	.16	$F(2, 71)=1.21\ p=.30, \eta^2=.03$
	(1.37)	(.87)	(.88)	
Surgency	.01	-.17	.11	$F(2, 71)=.56\ p=.58, \eta^2=.02$
	(1.19)	(1.11)	(.85)	
7-year CBQ	***N*=13**	***N*=21**	***N*=37**	
Negative Affect	.36[a]	.49[a]	-.42[b]	$F(2, 68)=7.74\ p=.001,$
	(1.04)	(.84)	(.91)	$\eta^2=.19$
Effortful Control	-.94[a]	-.12[b]	.40[b]	$F(2, 68)=11.60\ p<.001,$
	(1.11)	(.89)	(.77)	$\eta^2=.26$
Surgency	.29	.08	-.16	$F(2, 68)=1.09\ p=.35, \eta^2=.03$
	(1.32)	(1.06)	(.81)	

Groups denoted with different subscript letters (a, b, c) differed significantly with Bonferonni correction applied (p<.05). HR/LR indicates high-risk or low-risk group; ASD autism spectrum disorder; SD standard deviation; IBQ-R Infant Behavior Questionnaire-Revised; ECBQ Early Childhood Behavior Questionnire; CBQ Child Behavior Questionnaire; MANOVA Multivariate Analysis of Variance.

6.3.3 Changes in Negative Affect over time and its association with Effortful Control

The GEE revealed that the HR group (*M*=-.26, *SD*=.78) had higher levels of Negative Affect than the LR group (*M*=.25, *SD*=1.10) across visits, $X^2(1)=7.50$, $p=.01$. However, there was no significant effect of time on Negative Affect, $X^2(4)=.35$, $p=.99$. Furthermore, there was no significant group by time interaction, $X^2(4)=5.03$, $p=.28$. The mean Negative Affect scores for the HR and LR groups at each visit are presented in Figure 8. As this interaction was non-significant, further post-hoc analyses were not undertaken.

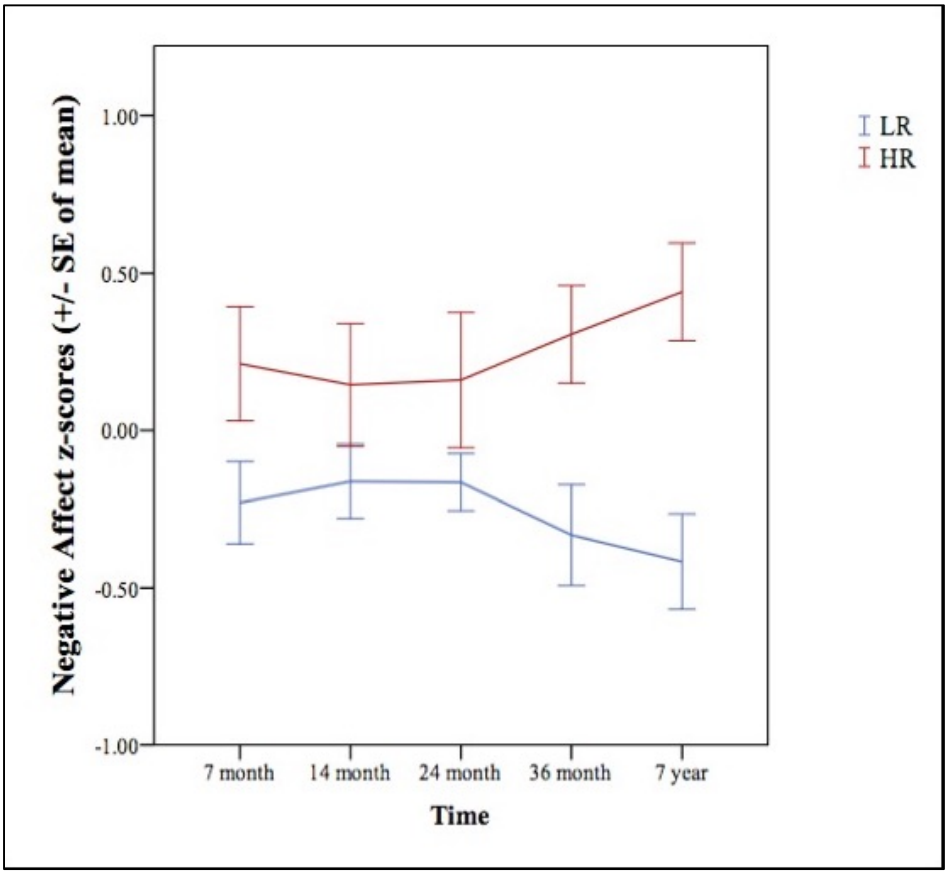

Figure 8. Mean Negative Affect scores for the HR and LR groups at each visit

There was a significant effect of Effortful control, which was negatively associated with Negative Affect (*B*=-.16, *SE*=.05 *p*=.001, 95% CI [-.26, -.07]). The association between Negative Affect and Effortful Control is illustrated in Figure 9. On the contrary, there was no significant association between Negative Affect and cognitive ability (*B*=-.001, *SE*=.004 *p*=.78, 95% CI [-.01, .01]).

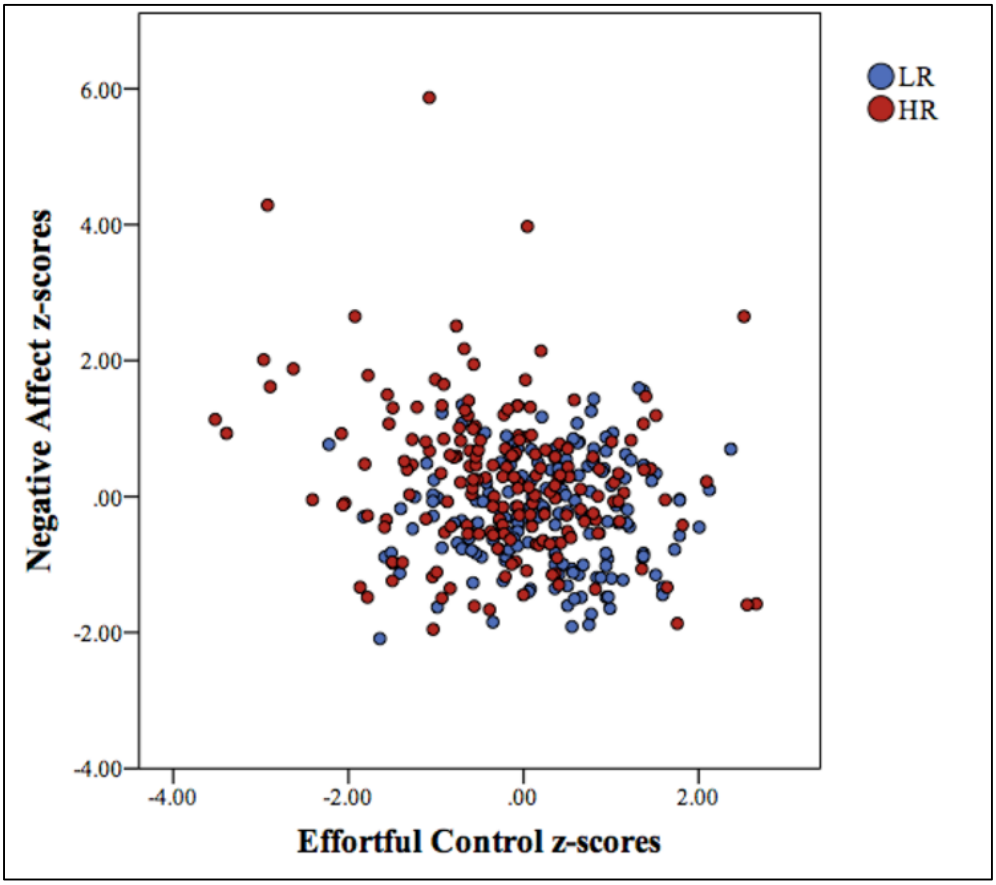

Figure 9. Association between Negative Affect and Effortful Control, with the HR and LR group scores marked.

6.3.4 Association between infant/toddler Negative Affect, Effortful Control and 7-year anxiety and ASD symptoms

Table 16 summarises the Pearson correlation coefficients showing the association between SCAS-P total score from the 7-year visit and Negative Affect,

Effortful Control and MSEL scores from visit 1-4. The 7-month visit was the first time that a significant association between Negative Affect and SCAS-P scores could be detected and the association remained significant at the 14-, 24- and 36-month visits. Effortful Control at the 36-month visit was associated with SCAS-P total score. MSEL SS were not significantly associated with SCAS-P scores at any time.

Post hoc power analyses revealed that the sample size at each time point had moderate to good power for detecting an association between Negative Affect, Effortful Control and anxiety, with a medium sized effect (r=.30). At the 7-month visit the sample (n=74) had a power of (1-β)=.76, critical t(72)=1.99 to detect a significant association between Negative Affect, Effortful control and anxiety symptoms. At the 14-month visit, the sample (n=72) had a power of (1-β)=.75, critical t(70)=1.99 to detect a significant association. At the 24-month visit, the sample (n=70) had a power of (1-β)=.74, critical t(68)=2.00 to detect a significant association. Finally, at the 36-month visit, the sample (n=72) had a power of (1-β)=.75, critical t(70)=1.99 to detect a significant association.

Finally, the association between ADOS CSS at 7 years and Negative Affect at 7-months (r(71)=-.02, p=.86), 14-months (r(68)=-.09, p=.47), 24-months (r(68)=-.06, p=.64) and 36-months (r(70)=.15, p=.21) was assessed, but was not significant at any point. Post hoc power analyses were carried out to examine whether the current sample had sufficient power to detect a significant association between Negative Affect, Effortful Control and ADOS CSS at the 7-year visit, with a medium sized effect. At the 7-month visit the sample (n=71) had a power of (1-β)=.74, critical t(69)=1.99 to detect a significant association. At the 14- and 24-month visits the sample (n=68) had a power

of (1-β)=.72, critical $t(66)$=2.00 to detect a significant association. Finally, at the 36-month visit, the sample (n=70) had a power of (1-β)=.74, critical $t(68)$=2.00 to detect a significant association.

Table 16: Correlation coefficients showing association between SCAS-P total score and Negative Affect, Effortful Control and MSEL scores at visits 1-4

Time/Measure	*N*	*r*	*p-value*
7-months			
Negative Affect	74	.25	.03*
Effortful Control	74	-.03	.83
MSEL SS	74	.15	.21
14-months			
Negative Affect	72	.30	.01*
Effortful Control	72	-.17	.16
MSEL SS	72	.04	.72
24-months			
Negative Affect	70	.49	<.001**
Effortful Control	70	-.06	.60
MSEL SS	70	-.02	.90
36-months			
Negative Affect	72	.38	.001*
Effortful Control	72	.34	.004*
MSEL SS	73	.05	.65

Group sizes are smaller for some visits due to missing data. SCAS-P denotes Spence Children's Anxiety Scale – Parent Version; MSEL Mullen Scales of Early Learning; SS Standard Score.

To follow up on the significant association between 7-month Negative Affect and anxiety, a linear regression was run with SCAS-P total score as the dependant variable and 7-month Negative Affect, risk group status and sex as predictors. This accounted for a significant proportion of the variance in SCAS-P total score, $F(3, 69)=6.97$, $p=.01$, $R^2=.21$. Risk group significantly predicted SCAS-P scores ($\beta=.29$, $t(72)=2.60$, $p=.01$), while Negative Affect was a marginally significant predictor ($\beta=.22$, $t(72)=1.95$, $p=.06$). Sex had a trend-level association ($\beta=.19$, $t(72)=1.79$, $p=.08$). Figure 10 illustrates the association between SCAS-P total score and 7-month Negative Affect. Post hoc power analyses revealed that, at the 7-months visit, the present sample had excellent power, $(1-\beta)=.95$, critical $F(3, 70)=2.57$, to detect a significant association with a medium sized effect ($R^2=.30$, $f^2=.25$).

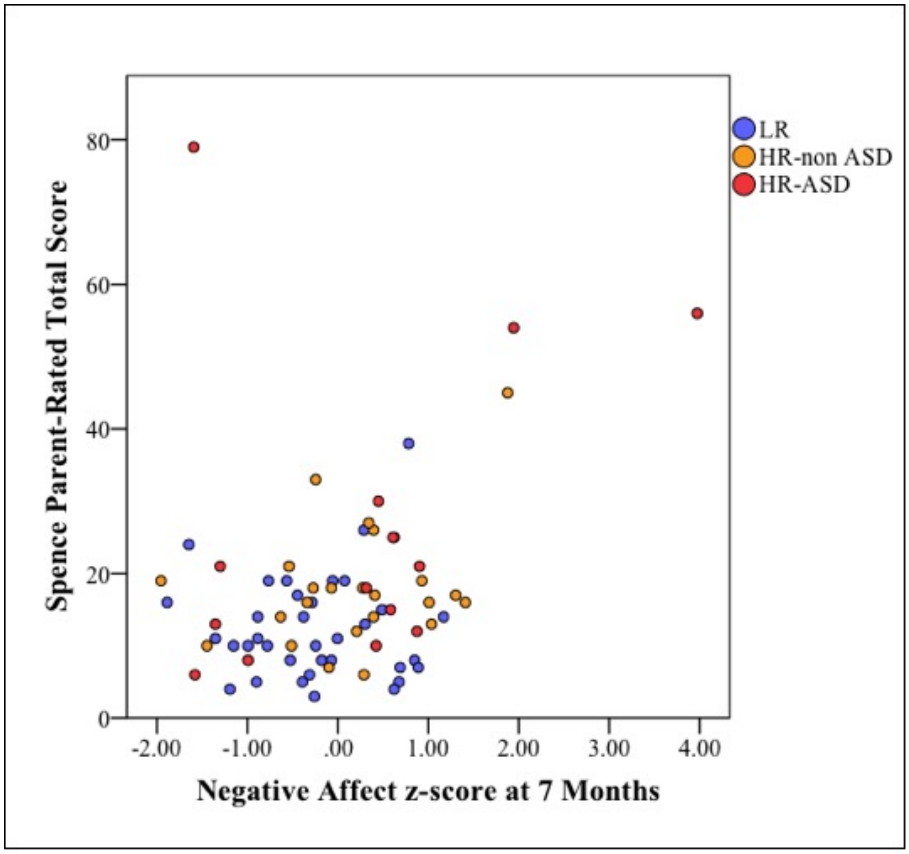

Figure 10. Scatter plot showing the association between 7-month Negative Affect and SCAS-P total scores with HR-ASD, HR-non ASD and LR groups marked.

6.4 Discussion

This chapter examined infant and toddler temperamental profiles and development among children at high-risk for ASD and their association with anxiety in middle childhood. Several main aims were addressed; to assess differences in temperamental characteristics of children at risk for ASD compared to LR controls, to examine whether levels of Negative Affect increased in HR children over time, and if Negative Affect in infancy was associated with anxiety symptoms in middle childhood. Even though group differences in temperamental characteristics fluctuated over time, by the age of 24 months, HR children who met diagnostic criteria for ASD exhibited elements of dysregulated temperament. In particular, they showed higher levels of Negative Affect and reduced Effortful Control, which persisted to the age of 7-years. While the HR group exhibited elevated levels of Negative Affect between the ages of 7 months and 7 years, neither the HR or LR groups exhibited changes in this trait over time. However, high Negative Affect was associated with reduced levels of Effortful control. Finally, Negative Affect in infancy was associated with anxiety symptoms at 7 years. The earlies time this association could be detected was at the 7-month visit and remained significant, regardless of group status or sex. This is the first study that has examined temperament in children at high-risk for ASD beyond the age of 36 months and to report on the association between high levels of Negative Affect in infancy and the development of anxiety in this population.

6.4.1 Temperamental characteristics of the HR and LR groups

Group differences on the three factors of Negative Affect, Effortful Control and Surgency were examined at the 7-, 14-, 24-, 36-month and 7-year visits. At the infant visits, it was the HR-non ASD group that exhibited a distinct temperamental profile

from the LR controls. The HR-non ASD group were reported to have reduced Surgency at 7 and 14 months, and lower Effortful Control at 7 months, than the LR group. On the other hand, the HR-ASD group did not manifest differences on any factor at either visit. This pattern is consistent with the temperamental profiles using the 36-month diagnostic outcomes reported in our cohort (Clifford et al., 2013). On the other hand, these findings differ from previous work that suggests atypicalities in temperament can be observed within the first year of life among children HR children who go on to develop ASD (e.g. Zwaigenbaum et al., 2005). It is also not fully clear why atypical temperament was observed in the HR-non ASD group on these visits, while the HR-ASD children did not differ from controls. As outlined by Clifford et al. (2013), it is possible that the reduced Surgency and Effortful control observed in the HR-non ASD group indicate that these temperamental characteristics are also present among children who manifest the BAP. However, this does not account for the absence of this pattern among the HR-ASD participants. It is possible that the modest sample size of the HR-ASD group (n=15) meant that there was insufficient statistical power to detect differences in that group. However, it is also possible that the items on the IBQ-R (Gartstein & Rothbart, 2003) do not best capture temperament in infants who go on to develop ASD. For example, infants with ASD may score more highly on the "Duration of Orienting" dimension, thus giving them a higher score of Effortful Control. However, this may not represent sustained attention as it does in typically developing infants and could be due to difficulties in flexibly disengaging attention, which is also observed among HR-ASD children in infancy (Elsabbagh et al., 2013). Furthermore, many items and dimensions that make up the Surgency factor involve approach to non-social stimuli (e.g. "How often did your baby move quickly towards new objects?"). It has been reported that children with ASD experience more reward from engaging with

non-social, than social, stimuli (Clifford et al., 2013; Dawson, Meltzoff, Osterling, Rinaldi, & Brown, 1998; Leekam, Lopez, & Moore, 2000). Thus, infants who go on to develop ASD may exhibit approach behaviours to non-social objects just as much as typically developing infants, but would differ if greater emphasis was placed on social stimuli (Clifford et al., 2013)

However, at the age of 24 months and older, the HR-ASD group manifested increased levels of Negative Affect and reduced Effortful Control compared to LR controls. It was unexpected, however, that the HR-ASD group did not differ significantly on Effortful Control at the 36-month visit, but did at 24 months and 7 years. To check whether this was due to the removal of items related to Perceptual Sensitivity, Soothability and Shyness, this analysis was repeated with the original factor structure (please see Appendix 5). There were no significant differences even when using the original factor structure. It is possible that use of the very short form of the CBQ (Putnam & Rothbart, 2006) altered the pattern of findings. Kochanska, Murray, and Harlan (2000) suggest that Effortful Control does not become fully stable until ~36 months of age and improves significantly between the ages of 22 and 33 months. Furthermore, Effortful Control is reported to be less stable than Negative Affect and Surgency (Putnam, Rothbart, & Gartstein, 2008). Therefore, it is possible that the HR-ASD group did indeed have improved Effortful Control ability at 36 months when this trait became more stable, but that difficulties became apparent again at 7-years due to increasing environmental and social demands, such as being in school.

Finally, while the HR-ASD group exhibited increased Negative Affect from the age of 24-months, this only became significantly elevated in the HR-non ASD group at the age of 7 years. However, it is important to note that the HR-non ASD group did

exhibit trend-level differences in Negative Affect compared to the LR group, with relatively strong effect sizes. This suggests that, with a larger sample size, the HR-non ASD group would have manifested increased Negative Affect earlier in childhood. This is in line with previous work by Garon et al. (2009), who suggested that Negative Affect manifests beyond clinical-level ASD and is part of the BAP as well. In particular, heightened negative emotionality distinguished the HR group from LR controls, but did not differentiate HR children who had ASD and those who did not.

6.4.2 Change in Negative Affect over time and its association to Effortful Control

The second aim of this chapter was to compare trajectories of Negative Affect in the HR and LR groups. Warren and Sroufe (2004) suggest that features of Negative Affect, such as fearfulness and shyness, peak within the first 14-18 months but start to decline at ~24 months of age. It is further suggested that, among a proportion of children (~15%), Negative Affect remains persistently high beyond this period, increasing their risk of developing mental health difficulties, such as anxiety. The decline in Negative Affect is attributed partly to the development of executive functioning abilities and attentional control, akin to characteristics associated with increased Effortful Control (Degnan & Fox, 2007). Given that the HR-ASD group manifested both high levels of Negative Affect and reduced Effortful Control, compared to LR controls at the 24-month visit, it was hypothesised that the LR controls would show a decrease in Negative Affect, while HR children would manifest consistently high levels over time. Overall, the HR group did have higher levels of Negative Affect compared to LR controls. However, neither group exhibited change in Negative Affectivity across time, from the age of 7-months to 7-years. This finding supports the hypothesis that the HR group would manifest persistently high levels of

Negative Affect across time. On the other hand, the LR group did not exhibit a decline in Negative Affect after 24 months or at any other time. Therefore, this finding is more consistent with reports by Putnam, Rothbart, and Gartstein (2008), who suggest that Negative Affect is continuous and stable across development.

One limitation of this approach was that trajectories were examined in the HR group as a whole and, due to the modest sample size of the HR-ASD group, it was not deemed possible to test development of Negative Affect separately for outcome groups. As the HR-ASD group manifested highest levels of Negative Affect and differed significantly from LR controls earlier than the HR-non ASD group did, it is possible that a distinct trajectory would be observed in this group. However, one study that compared the developmental pathways of temperament among high-risk siblings with ASD and with typical development also failed to find distinct trajectories for mood (Del Rosario, Gillespie-Lynch, Johnson, Sigman, & Hutman, 2014).

A further aim was to examine whether the trajectory of Negative Affect was associated with levels of Effortful Control. The Generalized Estimating Equation showed that there was a significant, negative association between Negative Affect and Effortful Control. This is in line with previous research, which suggests that heightened levels of Negative Affect are prevalent among children who have reduced ability to regulate their emotional states (Degnan & Fox, 2007; Nakagawa & Sukigara, 2012; Putnam, Rothbart, & Gartstein, 2008). Effortful Control begins to emerge after the age of 12 months (Rothbart, Ellis, Rosario Rueda, & Posner, 2003) and is not fully stable until ~36 months (Kochanska, Murray, & Harlan, 2000). Thus, it is possible that, even though the HR group manifested increased Negative Affect across time, group differences became more evident among the HR-ASD group when they started to

manifest reduced Effortful Control. However, this hypothesis needs to be tested further by examining trajectories of both Negative Affect and Effortful Control within the different diagnostic outcome groups.

6.4.3 The association between Negative Affect and Effortful Control in early development and anxiety symptoms during middle childhood

The final aim of this chapter was to examine the association between Negative Affect and Effortful Control in infancy and toddlerhood and anxiety symptoms at the 7-year visit in the HR and LR groups. A significant association was detected between Negative Affect at the 7-month visit and anxiety symptoms at the 7-year visit. Furthermore, the association between Negative Affect and anxiety remained significant even after taking risk group status and sex into account. This pattern was observed with Negative Affect at all subsequent visits and the association generally tended to increase in strength. On the other hand, there was no evidence of an association between Effortful Control and anxiety, except at the 36-month visit. At this time, increased Effortful Control was related to higher levels of anxiety, over and above Negative Affect, risk group status or sex. Similar to the lack of group difference in Effortful Control at the 36-month visit, this finding was unexpected as it was hypothesised that anxiety would be associated with reduced self-regulatory ability. Given that the CBQ very short form was used at this visit, it is difficult to determine whether this inconsistent finding represents a true association or if it is an artefact of using a distinct measure. Interestingly, there were no significant associations between Negative Affect and ADOS scores at 7-years. This is somewhat surprising, as Negative Affect was heightened within the HR group. However, by the 7-year visit, Negative Affect was heightened in both the HR-ASD and HR-non ASD groups, who did not differ from

each other. Therefore, it may be possible that Negative Affect is a feature of the BAP, but that it contributes specifically to emotional difficulties, rather than general ASD severity.

The association between infant Negative Affect and anxiety has been demonstrated widely among non-ASD populations (Fox & Pine, 2012) and children at heightened risk for ASD due to having Fragile X syndrome (Tonnsen, Malone, Hatton, & Roberts, 2013). This study is the first to show a similar association among children who are at high familial risk for ASD. Yet, in spite of the vast empirical evidence supporting this association, it is not fully clear how temperament relates to anxiety in both ASD and non-ASD populations. Two reviews (Nigg, 2006; Rettew & McKee, 2005) outlined several possible models that describe mechanisms by which atypical temperamental profiles contribute to psychopathology. From the proposed models, the pattern observed within this cohort and other studies suggests a risk/vulnerability model, whereby early atypicalities in Negative Affect serve as a risk factor for the development of anxiety. This model appears to be most fitting partly because it is possible to detect an association between Negative Affect at 7-months and anxiety in middle childhood. While it is challenging to accurately assess anxiety symptoms in young children, evidence suggests that the earliest time anxiety can be detected is the age of ~3 years (Egger & Angold, 2006). Thus, individual differences in Negative Affect can be observed much earlier than the onset of anxiety symptoms. Furthermore, similar to findings from other research (for review see Nigg, 2006), the association between Negative Affect and anxiety in this study is small to moderate in strength, suggesting that they are not simply extremes of the same trait. Although, this evidence

must be taken with caution as associations between any behaviour measured at 7-months and 7-years of age may be weak in strength.

The nature of the prevalence of Negative Affect among HR infants, particularly those who go on to develop ASD, is somewhat more difficult to characterise. Garon et al. (2016) suggest that children with the most severe ASD, which is diagnosed early in development, demonstrate lower levels of Negative Affect than those diagnosed later in development. This is possibly because children with severe ASD are more placid and less engaged with their surroundings. Therefore, it is less likely that Negative Affect is a risk factor for the development of ASD. Perhaps certain aspects of ASD, such as heightened sensory sensitivity, cause infants with the condition to experience more distress to subtle changes in their environment, thus exhibiting higher levels of Negative Affect. This hypothesis is supported by previous research that reports a significant association between Negative Affect and sensory modulation atypicalities in children with ASD (Brock et al., 2012). Furthermore, infants at-risk for ASD exhibit reduced habituation to auditory stimuli than LR controls (Guiraud et al., 2011), providing evidence that they may be more sensitive to sensory stimuli from an early age. However, the association between early temperamental profiles and specific features of ASD need to be studied prospectively to better understand how temperament contributes to ASD symptomatology.

On the contrary, aside from the 36-month visit, no association emerged between Effortful Control and anxiety symptoms. This is surprising as there was an association between Effortful Control and Negative Affect in this study and previous research has suggests that Effortful Control has both a direct association with anxiety (Cole, Zapp, Fettig, & Perez-Edgar, 2016) and that it moderates the association between Negative

Affect and anxiety (Lonigan & Vasey, 2009). However, these studies suggest that this association emerges because children with high levels of Negative Affect exhibit heightened bias to threatening stimuli and the presence of increased Effortful Control can help modulate these attentional patterns. However, threat bias has not been observed among children with ASD in this cohort (please see Chapter 5) or in other research (Hollocks, Ozsivadjian, Matthews, Howlin, & Simonoff, 2013; May, Cornish, & Rinehart, 2015). Therefore, these findings seem to suggest that reduced self-regulatory ability does indeed contribute to the development of higher Negative Affect, but having high levels of Negative Affect is sufficient to developing anxiety.

6.4.4 Strengths, limitations and implications for future work

This study is the first to prospectively examine the development of temperament in children at high-risk for ASD in middle childhood and the association between atypical temperamental profiles and anxiety symptoms. The findings from this study have important implications for both research and clinical practice. There is widespread evidence suggesting that Negative Affect in infancy and toddlerhood is associated with childhood anxiety (for review see Fox & Pine, 2012). The finding that Negative Affect was associated with anxiety symptoms, regardless of risk group status, suggests that the risk factors for anxiety are similar in children at risk for ASD as they are in non-ASD populations. Furthermore, Negative Affect at 7 months was the first time point that the association between this factor and anxiety could be detected. Thus, risk for anxiety in children at high-risk for ASD can be detected as early as infancy. These findings can assist in the development of targeted interventions for anxiety in children with ASD, as methods of reducing negative emotionality can be implemented before they progress to anxiety symptoms. Furthermore, high levels of Negative Affect were associated with

reduced Effortful Control, so one way of reducing negative emotionality could be through teaching the child better regulatory skills.

A limitation of this research is that measures of both temperament and anxiety were obtained through parent-report. However, given that measures of temperament were collected in infancy and toddlerhood, it would not have been possible to obtain self-report from the participants. However, Schwartz et al. (2009) obtained self-report temperament data from adolescents with ASD and report a similar pattern to the one observed in this study. In particular, adolescents with ASD reported higher levels of Negative Affect compared to typically developing controls. Future research would benefit from using observational or experimental measures of temperament in addition to parent-report questionnaires (Fox, Henderson, Marshall, Nichols, & Ghera, 2005).

Another limitation of this study is that temperament was the only risk factor that was studied, while many other important neurocognitive (e.g. infant threat bias) and environmental factors (e.g. parental psychopathology, family stress) contribute to the development of anxiety in children (Cole, Zapp, Fettig, & Perez-Edgar, 2016; Rapee, 2002). In particular, family stress due to having a sibling with a disability, could contribute to the development of anxiety in children with a family history of ASD. However, given the multitude of possible risk factors, it is not possible to address all in one study. Therefore, these findings suggest that high Negative Affect is one risk factor, but does not discount the impact of other factors in the development of anxiety in children at-risk for ASD.

As outlined in prior chapters, the sample size was a limitation for the analyses in this chapter, particularly as the sample varied at each visit. The post hoc power

analyses revealed that the present sample had weak to moderate power in detecting significant group differences on the temperamental factors. However, the power to detect an association between early temperament and 7-year anxiety levels was strong.

It is important for future research to examine the shared neurocognitive correlates between Negative Affect, anxiety and ASD in children at high-risk for ASD. Through such research, it will be possible to identify particular neural and cognitive mechanisms that contribute to all three conditions. This would aid in deepening our understanding of the shared aetiology of ASD and anxiety and identify specific features to focus on in targeted interventions.

Chapter 7
General Discussion

7.1 Overview of background and aims of thesis

The aim of this body of work was to examine the neurocognitive correlates and longitudinal predictors of co-occurring anxiety symptoms among children at increased familial risk for ASD. There is substantial evidence to suggest that co-occurring anxiety symptoms are highly prevalent among individuals with ASD (Salazar et al., 2015; Simonoff et al., 2008; White, Oswald, Ollendick, & Scahill, 2009). These symptoms emerge early in childhood and persist throughout development, often causing substantial difficulty in everyday functioning (White, Oswald, Ollendick, & Scahill, 2009). There is also evidence of heightened anxiety among first-degree relatives of children with ASD, particularly those who manifest aspects of the BAP (Hallett, Ronald, et al., 2013; Mazefsky, Folstein, & Lainhart, 2008; Schwichtenberg et al., 2013). Clinically, modified Cognitive Behaviour Therapy (CBT) has demonstrated significant utility in reducing anxiety symptoms among children and adolescents with ASD (Ung, Selles, Small, & Storch, 2015).

Yet, despite the high prevalence of anxiety symptoms and promising treatment options among individuals with ASD and their family members, the nature and function of co-occurring anxiety symptoms in this population remain unclear (Kerns & Kendall, 2012; Wood & Gadow, 2010). Reported prevalence rates are highly varied, ranging between 11-84% (White, Oswald, Ollendick, & Scahill, 2009). Furthermore, an increasing body of evidence suggests that co-occurring anxiety symptoms differ in their manifestation among individuals with ASD compared with non-ASD populations

(Kerns et al., 2014). In the ASD population, co-occurring anxiety is associated with the core features of ASD and is often 'atypical' in presentation, with the presence of unusual phobias and atypical cognitions (Hallett, Lecavalier, et al., 2013; Kerns & Kendall, 2012; Kerns et al., 2014; Rodgers, Glod, Connolly, & McConachie, 2012; Sukhodolsky et al., 2008). In addition to this, there are challenges in accurately measuring and conceptualising anxiety symptoms in the ASD population (Mazefsky, Kao, & Oswald, 2011; Rodgers et al., 2016). Consequently, there is a need for experimental research to examine whether the neural and cognitive correlates of anxiety are present among individuals with ASD and if they map on to self- and caregiver-reports of symptoms. It is also vital to examine the longitudinal predictors of anxiety to elucidate the developmental trajectories of anxiety symptoms in the ASD population and identify targets for early interventions.

This thesis was well-placed to address several of the issues outlined. Firstly, the high-risk sibling design allowed for exploration of anxiety symptoms and neurocognitive correlates among siblings who have clinical-level ASD and those who do not. Additionally, the prospective longitudinal design provided an opportunity to identify particular traits prevalent among high-risk infants that placed them at increased risk for developing anxiety. Therefore, the main aims of this thesis were threefold; to compare the prevalence of anxiety symptoms among the HR-ASD, HR-non ASD and LR groups, to examine the cognitive correlates (attentional bias to threat) of anxiety in this sample, and to investigate whether dysregulated temperament in infancy was a predictor of anxiety symptoms in middle childhood.

7.2 Summary of main findings

7.2.1 The prevalence of anxiety symptoms among high-risk children and their association with the core ASD symptoms

Chapter 3 examined the prevalence of co-occurring anxiety symptoms among children in the HR and LR groups using the Spence Children's Anxiety Scale parent- and child-report questionnaires (SCAS-P/C; Nauta et al., 2004; Spence, 1998). Results from the parent-report measure revealed a pattern of findings where the HR-ASD group had higher levels of anxiety across most subscales than the LR group. The HR-non ASD group, on the other hand, did not tend to differ from either the HR-ASD or LR groups, but they did exhibit heightened levels of separation anxiety. It was surprising that neither the HR-ASD nor HR-non ASD groups manifested heightened levels of social phobia, as this particular type of anxiety is often reported among individuals with ASD (e.g. Bellini, 2004). It is possible that this is due to the young age of the sample in this study (6-8 years); even though symptoms of social anxiety can be detected in young children, they become more readily observable in adolescence due to the escalation of social concerns that emerge with age (Costello, Mustillo, Erkanli, Keeler, & Angold, 2003; Kessler et al., 2005).

In the HR group, anxiety symptoms were significantly associated with each of the core symptoms of ASD. However, once social symptoms, communicative difficulties and RRBs were examined together, only the association between RRBs and anxiety remained significant. However, when examined in the HR-ASD and HR-non ASD groups separately, the association between anxiety and ASD symptoms was significant only in the HR-ASD group. This is likely due to the greater range of scores on measures of ASD severity (such as the SCQ) in the HR-ASD group. Furthermore,

the HR-atypical group (HR-non ASD children who manifested elevated, subclinical ASD traits) did not have heightened anxiety levels compared to the typically developing HR-non ASD participants. The findings contradict prior research, which suggest that, among siblings of children with ASD, anxiety is particularly heightened among those who manifest aspects of the BAP (e.g. Hallett, Ronald, et al., 2013). This could largely be due to the modest sample size, particularly of the HR-atypical group (*n*=7), which did not provide sufficient statistical power to detect a significant result. However, assignment to outcome group in this study was done somewhat differently to other studies examining anxiety in siblings. For example, some studies examine the prevalence of anxiety in "unaffected" siblings, but do not include measures of ASD severity (e.g. Shivers, Deisenroth, & Taylor, 2013). This could mean that children with elevated BAP features or even undiagnosed clinical-level ASD are considered unaffected. Hallett, Ronald, et al. (2013) used scores on the ADOS and ADI-R to classify participants as having ASD, BAP or being TD. While the present study used these measures as well, assignment to outcome group was done according to DSM-5 criteria (American Psychological Association, 2013) and children assigned to the HR-ASD group did not need to score above threshold on all diagnostic criteria. Furthermore, the HR-Atypical group included children who manifested developmental delay or other concerns reported by parents. These differences in diagnostic group assignment could mean that some children considered to have BAP in the study by Hallett, Lecavalier, et al. (2013) would have been assigned to the HR-ASD group in this study.

Unlike the findings from the parent-report measure, there were no significant group differences in self-reported anxiety symptoms. While the SCAS has been

suggested as a reliable measure of anxiety in children with ASD (Zainal et al., 2014), agreement in self- and parent-report was low to moderate in both the HR and LR groups. This was not surprising given that inter-rater agreement is not high when examining anxiety among typically developing children (Achenbach, McConaughy, & Howell, 1987). Furthermore, individuals with ASD are thought to under-report on their own anxiety symptoms and standard measures are suggested to be less sensitive in detecting clinical cases in this population (Mazefsky, Kao, & Oswald, 2011; White, Schry, & Maddox, 2012). The use of both self- and parent-reported anxiety scores in subsequent chapters was done to help clarify whether hypothesised neurocognitive correlates of anxiety (such as threat bias) mapped on to anxiety symptoms reported by either respondent.

7.2.2 The association between threat bias and anxiety among children at high familial risk for ASD

Biased cognitive processing, which favours elements of the environment that are perceived as threatening, is considered an important component of anxiety disorders, contributing to their aetiology and maintenance (Bar-Haim, Lamy, Pergamin, Bakermans-Kranenburg, & van IJzendoorn, 2007; Beck, Emery, & Greenberg, 1985; Eysenck, 1992). Thus, one of the aims of this thesis was to examine whether children at high-risk for ASD would manifest bias towards threatening stimuli and if this would be associated with anxiety symptoms.

7.2.2.1 Review to identify suitable threat bias tasks for children aged 6-8 years

In a recent meta-analysis, Dudeney, Sharpe, and Hunt (2015) suggested that the association between threat bias and anxiety increased with age. Furthermore, reduced ability to inhibit responding to threatening stimuli was suggested to be the cognitive

mechanism that yielded the strongest results among younger age groups. However, this study included participants aged up to 18 years and did not specify the age range of the youngest children. Therefore, it was deemed necessary to systematically review the literature to identify paradigms suitable for assessing threat processing among children as young as those tested in this study (6-8 years) or younger, given reports of reduced mental age among some children with ASD (Matson & Shoemaker, 2009).

The review in Chapter 4 highlighted the dearth in research examining cognitive processing related to anxiety in young children, particularly preschool aged groups. However, among the studies reviewed, those using reaction time (RT) paradigms, which compare RTs to detect threatening compared to non-threatening stimuli, reported a significant association between threat detection and anxiety symptoms. Thus, the use of such a task with the sample in this study was considered to be appropriate.

7.2.2.2 Emotional spatial cueing task

In Chapter 5, a modified, emotional, version of the spatial cueing task (Posner, Snyder, & Davidson, 1980) was used to measure attentional allocation to threat in the HR-ASD, HR-non ASD and LR groups. Importantly, this task was designed to address limitations in previous research examining threat bias in ASD, by using non-social threatening stimuli and comparing threatening with positive (as well as neutral) stimuli. Furthermore, this specific paradigm was selected because it allows for the measurement of both attentional orienting and disengagement. Prior research has suggested that delayed disengagement from threat may be a more precise description of threat bias than faster orienting to threat (Fox, Russo, Bowles, & Dutton, 2001; Yiend & Mathews, 2001). Examining both components of attention was considered especially important in ASD, as previous studies have suggested that children with ASD have general

difficulty in flexibly shifting attention and show delayed disengagement from threatening stimuli (Isomura, Ogawa, Shibasaki, & Masataka, 2015; Landry & Bryson, 2004), but did not examine whether this was associated with anxiety symptoms.

In spite of having the most severe parent-rated anxiety symptoms, the HR-ASD group did not exhibit enhanced orienting to or delayed disengagement from threatening stimuli. Instead, the HR-non ASD group exhibited elevated threat bias, higher than both the LR and HR-ASD groups. Threat bias was significantly associated with parent-reported anxiety symptoms, but not self-reported anxiety (although this did reach trend-level significance across all participants). The discrepancy in these findings may be indicative of differential cognitive mechanisms of anxiety among HR-ASD and HR-non ASD children. In the HR-non ASD group, the heightened threat bias could be indicative of the higher levels of separation anxiety observed in this group and the general trend of having elevated anxiety compared to LR controls. In the HR-ASD group, the absence of threat bias suggests that the mechanisms underlying anxiety may differ among children with ASD compared with non-ASD populations. Given that anxiety was significantly associated with the core features of ASD in the HR-ASD group, it is possible that the cognitive correlates may be more ASD-specific. Studies have suggested that factors such as intolerance of uncertainty and sensory modulation atypicalities are associated with anxiety among individuals with ASD (e.g. Wigham, Rodgers, South, McConachie, & Freeston, 2014). Perhaps, among individuals with ASD, anxiety is not associated with hypersensitivity to threat, and the factors that do relate to anxiety are not easily captured using the current visual stimuli.

7.2.3 The association between dysregulated temperament in infancy/toddlerhood and anxiety symptoms in middle childhood

The final chapter of this thesis examined the manifestation of dysregulated temperament among the HR children in infancy and toddlerhood and its association with anxiety symptoms at the 7-year follow-up. Multiple high-risk sibling studies have included measures of temperament and suggest that atypical temperamental profiles are characteristic of high-risk infants, particularly those who go on to meet diagnostic criteria for ASD at 36 months (Clifford et al., 2013; Del Rosario, Gillespie-Lynch, Johnson, Sigman, & Hutman, 2014; Garon et al., 2009; Garon et al., 2016; Zwaigenbaum et al., 2005). However, no study to date has examined temperament beyond the age of 36 months or how it relates to co-occurring psychopathology. This is highly relevant, as some of the traits observed among HR children, like increased levels of Negative Affect and reduced Effortful Control, have been suggested as early risk factors for anxiety disorders (e.g. Cole, Zapp, Fettig, & Perez-Edgar, 2016; Rapee, 2002). The aim of this chapter was threefold; to examine group differences in temperamental profiles from infancy until the age of 7 years, to compare developmental trajectories of Negative Affect and Effortful Control in the HR and LR groups, and to see whether these two factors were associated with the development of anxiety.

Firstly, the HR-ASD group presented with atypical temperament, heightened Negative Affect and reduced Effortful Control, compared with the LR group, from the age of 24 months. These group differences persisted until the age of 7 years (except for non-significant differences in Effortful Control at 36 months). On the other hand, the HR-non ASD group also showed heightened Negative Affect relative to the LR group, but this only reached trend level significance. At the 7-year visit, the HR-non ASD

group did present with higher Negative Affect than the LR group. The developmental trajectories of Negative Affect were examined and demonstrated that this factor is stable across time in both groups, and continually higher in the HR group. Heightened Negative Affect was also associated with contemporaneous Effortful Control across time. This finding is consistent with prior research, which suggests that Effortful Control represents the self-regulatory component of temperament and modulates the reactive components, such as Negative Affect (Rothbart & Deryberry, 1981). Therefore, the HR children may have been particularly vulnerable to high levels of Negative Affect due their reduced levels of Effortful Control.

Longitudinal studies examining early predictors of anxiety suggest that temperamental characteristics, such as high levels of Negative Affect and reduced ability to regulate such emotional states (i.e. Effortful Control) also contribute to the development of anxiety disorders in children (Degnan & Fox, 2007; Fox & Pine, 2012). Within the sample in this study, levels of Negative Affect at the age of 7 months were significantly associated with anxiety at 7 years. Effortful control was not associated with anxiety, except at the 36-month visit when the reverse finding emerged and high levels of Effortful Control predicted higher anxiety. Taken together, these findings suggest that reduced Effortful Control is necessary for the maintenance of high levels of Negative Affect. However, Negative Affect in itself is sufficient for the development of anxiety.

7.3 Implications for research and clinical practice

The findings from this thesis have several important implications for research, both for high-risk sibling studies and those examining co-occurring anxiety in ASD more broadly, as well as for clinical practice.

7.3.1 Implications for high-risk research in ASD

One of the important limitations of high-risk ASD studies to date has been that very few have examined the prevalence and manifestation of co-occurring mental health difficulties. Among the studies that have, the focus was solely on high-risk children who did not meet diagnostic criteria and those with ASD were excluded from analyses (Miller et al., 2015; Miller, Iosif, Young, Hill, & Ozonoff, 2016; Schwichtenberg et al., 2013). The present study, which included both the HR-ASD and HR-non ASD groups, found that both the severity of anxious symptoms and their correlates differed in the two groups. Given that the HR-ASD group had the highest levels of parent-rated anxiety, further examination of anxiety within this group is highly relevant. Taken together, the examination of differential prevalence and neurocognitive correlates of anxiety among high-risk siblings with and without ASD is highly warranted. Such research will be particularly important as the HR participants become older and move from childhood to adolescence, when anxiety symptoms become more readily observable (Beesdo, Knappe, & Pine, 2009). The HR-non ASD group manifested heightened threat bias and Negative Affect at the age of 7-years, which may signal the risk for developing more severe anxiety later in development (Perez-Edgar et al., 2011).

Additionally, the significant association between Negative Affect in infancy and anxiety at age 7-years suggests that temperament is an important factor to include in longitudinal designs aiming to investigate the development of anxiety in high-risk siblings. Tonnsen, Malone, Hatton, and Roberts (2013) report that among children with Fragile X syndrome (who are also at heightened risk for developing ASD), Negative Affect predicts anxiety, but not ASD symptoms, at 36 months of age. High-risk sibling

studies could extend the findings from this thesis to investigate whether Negative Affect uniquely predicts anxiety among HR children, or if it is related to ASD symptoms as well. Furthermore, given the differential patterns of anxiety in the HR-ASD and HR-non ASD groups, the examination of temperament separately in each group would be important to clarify whether developmental trajectories in the two groups are similar.

7.3.2 Implications for research examining co-occurring anxiety within ASD

Chapter 5 examined levels of threat bias in the HR-ASD, HR-non ASD and LR groups, as well as the association between threat bias and anxiety. An important aspect of the paradigm used was that it was designed to address limitations of prior research, by using non-social threat stimuli, comparing RTs to threatening stimuli with those to both positive and neutral stimuli, and examining multiple aspects of attention. In spite of these modifications, the HR-ASD group did not exhibit enhanced threat bias, despite having high anxiety. These findings largely agree with prior research, which was unable to detect an association between threat bias and anxiety among individuals with ASD, using socially threatening stimuli (Hollocks, Ozsivadjian, Matthews, Howlin, & Simonoff, 2013; May, Cornish, & Rinehart, 2015). These findings suggest that hypersensitivity threat in general may not underlie anxiety in ASD and that threat bias tasks are not helpful in examining the cognitive correlates of anxiety in this group. Alternatively, it is possible that the stimuli used need to be refined further to include ASD-specific threats, such as content relating to uncertainty, unexpected change or sensory arousal.

7.3.3 Clinical implications

The findings from this thesis, particularly the ones outlined above, have important clinical implications. The association between Negative Affect and anxiety suggests that temperament may be a useful target for early risk intervention. Interventions for pre-school aged children who manifest atypicalities in temperament have shown promise in reducing the risk of developing an anxiety disorder (e.g. Kennedy, Rapee, & Edwards, 2009). Proposed interventions provide parents with psychoeducation about the development and maintenance of anxiety disorders. They also teach parents strategies to restructure the child's anxious thinking styles, promote positive behaviours (e.g. reducing overprotection and increasing exposure to novel stimuli) and provide coping plans tailored to each child (Kennedy, Rapee, & Edwards, 2009). A particular advantage of such interventions is that they involve parent-child interaction and training for parents (McClowry, Rodriguez, & Koslowitz, 2008).

Recent research suggests that parent-mediated interventions, targeting risk markers of ASD, have yielded promising results, suggesting gains for both infants and parents (Bradshaw, Steiner, Gengoux, & Koegel, 2015). Perhaps such interventions could include training on reducing Negative Affect early in development.

Threat bias modification training, which is aimed at teaching children to control their attentional responding to threat, is reported to successfully reduce anxiety symptoms (Lau, 2013; Shechner et al., 2014). However, the findings from this study and others examining threat bias in ASD (Hollocks, Ozsivadjian, Matthews, Howlin, & Simonoff, 2013; May, Cornish, & Rinehart, 2015) suggest that this may not be a useful target for intervention among children with ASD. On the other hand, such an

intervention may be helpful in reducing anxiety symptoms among unaffected siblings of children with ASD, who do show heightened threat bias.

7.4 Limitations

While this thesis presented novel findings, it is important that they be considered in the context of several limitations. The modest size of the sample tested in this thesis has been discussed extensively in multiple chapters and post hoc power analyses suggested that there was only low to moderate power for most of the analyses undertaken. However, the results of this thesis, particularly the lack of threat bias in the HR-ASD group, must be taken with some caution, as it is possible that non-significant findings could have resulted from lack of statistical power. To ameliorate this issue, effect sizes were presented alongside each analysis to provide a sense of the strength of the associations reported in this small sample. Furthermore, it was not possible to examine the HR-ASD and HR-non ASD groups separately in some analyses (such as associations between temperament and anxiety). It was also not possible to examine the manifestation of anxiety in the HR-Atypical group, which was very small in size (n=7).

This thesis approached the examination of co-occurring anxiety symptoms from a familial or genetic risk perspective. This approach was deemed justifiable given that there is evidence of genetic risk for anxiety disorders (e.g. Waszczuk, Zavos, Gregory, & Eley, 2014), as well as evidence of increased prevalence of anxiety among siblings of children with ASD (e.g. Hallett, Ronald, et al., 2013). Consequently, the present study examined the cognitive and constitutional (e.g. temperament) factors associated with anxiety. However, there is vast evidence suggesting that environmental risk factors significantly contribute to the development of anxiety disorders (e.g. Rapee, 2002). This may be particularly relevant among children at high-risk for ASD, who

maybe be exposed to more environmental risk factors due to having an older sibling with a disability. Adjustment among siblings of children with ASD has been associated with multiple factors, including the presence of challenging behaviours in the probands, quality of the sibling relationship and parental wellbeing (e.g. Bitsika, Sharpley, & Mailli, 2014). Thus, while the present study adopted one particular approach, it is important to note that familial/genetic risk is likely not the only contributing factor to anxiety among high-risk siblings.

Anxiety symptoms were assessed using parent- and self-report questionnaires. There are numerous considerations when using questionnaire measures to assess anxiety among children with ASD. Multiple studies report that individuals with ASD tend to under-report anxiety symptoms and that self-report measures have reduced sensitivity in detecting clinical cases (Mazefsky, Kao, & Oswald, 2011; White, Schry, & Maddox, 2012). On the other hand, it can be difficult for parents to accurately report on their child's internal cognitions and emotional experiences (March, Parker, Sullivan, Stallings, & Conners, 1997). Parental psychopathology has also been suggested to influence parents' reports of their child's anxiety symptoms (Becker, Jensen-Doss, Kendall, Birmaher, & Ginsburg, 2016). This issue may be particularly relevant for high-risk studies, as familial risk for both ASD and anxiety could extend to parents as well as siblings. Unfortunately, the present study did not include a measure of parental psychopathology and future studies would benefit from including such measures to assess their contribution to parent-reported anxiety symptoms in offspring.

More broadly, it is presently not clear whether the current measures of childhood anxiety are able to accurately capture the manifestation of anxiety in ASD. Rodgers et al. (2016) suggest that the SCAS does not have sufficient content validity

for measuring anxiety among children with ASD. The authors developed a modified version of the SCAS, which includes subscales examining *Uncertainty* and *Performance Anxiety*, which were not part of the original scale. The modified scale has been reported to have good psychometric properties among children with ASD (Rodgers et al., 2016). Therefore, future studies could incorporate such measures to further validate their use and measure more relevant aspects of anxiety within ASD.

A further limitation of this thesis is that the participants examined were predominantly children who had average cognitive and verbal ability. Only two children in this sample met criteria for intellectual disability (IQ<70; Wechsler, 2011) or had reduced verbal capacity. The association between cognitive ability and anxiety among individuals with ASD is not fully understood. However, multiple studies report that individuals with ASD and intellectual disability have lower anxiety than those with average IQ (Hallett, Lecavalier, et al., 2013; Sukhodolsky et al., 2008). It is unclear whether this finding represents the true nature of anxiety among individuals with ASD and reduced cognitive ability, or if it is due to the difficulty measuring anxious symptoms in this population. In this context, it is important to note that the findings in this thesis apply to high-risk children with average cognitive ability, but that further research needs to be done among children with reduced IQ.

Finally, there are several more general limitations to high-risk sibling research that need to be considered. It is not fully clear whether HR children who themselves have ASD are truly representative of the general ASD population. Emerging evidence suggests that there may be distinct genetic pathways operating in simplex families, where only one individual has ASD, than in multiplex families, where more than one individual has ASD (Iossifov et al., 2012; Sanders et al., 2011). Correspondingly,

Taylor et al. (2015) suggest that behavioural phenotypes, such as severity of social difficulties and pragmatic language, are more severe among children from multiplex families. Oerlemans, Hartman, Franke, Buitelaar, and Rommelse (2016) report that cognitive functioning levels are similar across children with ASD from simplex and multiplex families. However, "unaffected" siblings from multiplex families have reduced performance on certain cognitive skills (e.g. verbal IQ) than siblings from simplex families. Thus, it is possible that the children in this study are not representative of children with ASD who do not have other siblings with the condition. Many of the measures used to ascertain information about anxiety and ASD severity are parent-report questionnaires and interviews. It is possible that having another child (or more children) with ASD influences parent-report such that they may report more severe symptoms in their child due to having more knowledge about mental health issues. Likewise, it is possible that parents may report less severe symptoms if the proband exhibits severe difficulties, as the younger child's symptoms may not be as obvious or worrisome in comparison.

7.5 Targets for future research

This thesis examined anxiety as a broad concept and the total SCAS-P and SCAS-C scores were used in analyses and focused on the non-social predictors of anxiety. However, the contribution of social functioning atypicalities to anxiety, particularly social phobia, warrants further investigation. Anxiety has been suggested to have a curvilinear relationship to social functioning in ASD (Bellini, 2004). White, Maddox, and Panneton (2015) reported that adolescents with ASD and fear of negative social evaluation spend more time fixating on threatening faces. However, Hollocks et al. (2014) did not find an association between overall anxiety symptoms and social

understanding. It is possible that individuals with ASD who have a certain degree of social interest or understanding are more likely to have higher social anxiety symptoms, while those with very low social motivation, as well as those with good social skills, experience less social anxiety. High-risk sibling studies are particularly well placed to address this, as much of the data collected in infant and toddler visits predominantly focus on measures relating to social factors, including social awareness, joint attention and false belief (Jones, Gliga, Bedford, Charman, & Johnson, 2014), allowing longitudinal exploration of the association between social attention and social anxiety.

It is also important to characterise the non-social correlates of anxiety among individuals with ASD. As previously outlined, factors such as distress to unexpected change and heightened sensitivity to sensory stimulation are considered important contributors to anxiety within ASD (Wigham, Rodgers, South, McConachie, & Freeston, 2014). However, information about these factors is currently obtained through the use of questionnaire measures. Future studies would benefit from coupling questionnaire measures with experimental tasks to enhance interpretation of these associations. A variety of methods, such as paradigms measuring physiological arousal to distressing stimuli or neural habituation to sensory stimuli could be implemented and their association with reports of anxiety measured. Findings from high-risk studies suggest that such measures could be implemented early in development and their longitudinal associations with anxiety could be examined. For example, Guiraud et al. (2011) report that high-risk infants exhibit reduced neural habituation to repeated sounds compared to LR controls.

Finally, in Chapter 3, significant sex differences in anxiety symptoms were reported in the HR-ASD group. Prior literature reports varied estimates of sex differences in anxiety symptoms in the ASD population (Lai, Lombardo, Pasco, Ruigrok, Wheelwright, Sadek, & Baron-Cohen, 2011; Solomon, Miller, Taylor, Hinshaw, & Carter, 2012). Unfortunately, this study did not have the statistical power to examine the neurocognitive correlates and longitudinal predictors of anxiety separately in males and females. However, such investigation is highly relevant, particularly as recent research suggests differential presentation of ASD symptoms among males and females (for review see Lai, Lombardo, Auyeung, Chakrabarti, & Baron-Cohen, 2015). The investigation of sex differences in the prevalence and manifestation of anxiety among individuals with ASD is an important area for future research.

Finally, most high-risk for ASD studies (including this one) use cognitive and neural measures aimed at investigating possible predictors of ASD symptoms and do not include measures relevant for co-occurring difficulties like anxiety. In addition to using parent-report measures of temperament, it may be helpful to also include observational measures of temperamental traits like fearfulness or behavioural inhibition (Fox, Henderson, Rubin, Calkins, & Schmidt, 2001; van Brakel, Muris, & Bögels, 2004). Similarly, threat bias paradigms could be implemented early in development through the use of eye-tracking tasks to examine whether early attention to threatening stimuli is associated with the later development of anxiety in HR infants, as it is in non-ASD children (Perez-Edgar et al., 2011).

7.6 Conclusion

This thesis was the first to examine the prevalence, neurocognitive correlates and developmental risk factors of co-occurring anxiety among children at increased familial risk for ASD. Firstly, the findings suggest that anxiety is highly elevated among high-risk children, particularly those who go on to meet diagnostic criteria for ASD. However, it also suggests that the underlying mechanisms associated with anxiety may differ in the two groups. Among high-risk siblings with ASD, increased anxiety is associated with the core symptoms of ASD, particularly RRBs. This does not appear to be true among unaffected high-risk siblings, who did not demonstrate a significant association between anxiety and ASD severity. On the other hand, the HR-non ASD children do exhibit heightened bias to threatening stimuli, while this bias was not observed among the HR-ASD group. Finally, among high-risk children, Negative Affect in infancy and toddlerhood is highly elevated compared to LR controls. Negative Affect at the age of 7 months was associated with anxiety severity in middle childhood. The findings from this thesis suggest that targeted interventions could be administered early in life to help reduce dysregulated temperament, thus lowering the risk for developing anxiety. However, both future research and clinical practice need to take into account that the nature and content of anxiety may differ in children with ASD and interventions need to be altered to meet their specific needs.

Appendices

Appendix 1: Summary of clinical measures using HR-ASD, HR-Atypical, HR-Typical and LR groups

Given that the 36-month outcomes included HR-ASD, HR-Atypical, HR-typically developing and LR groups, a similar grouping was formed using data from the 7-year follow-up. The HR-non ASD group was divided into two further groups: HR-Atypical (HR-Atyp) and HR-Typically Developing (HR-TD). Children in the HR-Atyp group (*n*=7) did not meet diagnostic criteria for ASD, but manifested sub-threshold clinical concern. They scored above threshold on at least one measure of ASD symptomatology and/or manifested developmental delay. Furthermore, the 3 children who lost diagnosis from the 36-month to 7-year visits were included in this group. The HR-TD group (*n*=20) scored below threshold on all measures of ASD symptomatology and had normative cognitive development.

Analyses of clinical measures (ADOS-2, ADI-R) and cognitive functioning (WASI-II) described in chapter 2 were repeated using HR-ASD, HR-Atyp, HR-TD and LR outcome groups. Group means are presented in Table 17.

Table 17: Summary of clinical scores of HR-ASD, HR-Atyp, HR-TD and LR groups

Measure	HR-ASD	HR-Atyp	HR-TD	LR	ANOVA/MANOVA
ADI-R	*N*=14	*N*=7	*N*=20	N/A	
Social	13.14 (4.69)[a]	9.86 (6.82)[a]	2.20 (2.55)[b]	N/A	$F(3, 38)=28.84, p<.001, \eta^2=.603$
Comm.	10.43 (4.59)[a]	8.29 (6.63)[a]	2.80 (2.14)[b]	N/A	$F(3, 38)=15.56, p<.001, \eta^2=.450$
RRB	3.57 (1.74)[a]	1.14 (219)[b]	.45 (.95)[b]	N/A	$F(3, 38)=18.34, p<.001, \eta^2=.491$
ADOS-2	*N*=15	*N*=7	*N*=20	*N*=34	
CSS Total	6.33 (2.92)[a]	4.14 (1.57)[b]	1.95 (.83)[c]	1.70 (1.19)[c]	$F(3, 71)=31.22, p<.001, \eta^2=.569$
CSS SA	6.60 (2.59)[a]	4.57 (1.90)[a]	2.50 (1.15)[b]	2.18 (1.70)[b]	$F(3, 71)=23.17, p<.001, \eta^2=.495$
CSS RRB	6.13 (2.70)[a]	5.57 (3.36)[a]	2.20 (2.19)[b]	1.12 (.70)[b]	$F(3, 71)=27.43, p<.001, \eta^2=.537$
WASI-II	*N*=14	*N*=7	*N*=20	*N*=35	
FSIQ	109.79 (21.36)	105.43 (10.28)	110.40 (14.36)	117.06 (11.61)	n.s
VIQ	110.14 (25.87)	105.57 (9.64)	114.05 (15.76)	119.77 (13.93)	n.s
PIQ	109.57 (18.26)	104.29 (11.31)	104.29 (11.31)	110.34 (12.05)	n.s

Group sizes are smaller for some variables due to missing data. Groups denoted with different subscript letters (a, b, c) differed significantly with Bonferonni correction applied (p<.05). HR/LR indicates high-risk or low-risk group; ASD autism spectrum disorder; SD standard deviation; ADI Autism Diagnostic Interview – Revised; RRB Restricted Repetitive Behaviour; ADOS Autism Diagnostic Observation Schedule; CSS Calibrated Severity Score; SA Social Affect; WASI-II Wechsler Abbreviated Scales of Intelligence- 2nd Edition; FSIQ Full Scale IQ; VIQ Verbal IQ; PIQ Perceptual IQ.

Appendix 2: Anxiety prevalence when the HR-non ASD group is split into the HR-Atypical and HR-Typically Developing

Table 18: SCAS-P and SCAS-C scores for the HR-ASD, HR-Atyp, HR-TD and LR groups

SCAS subscale (SD)	HR-ASD	HR-Atyp	HR-TD	LR	MANOVA
SCAS-P	*N*=15	*N*=7	*N*=19	*N*=36	
Total	26.20 (20.86)[a]	17.86 (2.55)	18.79 (9.71)	12.22 (7.27)[b]	$F(3, 73)=5.42, p=.002, \eta^2=.182$
Separation Anxiety	6.27 (4.20)[a]	5.71 (2.56)	4.84 (2.91)	2.94 (2.14)[b]	$F(3, 73)=5.84, p=.001, \eta^2=.194$
OCD	2.27 (3.41)[a]	.71 (.76)	1.26 (1.73)	0.67 (0.99)[b]	$F(3, 73)=2.74, p=.049, \eta^2=.101$
Social Phobia	5.07 (5.65)	2.86 (1.07)	4.63 (2.77)	2.64 (2.98)	$F(3, 73)=2.42, p=.073, \eta^2=.090$
Physical Injury/Fears	4.53 (2.85)	5.43 (2.30)	3.16 (2.17)	2.97 (2.08)	$F(3, 73)=3.46, p=.021, \eta^2=.124$
Panic/Agoraphobia	2.93 (4.62)[a]	.14 (.38)[b]	.95 (1.03)	.053 (.094)[b]	$F(3, 73)=4.80, p=.004, \eta^2=.165$

Generalised Anxiety	5.13 (3.66)[a]	3.00 (.82)	3.95 (2.22)	2.47 (1.40)[b]	$F(3, 73)=5.77, p=.001, \eta^2=.192$
SCAS-C	*N*=11	*N*=7	*N*=19	*N*=32	
Total	23.82 (10.59)	27.29 (11.00)	23.63 (10.57)	23.75 (11.44)	n.s
Separation Anxiety	6.45 (4.06)	6.71 (2.43)	6.00 (3.61)	5.09 (3.15)	n.s
OCD	1.82 (1.66)	4.00 (3.00)	2.53 (2.20)	2.41 (2.70)	n.s
Social Phobia	3.64 (2.94)	4.57 (2.37)	4.05 (2.78)	4.56 (2.86)	n.s
Physical Injury/Fears	4.82 (3.25)	5.86 (4.06)	3.68 (2.87)	3.94 (3.18)	n.s
Panic/Agoraphobia	2.64 (2.62)	1.86 (1.77)	2.58 (2.19)	2.56 (3.51)	n.s
Generalised Anxiety	4.45 (2.51)	4.29 (1.98)	4.79 (1.81)	5.19 (2.48)	n.s

Group sizes are smaller for some variables due to missing data. Groups denoted with different subscript letters (a, b, c) differed significantly with Bonferonni correction applied (p<.05). HR/LR indicates high-risk or low-risk group; ASD autism spectrum disorder; SD standard deviation; SCAS-P Spence Children's Anxiety Scale-Parent report; SCAS-C Spence Children's Anxiety Scale-Child report; OCD Obsessive Compulsive Disorder

Appendix 3: Results of pilot study of the Emotional Spatial Cueing task

Prior to administering the Emotional Spatial Cueing task to the HR and LR participants in this study, the task was piloted in a group of children and adults to ensure that the disengagement effect could be observed and that children were able to complete the task.

Five children aged 4-8 years and 4 adults aged 23-29 years were included in the pilot phase. Their performance across conditions was examined to determine whether the basic engagement and disengagement effects could be observed.

To examine performance on the task in the child and adult groups, a 2 (group: child, adult) by 3 (cue: threatening, neutral, positive) by 2 (congruency: congruent, incongruent) repeated measures ANOVA was used. Participants' RTs across trials are presented in Figure 11. Post-hoc analyses were used where significant differences emerged, with Bonferonni correction applied to account for multiple testing.

Overall, there was a trend-level effect of cue ($F(2, 14)=2.88$, $p=.09$, $\eta^2=.29$). There was also a marginally significant effect of congruency ($F(2, 7)=5.65$, $p=.05$, $\eta^2=.48$). Post-hoc analyses revealed that, overall, participants had longer RTs on incongruent trials ($M=497.96$, $SD=53.42$) than congruent trials ($M=470.53$, $SD=46.38$). There was also a significant effect of age ($F(1, 7)=3.10$, $p=.02$, $\eta^2=.33$), where adults ($M=412.20$, $SD=66.25$) had shorter RTs than children ($M=556.29$, $SD=74.07$). On the contrary, there were no significant interactions between cue and age ($F(1, 14)=.64$, $p=.54$, $\eta^2=.08$) or congruency and age ($F(1, 7)=.83$, $p=.39$, $\eta^2=.11$).

These findings suggest that the disengagement effect worked, given the longer RTs on incongruent trials. Furthermore, the trend-level effect of cue could possibly become significant with a larger sample size, suggesting that the manipulation of emotionally-valanced stimuli was successful. Children had longer RTs than adults, but their susceptibility to the task did not differ from adults, as they responded similarly to both congruency and cue.

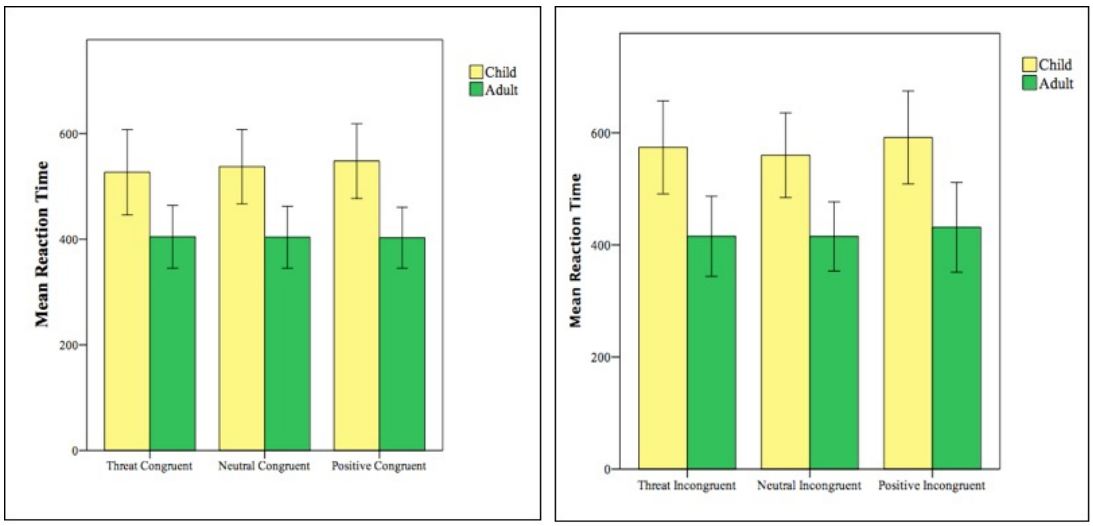

Figure 11. Child and Adult reaction times in congruent (left) and incongruent (right) trials on the Emotional Spatial Cueing task.

Appendix 4: Prevalence of anxiety, when co-varying for FSIQ

To examine group and sex differences on anxiety symptoms, a 3 (group: HR-ASD, HR-non ASD, LR) x 2 (sex: male, female) ANCOVA was run on the SCAS-P total score, co-varying for IQ. Planned comparisons between each pair of groups were used where significant differences emerged, with Bonferonni correction applied for multiple testing. If a significant group x sex interaction emerged, follow up independent samples t-tests were run within each group to examine sex differences on anxiety scores, with Bonferonni correction applied for family-wise error related to multiple testing. As there were significant sex differences and a group x sex interaction on anxiety scores (see Chapter 3), sex was also co-varied for in further analyses.

Parent-report of anxiety symptoms, SCAS-P total score, revealed significant differences among groups, $F(2, 64)=8.45$, $p=.001$, $\eta^2=.21$. The HR-ASD group had substantially higher total SCAS-P scores than the LR group ($p<.001$, $d=.89$), whereas the HR-non ASD group did not differ from either the HR-ASD ($p=.22$, $d=.52$) or LR ($p=.14$, $d=.72$) groups.

There were significant sex differences in total anxiety levels $F(1, 64)=9.67$ $p=.003$, $d=.42$. Females ($M=18.50$, $SD=13.96$) had higher anxiety than males ($M=13.65$, $SD=8.55$). There was also a significant group x sex interaction on the total anxiety score $F(2, 64)=8.47$, $p=.001$, $\eta^2=.23$. To follow up on this interaction, independent samples t-tests were run within each group to examine sex differences on total anxiety. Bonferonni correction was applied to the *p*-value to account for family wise error related to multiple testing (.05/6=.008). The only significant difference emerged in the HR-ASD group, where females ($M=38.88$, $SD=21.50$) had significantly

higher anxiety levels than males (M=11.71, SD=4.11), $t(13)$=-3.28, p=.001, d=1.76, but there were no sex differences in the LR or HR-non ASD groups.

Group differences in threat bias, co-varying for FSIQ and sex

A 3 (Group: HR-ASD, HR-non ASD, LR) x 6 (Index) MANCOVA was run, co-varying for FSIQ and sex. Only one significant difference emerged, $F(2, 56)$=7.52, p=.001, η^2=.21, on the threat-positive engagement index. Follow-up analyses revealed that the HR-non ASD group took significantly longer to engage with threatening stimuli (compared to positive stimuli) than both the HR-ASD (p=.002, d=1.25) and the LR (p=.02, d=.82) groups.

Appendix 5: Additional temperament analyses

Association between SCAS-P total score and AOSI/ADOS scores at visits 1-3

First-order Pearson correlations were run between SCAS-P total score, AOSI total scores and ADOS CS scores. There was no significant association between SCAS-P and AOSI scores at 7-months ($r(74)=-.17$, $p=.16$), 14-months ($r(73)=.03$, $p=.79$) or 36 months ($r(73)=-.07$, $p=.58$). As the ADOS was not administered to children in the LR group at 24 months, the association between SCAS-P and ADOS CS scores for this visit are presented only in the HR group. There was no significant association between SCAS-P and ADOS CS at 24-months ($r(38)=-.11$, $p=.50$).

Regression analyses between temperament and anxiety, co-varying for risk group and sex

Follow-up regression analyses were run for each visit, with SCAS-P total score as the dependant variable and Negative Affect, risk group status and sex as predictors.

At the 14-month visit, this accounted for a significant proportion of the variance in SCAS-P total score, $F(3, 67)=7.32$, $p<.001$, $R^2=.25$. Both risk group ($\beta=.32$, $t(70)=3.01$, $p=.004$) and Negative Affect ($\beta=.27$, $t(70)=2.59$, $p=.02$) significantly predicted SCAS-P total score. Sex had a trend level association with SCAS-P total score ($\beta=.19$, $t(70)=1.78$, $p=.08$)

At the 24-month visit, this accounted for a significant proportion of the variance in SCAS-P total score, $F(3, 65)=11.49$, $p<.001$, $R^2=.35$. This time, risk group ($\beta=.24$,

$t(68)=2.40$, $p=.02$) and Negative Affect ($\beta=.43$, $t(68)=4.19$, $p<.001$) and sex ($\beta=.22$, $t(68)=2.15$, $p=.04$) significantly predicted SCAS-P total score.

Finally, at the 36-month visit, Effortful Control was also added as a predictor given its significant association with SCAS-P total score. Given the association between Negative Affect and Effortful Control, collinearity diagnostics were assessed and indicated that there was no risk of multi-collinearity (Durbin Watson= 1.97). This model accounted for a significant portion of SCAS-P total score, $F(4, 66)=6.50$, $p<.001$, $R^2=.28$. Risk group ($\beta=.35$, $t(70)=2.98$, $p=.004$) and Effortful Control significantly predicted SCAS-P scores ($\beta=.32$, $t(70)=2.65$, $p=.01$), while Negative Affect ($\beta=.12$, $t(70)=1.07$, $p=.29$) and sex ($\beta=.06$, $t(70)=.49$, $p=.63$) did not.

36-month group differences in temperament and association with anxiety, using the original factor structure

Finally, as 36-month Effortful Control scores were unexpected, analyses were run again with the original factor structure.

Table 19: Group differences in 36 month CBQ scores using original factor structure

36-month CBQ	**HR-ASD**	**HR-non ASD**	**LR**	**MANOVA/ANOVA**
	***N*=15**	***N*=23**	***N*=36**	
Negative Affect	.43[a] (1.18)	.23 (.80)	-.33[b] (.95)	$F(2, 71)=6.26$ $p=.003$, $\eta^2=.15$
Effortful Control	.03 (1.37)	-.25 (.87)	.16 (.88)	$F(2, 71)=.46$ $p=.63$, $\eta^2=.15$
Surgency	.01 (1.19)	-.17 (1.11)	.11 (.85)	$F(2, 71)=.2.41$ $p=.10$, $\eta^2=.06$

Groups denoted with different subscript letters (a, b, c) differed significantly with Bonferonni correction applied (p<.05). HR/LR indicates high-risk or low-risk group; ASD autism spectrum disorder

Both Negative Affect ($r(72)=.43$, $p<.001$) and Effortful Control ($r(.72)=.39$, $p=.001$) were significantly correlated with SCAS-P total score.

This model accounted for a significant portion of SCAS-P total score, $F(4, 67)=7.99$, $p<.001$, $R2=.31$. Risk group ($\beta=.31$, $t(70)=2.78$, $p=.01$) and Effortful Control significantly predicted SCAS-P scores ($\beta=.34$, $t(71)=2.92$, $p=.01$), while Negative Affect ($\beta=.18$, $t(71)=1.45$, $p=.15$) and sex ($\beta=.03$, $t(71)=.23$, $p=.82$) did not.

References

Ablow, J. C., Measelle, J. R., Kraemer, H. C., Harrington, R., Luby, J., Smider, N., Dierker, L., Clark, V., Dubicka, B., Heffelfinger, A. M. Y., Essex, M. J., & Kupfer, D. J. (1999). The macarthur three-city outcome study: Evaluating multi-informant measures of young children's symptomatology. *Journal of the American Academy of Child and Adolescent Psychiatry, 38*(12), 1580-1590. doi:10.1097/00004583-199912000-00020

Achenbach, T. M. (2011). Commentary: Definitely more than measurement error: But how should we understand and deal with informant discrepancies? *Journal of Clinical Child and Adolescent Psychology, 40*(1), 80-86. doi:10.1080/15374416.2011.533416

Achenbach, T. M., McConaughy, S. H., & Howell, C. T. (1987). Child/adolescent behavioral and emotional problems: Implications of cross-informant correlations for situational specificity. *Psychological Bulletin, 101*(2), 213-232.

Adrien, J. L., Lenoir, P., Martineau, J., Perrot, A., Hameury, L., Larmande, C., & Sauvage, D. (1993). Blind ratings of early symptoms of autism based upon family home movies. *Journal of the American Academy of Child and Adolescent Psychiatry, 32*(3), 617-626. doi:10.1097/00004583-199305000-00019

Airaksinen, E., Larsson, M., & Forsell, Y. (2005). Neuropsychological functions in anxiety disorders in population-based samples: Evidence of episodic memory

dysfunction. *Journal of Psychiatric Research, 39*(2), 207-214. doi:10.1016/j.jpsychires.2004.06.001

American Psychiatric Association. (1980). *Diagnostic and statistical manual of mental disorders (3rd ed)*. Washington, DC: American Psychiatric Association.

American Psychiatric Association. (1994). *Diagnostic and statistical manual of mental disorders 4th edition (dsm-iv)*. Washington, DC: American Psychiatric Association.

American Psychological Association. (2013). *Diagnostic and statistical manual of mental disorders* (5th ed.). Arlington, VA: American Psychiatric Publishing.

Arch, J. J., & Craske, M. G. (2008). Acceptance and commitment therapy and cognitive behavioral therapy for anxiety disorders: Different treatments, similar mechanisms? *Clinical Psychology: Science and Practice, 15*(4), 263-279. doi:10.1111/j.1468-2850.2008.00137.x

Asperger, H. (1944). Die „autistischen psychopathen" im kindesalter. *Archiv für Psychiatrie und Nervenkrankheiten, 117*(1), 76-136. doi:10.1007/BF01837709

Bailey, A., Le Couteur, A., Gottesman, I., Bolton, P., Simonoff, E., Yuzda, E., & Rutter, M. (1995). Autism as a strongly genetic disorder: Evidence from a british twin study. *Psychological Medicine, 25*(1), 63-77. doi:10.1017/S0033291700028099

Baird, G., Charman, T., Baron-Cohen, S., Cox, A., Swettenham, J., Wheelwright, S., & Drew, A. (2000). A screening instrument for autism at 18 months of age: A 6-year follow-up study. *Journal of the American Academy of Child and*

Adolescent Psychiatry, 39(6), 694-702. doi:10.1097/00004583-200006000-00007

Baird, G., Simonoff, E., Pickles, A., Chandler, S., Loucas, T., Meldrum, D., & Charman, T. (2006). Prevalence of disorders of the autism spectrum in a population cohort of children in south thames: The special needs and autism project (snap). *Lancet, 368*(9531), 210-215. doi:10.1016/s0140-6736(06)69041-7

Ballinger, G. A. (2004). Using generalized estimating equations for longitudinal data analysis. *Organizational Research Methods, 7*(2), 127-150.

Bar-Haim, Y., Lamy, D., Pergamin, L., Bakermans-Kranenburg, M. J., & van IJzendoorn, M. H. (2007). Threat-related attentional bias in anxious and nonanxious individuals: A meta-analytic study. *Psychological Bulletin, 133*(1), 1-24. doi:10.1037/0033-2909.133.1.1

Bar-Haim, Y., Morag, I., & Glickman, S. (2011). Training anxious children to disengage attention from threat: A randomized controlled trial. *Journal of Child Psychology and Psychiatry and Allied Disciplines, 52*(8), 861-869. doi:10.1111/j.1469-7610.2011.02368.x

Barger, B. D., Campbell, J. M., & McDonough, J. D. (2013). Prevalence and onset of regression within autism spectrum disorders: A meta-analytic review. *Journal of Autism and Developmental Disorders, 43*(4), 817-828. doi:10.1007/s10803-012-1621-x

Baron-Cohen, S., Leslie, A. M., & Frith, U. (1985). Does the autistic child have a "theory of mind" ? *Cognition, 21*(1), 37-46. doi:10.1016/0010-0277(85)90022-8

Baron-Cohen, S., Ring, H. A., Bullmore, E. T., Wheelwright, S., Ashwin, C., & Williams, S. C. (2000). The amygdala theory of autism. *Neuroscience and Biobehavioral Reviews, 24*(3), 355-364.

Beck, A. T. (1991). *Cognitive therapy and the emotional disorders*: Penguin.

Beck, A. T., & Clark, D. A. (1997). An information processing model of anxiety: Automatic and strategic processes. *Behaviour Research and Therapy, 35*(1), 49-58. doi:10.1016/S0005-7967(96)00069-1

Beck, A. T., Emery, G., & Greenberg, R. L. (1985). *Anxiety disorders and phobias: A cognitive perspective*: Basic Books.

Becker, E. M., Jensen-Doss, A., Kendall, P. C., Birmaher, B., & Ginsburg, G. S. (2016). All anxiety is not created equal: Correlates of parent/youth agreement vary across subtypes of anxiety. *Journal of Psychopathology and Behavioral Assessment*, 1-10. doi:10.1007/s10862-016-9544-z

Bedford, R., Elsabbagh, M., Gliga, T., Pickles, A., Senju, A., Charman, T., Johnson, M. H., & team, B. (2012). Precursors to social and communication difficulties in infants at-risk for autism: Gaze following and attentional engagement. *Journal of Autism and Developmental Disorders, 42*(10), 2208-2218. doi:10.1007/s10803-012-1450-y

Bedford, R., Pickles, A., Gliga, T., Elsabbagh, M., Charman, T., & Johnson, M. H. (2014). Additive effects of social and non-social attention during infancy relate to later autism spectrum disorder. *Dev Sci, 17*. doi:10.1111/desc.12139

Beesdo, K., Knappe, S., & Pine, D. S. (2009). Anxiety and anxiety disorders in children and adolescents: Developmental issues and implications for dsm-v. *The Psychiatric clinics of North America, 32*(3), 483-524. doi:10.1016/j.psc.2009.06.002

Bellini, S. (2004). Social skill deficits and anxiety in high-functioning adolescents with autism spectrum disorders. *Focus on Autism and Other Developmental Disabilities, 19*(2), 78-86. doi:10.1177/10883576040190020201

Ben-Sasson, A., Cermak, S. A., Orsmond, G. I., Tager-Flusberg, H., Kadlec, M. B., & Carter, A. S. (2008). Sensory clusters of toddlers with autism spectrum disorders: Differences in affective symptoms. *Journal of Child Psychology and Psychiatry and Allied Disciplines, 49*(8), 817-825. doi:10.1111/j.1469-7610.2008.01899.x

Benson, P. R., & Karlof, K. L. (2008). Child, parent, and family predictors of latter adjustment in siblings of children with autism. *Research in Autism Spectrum Disorders, 2*(4), 583-600. doi:10.1016/j.rasd.2007.12.002

Berry, A., & Cooper, M. (2012). Anxious children's ability to generate alternative attributions for ambiguous situations. *Behavioural and Cognitive Psychotherapy, 40*(1), 89-103. doi:10.1017/S1352465811000518

Berthoz, S., Lalanne, C., Crane, L., & Hill, E. L. (2013). Investigating emotional impairments in adults with autism spectrum disorders and the broader autism phenotype. *Psychiatry Research, 208*(3), 257-264. doi:10.1016/j.psychres.2013.05.014

Birmaher, B., Ehmann, M., Axelson, D. A., Goldstein, B. I., Monk, K., Kalas, C., Kupfer, D., Gill, M. K., Leibenluft, E., Bridge, J., Guyer, A., Egger, H. L., & Brent, D. A. (2009). Schedule for affective disorders and schizophrenia for school-age children (k-sads-pl) for the assessment of preschool children- a preliminary psychometric study. *Journal of Psychiatric Research, 43*(7), 680-686. doi:10.1016/j.jpsychires.2008.10.003

Birmaher, B., Khetarpal, S., Brent, D., Cully, M., Balach, L., Kaufman, J., & Neer, S. M. (1997). The screen for child anxiety related emotional disorders (scared): Scale construction and psychometric characteristics. *Journal of the American Academy of Child and Adolescent Psychiatry, 36*(4), 545-553. doi:10.1097/00004583-199704000-00018

Bishop, S., Duncan, J., Brett, M., & Lawrence, A. D. (2004). Prefrontal cortical function and anxiety: Controlling attention to threat-related stimuli. *Nature Neuroscience, 7*(2), 184-188. doi:10.1038/nn1173

Bishop, S. J. (2008). Neural mechanisms underlying selective attention to threat. *Annals of the New York Academy of Sciences, 1129*, 141-152. doi:10.1196/annals.1417.016

Bitsika, V., Sharpley, C. F., Andronicos, N. M., & Agnew, L. L. (2015). Agreement between self- vs parent-ratings of general anxiety disorder symptoms and

salivary cortisol in boys with an asd. *Journal of Developmental and Physical Disabilities, 27*(4), 467-477. doi:10.1007/s10882-015-9431-7

Bitsika, V., Sharpley, C. F., & Mailli, R. (2014). The influence of gender, age, psychological resilience and family interaction factors upon anxiety and depression in non-autism spectrum disorder siblings of children with an autism spectrum disorder. *British Journal of Guidance & Counselling, 43*(2), 216-228. doi:10.1080/03069885.2014.950944

Bleuler, E. (1950). *Dementia praecox or the group of schizophrenias*. Oxford, England: International Universities Press.

Bogels, S. M., & Brechman-Toussaint, M. L. (2006). Family issues in child anxiety: Attachment, family functioning, parental rearing and beliefs. *Clinical Psychology Review, 26*(7), 834-856. doi:10.1016/j.cpr.2005.08.001

Bolton, P., Macdonald, H., Pickles, A., Rios, P., Goode, S., Crowson, M., Bailey, A., & Rutter, M. (1994). A case-control family history study of autism. *Journal of Child Psychology and Psychiatry, 35*(5), 877-900. doi:10.1111/j.1469-7610.1994.tb02300.x

Bolton, P. F., Pickles, A., Murphy, M., & Rutter, M. (1998). Autism, affective and other psychiatric disorders: Patterns of familial aggregation. *Psychological Medicine, 28*(2), 385-395.

Boucher, J. (2003). Language development in autism. *International Congress Series, 1254*, 247-253. doi:10.1016/S0531-5131(03)00976-2

Boucher, J. (2012). Research review: Structural language in autistic spectrum disorder - characteristics and causes. *Journal of Child Psychology and Psychiatry and Allied Disciplines, 53*(3), 219-233. doi:10.1111/j.1469-7610.2011.02508.x

Bradshaw, J., Steiner, A. M., Gengoux, G., & Koegel, L. K. (2015). Feasibility and effectiveness of very early intervention for infants at-risk for autism spectrum disorder: A systematic review. *Journal of Autism and Developmental Disorders, 45*(3), 778-794. doi:10.1007/s10803-014-2235-2

Brian, J., Bryson, S. E., Smith, I. M., Roberts, W., Roncadin, C., Szatmari, P., & Zwaigenbaum, L. (2015). Stability and change in autism spectrum disorder diagnosis from age 3 to middle childhood in a high-risk sibling cohort. *Autism*.

Briggs-Gowan, M. J., Pollak, S. D., Grasso, D., Voss, J., Mian, N. D., Zobel, E., McCarthy, K. J., Wakschlag, L. S., & Pine, D. S. (2015). Attention bias and anxiety in young children exposed to family violence. *Journal of Child Psychology and Psychiatry, 56*(11), 1194-1201. doi:10.1111/jcpp.12397

Brock, M. E., Freuler, A., Baranek, G. T., Watson, L. R., Poe, M. D., & Sabatino, A. (2012). Temperament and sensory features of children with autism. *Journal of Autism and Developmental Disorders, 42*(11), 2271-2284. doi:10.1007/s10803-012-1472-5

Brown, H. M., Eley, T. C., Broeren, S., Macleod, C., Rinck, M., Hadwin, J. A., & Lester, K. J. (2014). Psychometric properties of reaction time based experimental paradigms measuring anxiety-related information-processing biases in children. *Journal of Anxiety Disorders, 28*(1), 97-107. doi:10.1016/j.janxdis.2013.11.004

Brown, H. M., McAdams, T. A., Lester, K. J., Goodman, R., Clark, D. M., & Eley, T. C. (2013). Attentional threat avoidance and familial risk are independently associated with childhood anxiety disorders. *Journal of Child Psychology and Psychiatry and Allied Disciplines, 54*(6), 678-685. doi:10.1111/jcpp.12024

Brozina, K., & Abela, J. R. (2006). Behavioural inhibition, anxious symptoms, and depressive symptoms: A short-term prospective examination of a diathesis-stress model. *Behaviour Research and Therapy, 44*(9), 1337-1346. doi:10.1016/j.brat.2005.09.010

Bryson, S. E., Zwaigenbaum, L., Brian, J., Roberts, W., Szatmari, P., Rombough, V., & McDermott, C. (2007). A prospective case series of high-risk infants who developed autism. *Journal of Autism and Developmental Disorders, 37*. doi:10.1007/s10803-006-0328-2

Bryson, S. E., Zwaigenbaum, L., McDermott, C., Rombough, V., & Brian, J. (2008). The autism observation scale for infants: Scale development and reliability data. *Journal of Autism and Developmental Disorders, 38*(4), 731-738. doi:10.1007/s10803-007-0440-y

Castillo, M. D., & Leandro, P. G. (2010). Interpretation bias in anxiety a synthesis of studies with children and adolescents. *Procedia - Social and Behavioral Sciences, 5*, 1105-1111. doi:10.1016/j.sbspro.2010.07.243

Cerda, M., Sagdeo, A., & Galea, S. (2008). Comorbid forms of psychopathology: Key patterns and future research directions. *Epidemiologic Reviews, 30*, 155-177. doi:10.1093/epirev/mxn003

Chamberlain, S. R., Blackwell, A. D., Fineberg, N. A., Robbins, T. W., & Sahakian, B. J. (2005). The neuropsychology of obsessive compulsive disorder: The importance of failures in cognitive and behavioural inhibition as candidate endophenotypic markers. *Neuroscience and Biobehavioral Reviews, 29*(3), 399-419. doi:10.1016/j.neubiorev.2004.11.006

Charman, T. (2003). Why is joint attention a pivotal skill in autism? *Philosophical Transactions of the Royal Society B: Biological Sciences, 358*(1430), 315-324. doi:10.1098/rstb.2002.1199

Charman, T., Young, G. S., Brian, J., Carter, A., Carver, L. J., Chawarska, K., Curtin, S., Dobkins, K., Elsabbagh, M., Georgiades, S., Hertz-Picciotto, I., Hutman, T., Iverson, J. M., Jones, E. J., Landa, R., Macari, S., Messinger, D. S., Nelson, C. A., Ozonoff, S., Saulnier, C., Stone, W. L., Tager-Flusberg, H., Webb, S. J., Yirmiya, N., & Zwaigenbaum, L. (2016). Non-asd outcomes at 36 months in siblings at familial risk for autism spectrum disorder (asd): A baby siblings research consortium (bsrc) study. *Autism Research*. doi:10.1002/aur.1669

Cheie, L., & Visu-Petra, L. (2012). Relating individual differences in trait-anxiety to memory functioning in young children. *Journal of Individual Differences, 33*(2), 109-118. doi:10.1027/1614-0001/a000079

Cheie, L., Visu-Petra, L., & Miclea, M. (2012). Trait anxiety, visual search and memory for facial identities in preschoolers: An investigation using taskirrelevant emotional information. *Procedia - Social and Behavioral Sciences, 33*, 622-626. doi:10.1016/j.sbspro.2012.01.196

Chorpita, B. F., Moffitt, C. E., & Gray, J. (2005). Psychometric properties of the revised child anxiety and depression scale in a clinical sample. *Behaviour Research and Therapy, 43*(3), 309-322. doi:10.1016/j.brat.2004.02.004

Choudhury, M. S., Pimentel, S. S., & Kendall, P. C. (2003). Childhood anxiety disorders: Parent-child (dis)agreement using a structured interview for the dsm-iv. *Journal of the American Academy of Child and Adolescent Psychiatry, 42*(8), 957-964. doi:10.1097/01.CHI.0000046898.27264.A2

Chuang, I. C., Tseng, M.-H., Lu, L., & Shieh, J.-Y. (2012). Sensory correlates of difficult temperament characteristics in preschool children with autism. *Research in Autism Spectrum Disorders, 6*(3), 988-995. doi:10.1016/j.rasd.2012.01.002

Cisler, J. M., Bacon, A. K., & Williams, N. L. (2009). Phenomenological characteristics of attentional biases towards threat: A critical review. *Cognitive Therapy and Research, 33*(2), 221-234. doi:10.1007/s10608-007-9161-y

Clark, D. A., & Beck, A. T. (2010). Cognitive theory and therapy of anxiety and depression: Convergence with neurobiological findings. *Trends in Cognitive Sciences, 14*(9), 418-424. doi:10.1016/j.tics.2010.06.007

Clauss, J. A., Cowan, R. L., & Blackford, J. U. (2011). Expectation and temperament moderate amygdala and dorsal anterior cingulate cortex responses to fear faces. *Cognitive, Affective & Behavioral Neuroscience, 11*(1), 13-21. doi:10.3758/s13415-010-0007-9

Clifford, S. M., Hudry, K., Elsabbagh, M., Charman, T., Johnson, M. H., & The BASIS Team. (2013). Temperament in the first 2 years of life in infants at high-risk for autism spectrum disorders. *Journal of Autism and Developmental Disorders, 43*(3), 673-686. doi:10.1007/s10803-012-1612-y

Cohen, J. (1973). Eta-squared and partial eta-squared in fixed factor anova designs. *Educational and Psychological Measurement, 33*(1), 107-112. doi:10.1177/001316447303300111

Cohen, L. J., Hollander, E., DeCaria, C. M., Stein, D. J., Simeon, D., Liebowitz, M. R., & Aronowitz, B. R. (1996). Specificity of neuropsychological impairment in obsessive-compulsive disorder: A comparison with social phobic and normal control subjects. *Journal of Neuropsychiatry and Clinical Neurosciences, 8*(1), 82-85. doi:10.1176/jnp.8.1.82

Cole, C. E., Zapp, D. J., Fettig, N. B., & Perez-Edgar, K. (2016). Impact of attention biases to threat and effortful control on individual variations in negative affect and social withdrawal in very young children. *Journal of Experimental Child Psychology, 141*, 210-221. doi:10.1016/j.jecp.2015.09.012

Constantino, J. N. (2012). *Social responsiveness scale, second edition (srs-2)*. Los Angeles, CA: Western Psychological Services.

Constantino, J. N., Zhang, Y., Frazier, T., Abbacchi, A. M., & Law, P. (2010). Sibling recurrence and the genetic epidemiology of autism. *The American Journal of Psychiatry, 167*(11), 1349-1356. doi:10.1176/appi.ajp.2010.09101470

Coombes, S. A., Higgins, T., Gamble, K. M., Cauraugh, J. H., & Janelle, C. M. (2009). Attentional control theory: Anxiety, emotion, and motor planning. *Journal of Anxiety Disorders, 23*(8), 1072-1079. doi:10.1016/j.janxdis.2009.07.009

Costello, E., Mustillo, S., Erkanli, A., Keeler, G., & Angold, A. (2003). Prevalence and development of psychiatric disorders in childhood and adolescence. *Archives of General Psychiatry, 60*(8), 837-844. doi:10.1001/archpsyc.60.8.837

Costello, E. J., Egger, H. L., & Angold, A. (2005). The developmental epidemiology of anxiety disorders: Phenomenology, prevalence, and comorbidity. *Child and Adolescent Psychiatric Clinics of North America, 14*(4), 631-648, vii. doi:10.1016/j.chc.2005.06.003

Cote, S. M., Boivin, M., Liu, X., Nagin, D. S., Zoccolillo, M., & Tremblay, R. E. (2009). Depression and anxiety symptoms: Onset, developmental course and risk factors during early childhood. *Journal of Child Psychology and Psychiatry and Allied Disciplines, 50*(10), 1201-1208. doi:10.1111/j.1469-7610.2009.02099.x

Cramer, S. C., Sur, M., Dobkin, B. H., O'Brien, C., Sanger, T. D., Trojanowski, J. Q., Rumsey, J. M., Hicks, R., Cameron, J., Chen, D., Chen, W. G., Cohen, L. G., deCharms, C., Duffy, C. J., Eden, G. F., Fetz, E. E., Filart, R., Freund, M., Grant, S. J., Haber, S., Kalivas, P. W., Kolb, B., Kramer, A. F., Lynch, M., Mayberg, H. S., McQuillen, P. S., Nitkin, R., Pascual-Leone, A., Reuter-Lorenz, P., Schiff, N., Sharma, A., Shekim, L., Stryker, M., Sullivan, E. V., & Vinogradov, S. (2011). Harnessing neuroplasticity for clinical applications. *Brain, 134*(6), 1591-1609. doi:10.1093/brain/awr039

Davis, T. E., Fodstad, J. C., Jenkins, W. S., Hess, J. A., Moree, B. N., Dempsey, T., & Matson, J. L. (2010). Anxiety and avoidance in infants and toddlers with autism spectrum disorders: Evidence for differing symptom severity and presentation. *Research in Autism Spectrum Disorders, 4*(2), 305-313. doi:10.1016/j.rasd.2009.10.002

Dawson, G., Meltzoff, A. N., Osterling, J., Rinaldi, J., & Brown, E. (1998). Children with autism fail to orient to naturally occurring social stimuli. *Journal of Autism and Developmental Disorders, 28*(6), 479-485.

de Bruin, E. I., Ferdinand, R. F., Meester, S., de Nijs, P. F., & Verheij, F. (2007). High rates of psychiatric co-morbidity in pdd-nos. *Journal of Autism and Developmental Disorders, 37*(5), 877-886. doi:10.1007/s10803-006-0215-x

De Giacomo, A., & Fombonne, E. (1998). Parental recognition of developmental abnormalities in autism. *European Child and Adolescent Psychiatry, 7*(3), 131-136.

De Pauw, S. S. W., & Mervielde, I. (2010). Temperament, personality and developmental psychopathology: A review based on the conceptual dimensions underlying childhood traits. *Child Psychiatry and Human Development, 41*(3), 313-329. doi:10.1007/s10578-009-0171-8

De Pauw, S. S. W., Mervielde, I., Van Leeuwen, K. G., & De Clercq, B. J. (2011). How temperament and personality contribute to the maladjustment of children with autism. *Journal of Autism and Developmental Disorders, 41*(2), 196-212. doi:10.1007/s10803-010-1043-6

DeCicco, J. M., O'Toole, L. J., & Dennis, T. A. (2014). The late positive potential as a neural signature for cognitive reappraisal in children. *Developmental Neuropsychology, 39*(7), 497-515. doi:10.1080/87565641.2014.959171

DeCicco, J. M., Solomon, B., & Dennis, T. A. (2012). Neural correlates of cognitive reappraisal in children: An erp study. *Developmental Cognitive Neuroscience, 2*(1), 70-80. doi:10.1016/j.dcn.2011.05.009

Degnan, K. A., & Fox, N. A. (2007). Behavioral inhibition and anxiety disorders: Multiple levels of a resilience process. *Development and Psychopathology, 19*(3), 729-746. doi:10.1017/S0954579407000363

Del Rosario, M., Gillespie-Lynch, K., Johnson, S., Sigman, M., & Hutman, T. (2014). Parent-reported temperament trajectories among infant siblings of children with autism. *Journal of Autism and Developmental Disorders, 44*(2), 381-393. doi:10.1007/s10803-013-1876-x

Dennis, T. A., & Hajcak, G. (2009). The late positive potential: A neurophysiological marker for emotion regulation in children. *Journal of Child Psychology and Psychiatry and Allied Disciplines, 50*(11), 1373-1383. doi:10.1111/j.1469-7610.2009.02168.x

Dodd, H. F., Hudson, J. L., Morris, T. M., & Wise, C. K. (2012). Interpretation bias in preschool children at risk for anxiety: A prospective study. *Journal of Abnormal Psychology, 121*(1), 28-38. doi:10.1037/a0024589

Dodd, H. F., Hudson, J. L., Williams, T., Morris, T., Lazarus, R. S., & Byrow, Y. (2015). Anxiety and attentional bias in preschool-aged children: An eyetracking

study. *Journal of Abnormal Child Psychology, 43*(6), 1055-1065. doi:10.1007/s10802-014-9962-x

Dodd, H. F., & Porter, M. A. (2011). There's that scary picture: Attention bias to threatening scenes in williams syndrome. *Neuropsychologia, 49*(2), 247-253. doi:10.1016/j.neuropsychologia.2010.11.023

Downs, S. H., & Black, N. (1998). The feasibility of creating a checklist for the assessment of the methodological quality both of randomised and non-randomised studies of health care interventions. *Journal of Epidemiology and Community Health, 52*(6), 377-384.

Drumm, E., Bryson, S., Zwaigenbaum, L., & Brian, J. (2015). Language-related abilities in 'unaffected' school-aged siblings of children with asd. *Research in Autism Spectrum Disorders, 18*, 83-96. doi:10.1016/j.rasd.2015.07.007

Dudeney, J., Sharpe, L., & Hunt, C. (2015). Attentional bias towards threatening stimuli in children with anxiety: A meta-analysis. *Clinical Psychology Review, 40*, 66-75. doi:10.1016/j.cpr.2015.05.007

Dykens, E. M. (2003). Anxiety, fears, and phobias in persons with williams syndrome. *Developmental Neuropsychology, 23*(1-2), 291-316. doi:10.1080/87565641.2003.9651896

Dyson, M. W., Klein, D. N., Olino, T. M., Dougherty, L. R., & Durbin, C. E. (2011). Social and non-social behavioral inhibition in preschool-age children: Differential associations with parent-reports of temperament and anxiety. *Child*

Psychiatry and Human Development, 42(4), 390-405. doi:10.1007/s10578-011-0225-6

Egger, H. L., & Angold, A. (2006). Common emotional and behavioral disorders in preschool children: Presentation, nosology, and epidemiology. *Journal of Child Psychology and Psychiatry, and Allied Disciplines, 47*(3-4), 313-337. doi:10.1111/j.1469-7610.2006.01618.x

Eisenberg, N., & Morris, A. S. (2002). Children's emotion-related regulation. In R. V. K. (Ed.) (Ed.), *Advances in child development and behavior*. Amsterdam/Boston: Academic Pres.

Eisenberg, N., Valiente, C., Spinrad, T. L., Liew, J., Zhou, Q., Losoya, S. H., Reiser, M., & Cumberland, A. (2009). Longitudinal relations of children's effortful control, impulsivity, and negative emotionality to their externalizing, internalizing, and co-occurring behavior problems. *Developmental Psychology, 45*(4), 988-1008. doi:10.1037/a0016213

Eley, T. C., Gregory, A. M., Lau, J. Y., McGuffin, P., Napolitano, M., Rijsdijk, F. V., & Clark, D. M. (2008). In the face of uncertainty: A twin study of ambiguous information, anxiety and depression in children. *Journal of Abnormal Child Psychology, 36*(1), 55-65. doi:10.1007/s10802-007-9159-7

Ellis, A. (1984). Expanding the abcs of ret. *Journal of Rational Emotive Therapy, 2*(2), 20-24. doi:10.1007/BF02281207

Elsabbagh, M., Fernandes, J., Jane Webb, S., Dawson, G., Charman, T., Johnson, M. H., & British Autism Study of Infant Siblings, T. (2013). Disengagement of

visual attention in infancy is associated with emerging autism in toddlerhood. *Biological Psychiatry, 74*(3), 189-194. doi:10.1016/j.biopsych.2012.11.030

Elsabbagh, M., Holmboe, K., Gliga, T., Mercure, E., Hudry, K., Charman, T., Baron-Cohen, S., Bolton, P., Johnson, M. H., & Team, B. (2011). Social and attention factors during infancy and the later emergence of autism characteristics. *Progress in Brain Research, 189*, 195-207. doi:10.1016/B978-0-444-53884-0.00025-7

Elsabbagh, M., & Johnson, M. H. (2010). Getting answers from babies about autism. *Trends in Cognitive Sciences, 14*. doi:10.1016/j.tics.2009.12.005

Elsabbagh, M., Mercure, E., Hudry, K., Chandler, S., Pasco, G., Charman, T., Pickles, A., Baron-Cohen, S., Bolton, P., Johnson, M. H., & Team, B. (2012). Infant neural sensitivity to dynamic eye gaze is associated with later emerging autism. *Current Biology, 22*(4), 338-342. doi:10.1016/j.cub.2011.12.056

Emde, R. N., Plomin, R., Robinson, J. A., Corley, R., DeFries, J., Fulker, D. W., Reznick, J. S., Campos, J., Kagan, J., & Zahn-Waxler, C. (1992). Temperament, emotion, and cognition at fourteen months: The macarthur longitudinal twin study. *Child Development, 63*(6), 1437-1455.

Etkin, A., & Wager, T. D. (2007). Functional neuroimaging of anxiety: A meta-analysis of emotional processing in ptsd, social anxiety disorder, and specific phobia. *American Journal of Psychiatry, 164*(10), 1476-1488. doi:10.1176/appi.ajp.2007.07030504

Euser, A. M., Zoccali, C., Jager, K. J., & Dekker, F. W. (2009). Cohort studies: Prospective versus retrospective. *Nephron Clinical Practice, 113*(3), c214-c217.

Evans, D. W., Canavera, K., Kleinpeter, F. L., Maccubbin, E., & Taga, K. (2005). The fears, phobias and anxieties of children with autism spectrum disorders and down syndrome: Comparisons with developmentally and chronologically age matched children. *Child Psychiatry and Human Development, 36*(1), 3-26. doi:10.1007/s10578-004-3619-x

Evans, D. W., Lewis, M. D., & Iobst, E. (2004). The role of the orbitofrontal cortex in normally developing compulsive-like behaviors and obsessive-compulsive disorder. *Brain and Cognition, 55*(1), 220-234. doi:10.1016/s0278-2626(03)00274-4

Eysenck, M. W. (1992). *Anxiety: The cognitive perspective*: Lawrence Erlbaum.

Eysenck, M. W., Derakshan, N., Santos, R., & Calvo, M. G. (2007). Anxiety and cognitive performance: Attentional control theory. *Emotion, 7*(2), 336-353. doi:10.1037/1528-3542.7.2.336

Farrugia, S., & Hudson, J. (2006). Anxiety in adolescents with asperger syndrome: Negative thoughts, behavioral problems, and life interference. *Focus on Autism and Other Developmental Disabilities, 21*(1), 25-35. doi:10.1177/10883576060210010401

Faul, F., Erdfelder, E., Buchner, A., & Lang, A. G. (2009). Statistical power analyses using g*power 3.1: Tests for correlation and regression analyses. *Behavior Research Methods, 41*(4), 1149-1160. doi:10.3758/brm.41.4.1149

Faul, F., Erdfelder, E., Lang, A. G., & Buchner, A. (2007). G*power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods, 39*(2), 175-191.

Ferraro, F. R. (2016). No evidence of reaction time slowing in autism spectrum disorder. *Autism, 20*(1), 116-122. doi:10.1177/1362361314559986

Field, A. P., & Lester, K. J. (2010). Is there room for 'development' in developmental models of information processing biases to threat in children and adolescents? *Clinical Child and Family Psychology Review, 13*(4), 315-332. doi:10.1007/s10567-010-0078-8

Fischer, R., & Milfont, T. L. (2015). Standardization in psychological research. *International Journal of Psychological Research, 3*(1), 88-96.

Fisman, S., Wolf, L., Ellison, D., Gillis, B., Freeman, T. O. M., & Szatmari, P. (1996). Risk and protective factors affecting the adjustment of siblings of children with chronic disabilities. *Journal of the American Academy of Child and Adolescent Psychiatry, 35*(11), 1532-1541. doi:10.1097/00004583-199611000-00023

Foley, D. L., Rutter, M., Angold, A., Pickles, A., Maes, H. M., Silberg, J. L., & Eaves, L. J. (2005). Making sense of informant disagreement for overanxious disorder. *Journal of Anxiety Disorders, 19*(2), 193-210. doi:10.1016/j.janxdis.2004.01.006

Fombonne, E., Quirke, S., & Hagen, A. (2011). *Epidemiology of pervasive developmental disorders*. New York, NY: Oxford University Press.

Forster, S., Nunez Elizalde, A. O., Castle, E., & Bishop, S. J. (2015). Unraveling the anxious mind: Anxiety, worry, and frontal engagement in sustained attention versus off-task processing. *Cerebral Cortex, 25*(3), 609-618. doi:10.1093/cercor/bht248

Fox, E., Russo, R., Bowles, R., & Dutton, K. (2001). Do threatening stimuli draw or hold visual attention in subclinical anxiety? *Journal of Experimental Psychology: General, 130*(4), 681-700. doi:10.1037/0096-3445.130.4.681

Fox, N. A., Henderson, H. A., Marshall, P. J., Nichols, K. E., & Ghera, M. M. (2005). Behavioral inhibition: Linking biology and behavior within a developmental framework. *Annual Review of Psychology, 56*, 235-262. doi:10.1146/annurev.psych.55.090902.141532

Fox, N. A., Henderson, H. A., Rubin, K. H., Calkins, S. D., & Schmidt, L. A. (2001). Continuity and discontinuity of behavioral inhibition and exuberance: Psychophysiological and behavioral influences across the first four years of life. *Child Development, 72*(1), 1-21. doi:10.1111/1467-8624.00262

Fox, N. A., & Pine, D. S. (2012). Temperament and the emergence of anxiety disorders. *Journal of the American Academy of Child and Adolescent Psychiatry, 51*(2), 125-128. doi:10.1016/j.jaac.2011.10.006

Franz, L., Angold, A., Copeland, W., Costello, E. J., Towe-Goodman, N., & Egger, H. (2013). Preschool anxiety disorders in pediatric primary care: Prevalence and comorbidity. *Journal of the American Academy of Child and Adolescent Psychiatry, 52*(12), 1294-1303 e1291. doi:10.1016/j.jaac.2013.09.008

Fujii, Y., Kitagawa, N., Shimizu, Y., Mitsui, N., Toyomaki, A., Hashimoto, N., Kako, Y., Tanaka, T., Asakura, S., Koyama, T., & Kusumi, I. (2013). Severity of generalized social anxiety disorder correlates with low executive functioning. *Neuroscience Letters, 543*, 42-46. doi:10.1016/j.neulet.2013.02.059

Fulcher, E. P., Mathews, A., & Hammerl, M. (2008). Rapid acquisition of emotional information and attentional bias in anxious children. *Journal of Behavior Therapy and Experimental Psychiatry, 39*(3), 321-339. doi:10.1016/j.jbtep.2007.08.003

Gadow, K., & Sprafkin, J. (2002). *Child symptom inventory - 4 screening and norms manual*. Stony Brook, NY: Checkmate Plus.

Gaffrey, M. S., Barch, D. M., & Luby, J. L. (2016). Amygdala reactivity to sad faces in preschool children: An early neural marker of persistent negative affect. *Developmental Cognitive Neuroscience, 17*, 94-100. doi:10.1016/j.dcn.2015.12.015

Gaigg, S. B. (2012). The interplay between emotion and cognition in autism spectrum disorder: Implications for developmental theory. *Frontiers in Integrative Neuroscience, 6*, 113. doi:10.3389/fnint.2012.00113

Gaigg, S. B., & Bowler, D. M. (2007). Differential fear conditioning in asperger's syndrome: Implications for an amygdala theory of autism. *Neuropsychologia, 45*(9), 2125-2134. doi:10.1016/j.neuropsychologia.2007.01.012

Gamble, A. L., & Rapee, R. M. (2009). The time-course of attentional bias in anxious children and adolescents. *Journal of Anxiety Disorders, 23*(7), 841-847. doi:10.1016/j.janxdis.2009.04.001

Gamliel, I., Yirmiya, N., Jaffe, D. H., Manor, O., & Sigman, M. (2009). Developmental trajectories in siblings of children with autism: Cognition and language from 4 months to 7 years. *Journal of Autism and Developmental Disorders, 39*(8), 1131-1144. doi:10.1007/s10803-009-0727-2

Gammer, I., Bedford, R., Elsabbagh, M., Garwood, H., Pasco, G., & Tucker, L. (2015). Behavioural markers for autism in infancy: Scores on the autism observational scale for infants in a prospective study of at-risk siblings. *Infant Behavior & Development, 38*. doi:10.1016/j.infbeh.2014.12.017

Garaas, T. W., & Pomplun, M. (2008). Inspection time and visual–perceptual processing. *Vision Research, 48*(4), 523-537. doi:10.1016/j.visres.2007.11.011

Garon, N., Bryson, S. E., Zwaigenbaum, L., Smith, I. M., Brian, J., Roberts, W., & Szatmari, P. (2009). Temperament and its relationship to autistic symptoms in a high-risk infant sib cohort. *Journal of Abnormal Child Psychology, 37*(1), 59-78. doi:10.1007/s10802-008-9258-0

Garon, N., Zwaigenbaum, L., Bryson, S., Smith, I. M., Brian, J., Roncadin, C., Vaillancourt, T., Armstrong, V., Sacrey, L. A., & Roberts, W. (2016). Temperament and its association with autism symptoms in a high-risk population. *Journal of Abnormal Child Psychology, 44*(4), 757-769. doi:10.1007/s10802-015-0064-1

Gartstein, M. A., Bridgett, D. J., Rothbart, M. K., Robertson, C., Iddins, E., Ramsay, K., & Schlect, S. (2010). A latent growth examination of fear development in infancy: Contributions of maternal depression and the risk for toddler anxiety. *Developmental Psychology, 46*(3), 651-668. doi:10.1037/a0018898

Gartstein, M. A., & Rothbart, M. K. (2003). Studying infant temperament via the revised infant behavior questionnaire. *Infant Behavior and Development, 26*(1), 64-86. doi:10.1016/S0163-6383(02)00169-8

Gillberg, C., Ehlers, S., Schaumann, H., Jakobsson, G., Dahlgren, S. O., Lindblom, R., Bagenholm, A., Tjuus, T., & Blidner, E. (1990). Autism under age 3 years: A clinical study of 28 cases referred for autistic symptoms in infancy. *Journal of Child Psychology and Psychiatry and Allied Disciplines, 31*(6), 921-934.

Gillberg, I. C., Billstedt, E., Wentz, E., Anckarsater, H., Rastam, M., & Gillberg, C. (2010). Attention, executive functions, and mentalizing in anorexia nervosa eighteen years after onset of eating disorder. *Journal of Clinical and Experimental Neuropsychology, 32*(4), 358-365. doi:10.1080/13803390903066857

Gillott, A., Furniss, F., & Walter, A. (2001). Anxiety in high-functioning children with autism. *Autism, 5*(3), 277-286.

Gillott, A., & Standen, P. J. (2007). Levels of anxiety and sources of stress in adults with autism. *Journal of Intellectual Disabilities, 11*(4), 359-370.

Gjone, H., & Stevenson, J. (1997). A longitudinal twin study of temperament and behavior problems: Common genetic or environmental influences? *Journal of*

the American Academy of Child and Adolescent Psychiatry, 36(10), 1448-1456. doi:10.1097/00004583-199710000-00028

Gliga, T., Bedford, R., Charman, T., & Johnson, M. H. (2015). Enhanced visual search in infancy predicts emerging autism symptoms. *Current Biology, 25*(13), 1727-1730. doi:10.1016/j.cub.2015.05.011

Gotham, K., Pickles, A., & Lord, C. (2009). Standardizing ados scores for a measure of severity in autism spectrum disorders. *Journal of Autism and Developmental Disorders, 39*(5), 693-705. doi:10.1007/s10803-008-0674-3

Green, J., Charman, T., Pickles, A., Wan, M. W., Elsabbagh, M., Slonims, V., Taylor, C., McNally, J., Booth, R., Gliga, T., Jones, E. J. H., Harrop, C., Bedford, R., & Johnson, M. H. (2015). Parent-mediated intervention versus no intervention for infants at high risk of autism: A parallel, single-blind, randomised trial. *The Lancet Psychiatry, 2*(2), 133-140. doi:10.1016/S2215-0366(14)00091-1

Green, S. A., & Ben-Sasson, A. (2010). Anxiety disorders and sensory over-responsivity in children with autism spectrum disorders: Is there a causal relationship? *Journal of Autism and Developmental Disorders, 40*(12), 1495-1504. doi:10.1007/s10803-010-1007-x

Green, S. A., Ben-Sasson, A., Soto, T. W., & Carter, A. S. (2012). Anxiety and sensory over-responsivity in toddlers with autism spectrum disorders: Bidirectional effects across time. *Journal of Autism and Developmental Disorders, 42*(6), 1112-1119. doi:10.1007/s10803-011-1361-3

Grills, A. E., & Ollendick, T. H. (2003). Multiple informant agreement and the anxiety disorders interview schedule for parents and children. *Journal of the American Academy of Child and Adolescent Psychiatry, 42*(1), 30-40. doi:10.1097/00004583-200301000-00008

Grondhuis, S. N., & Aman, M. G. (2012). Assessment of anxiety in children and adolescents with autism spectrum disorders. *Research in Autism Spectrum Disorders, 6*(4), 1345-1365. doi:10.1016/j.rasd.2012.04.006

Guiraud, J., Kushnerenko, E., Tomalski, P., Davies, K., Ribeiro, H., Johnson, M. H., & The BASIS Team. (2011). Differential habituation to repeated sounds in infants at high risk for autism. *Neuroreport, 22*(16), 845-849. doi:10.1097/WNR.0b013e32834c0bec

Hadwin, J. A., & Field, A. P. (2010). An introduction to the study of information processing biases in childhood anxiety: Theoretical and methodological issues *Information processing biases and anxiety* (pp. 1-17): John Wiley & Sons, Ltd.

Hadwin, J. A., Garner, M., & Perez-Olivas, G. (2006). The development of information processing biases in childhood anxiety: A review and exploration of its origins in parenting. *Clinical Psychology Review, 26*(7), 876-894. doi:10.1016/j.cpr.2005.09.004

Hajcak, G., & Dennis, T. A. (2009). Brain potentials during affective picture processing in children. *Biological Psychology, 80*(3), 333-338. doi:10.1016/j.biopsycho.2008.11.006

Hallett, V., Lecavalier, L., Sukhodolsky, D. G., Cipriano, N., Aman, M. G., McCracken, J. T., McDougle, C. J., Tierney, E., King, B. H., Hollander, E., Sikich, L., Bregman, J., Anagnostou, E., Donnelly, C., Katsovich, L., Dukes, K., Vitiello, B., Gadow, K., & Scahill, L. (2013). Exploring the manifestations of anxiety in children with autism spectrum disorders. *Journal of Autism and Developmental Disorders, 43*(10), 2341-2352. doi:10.1007/s10803-013-1775-1

Hallett, V., Ronald, A., Colvert, E., Ames, C., Woodhouse, E., Lietz, S., Garnett, T., Gillan, N., Rijsdijk, F., Scahill, L., Bolton, P., & Happé, F. (2013). Exploring anxiety symptoms in a large-scale twin study of children with autism spectrum disorders, their co-twins and controls. *Journal of Child Psychology and Psychiatry, 54*(11), 1176-1185. doi:10.1111/jcpp.12068

Hallett, V., Ronald, A., & Happé, F. (2009). Investigating the association between autistic-like and internalizing traits in a community-based twin sample. *Journal of the American Academy of Child and Adolescent Psychiatry, 48*(6), 618-627. doi:10.1097/CHI.0b013e31819f7116

Hallett, V., Ronald, A., Rijsdijk, F., & Happé, F. (2010). Association of autistic-like and internalizing traits during childhood: A longitudinal twin study. *American Journal of Psychiatry, 167*(7), 809-817. doi:10.1176/appi.ajp.2009.09070990

Hallmayer, J., Cleveland, S., Torres, A., & et al. (2011). Genetic heritability and shared environmental factors among twin pairs with autism. *Archives of General Psychiatry, 68*(11), 1095-1102. doi:10.1001/archgenpsychiatry.2011.76

Hamblin, R. J., Salloum, A., Andel, R., Nadeau, J. M., McBride, N. M., Lewin, A. B., & Storch, E. A. (2016). Predictors of parent -child agreement on child anxiety

diagnoses on the adis-iv-c/p. *Psychiatry Research*. doi:10.1016/j.psychres.2016.07.041

Happe, F., Ronald, A., & Plomin, R. (2006). Time to give up on a single explanation for autism. *Nature Neuroscience, 9*(10), 1218-1220. doi:10.1038/nn1770

Harms, M. B., Martin, A., & Wallace, G. L. (2010). Facial emotion recognition in autism spectrum disorders: A review of behavioral and neuroimaging studies. *Neuropsychology Review, 20*(3), 290-322. doi:10.1007/s11065-010-9138-6

Harsanyi, A., Csigo, K., Rajkai, C., Demeter, G., Nemeth, A., & Racsmany, M. (2014). Two types of impairments in ocd: Obsessions, as problems of thought suppression; compulsions, as behavioral-executive impairment. *Psychiatry Research, 215*(3), 651-658. doi:10.1016/j.psychres.2013.11.014

Hartley, S. L., & Sikora, D. M. (2009). Sex differences in autism spectrum disorder: An examination of developmental functioning, autistic symptoms, and coexisting behavior problems in toddlers. *Journal of Autism and Developmental Disorders, 39*(12), 1715-1722. doi:10.1007/s10803-009-0810-8

Hastings, R. P., & Petalas, M. A. (2014). Self-reported behaviour problems and sibling relationship quality by siblings of children with autism spectrum disorder. *Child: Care, Health and Development, 40*(6), 833-839. doi:10.1111/cch.12131

Herrington, J. D., Miller, J. S., Pandey, J., & Schultz, R. T. (2016). Anxiety and social deficits have distinct relationships with amygdala function in autism spectrum disorder. *Social Cognitive and Affective Neuroscience, 11*(6), 907-914.

Hezel, D. M., & McNally, R. J. (2014). Theory of mind impairments in social anxiety disorder. *Behavior Therapy, 45*(4), 530-540. doi:10.1016/j.beth.2014.02.010

Hill, E. L. (2004). Executive dysfunction in autism. *Trends in Cognitive Sciences, 8*(1), 26-32. doi:10.1016/j.tics.2003.11.003

Hirshfeld-Becker, D. R., Micco, J. A., Mazursky, H., Bruett, L., & Henin, A. (2011). Applying cognitive-behavioral therapy for anxiety to the younger child. *Child and Adolescent Psychiatric Clinics of North America, 20*(2), 349-368. doi:10.1016/j.chc.2011.01.008

Hodge, D., Hoffman, C. D., & Sweeney, D. P. (2011). Increased psychopathology in parents of children with autism: Genetic liability or burden of caregiving? *Journal of Developmental and Physical Disabilities, 23*(3), 227-239. doi:10.1007/s10882-010-9218-9

Hofmann, S. G., & Smits, J. A. J. (2008). Cognitive-behavioral therapy for adult anxiety disorders: A meta-analysis of randomized placebo-controlled trials. *The Journal of clinical psychiatry, 69*(4), 621-632.

Hollocks, M. J., Howlin, P., Papadopoulos, A. S., Khondoker, M., & Simonoff, E. (2014). Differences in hpa-axis and heart rate responsiveness to psychosocial stress in children with autism spectrum disorders with and without co-morbid anxiety. *Psychoneuroendocrinology, 46*, 32-45. doi:10.1016/j.psyneuen.2014.04.004

Hollocks, M. J., Jones, C. R., Pickles, A., Baird, G., Happé, F., Charman, T., & Simonoff, E. (2014). The association between social cognition and executive

functioning and symptoms of anxiety and depression in adolescents with autism spectrum disorders. *Autism Research, 7*(2), 216-228. doi:10.1002/aur.1361

Hollocks, M. J., Ozsivadjian, A., Matthews, C. E., Howlin, P., & Simonoff, E. (2013). The relationship between attentional bias and anxiety in children and adolescents with autism spectrum disorders. *Autism Research, 6*(4), 237-247. doi:10.1002/aur.1285

Hollocks, M. J., Pickles, A., Howlin, P., & Simonoff, E. (2016). Dual cognitive and biological correlates of anxiety in autism spectrum disorders. *Journal of Autism and Developmental Disorders*. doi:10.1007/s10803-016-2878-2

Hoogenhout, M., & Malcolm-Smith, S. (2014). Theory of mind in autism spectrum disorder: Does dsm classification predict development? *Research in Autism Spectrum Disorders, 8*(6), 597-607. doi:10.1016/j.rasd.2014.02.005

Hughes, C., Plumet, M. H., & Leboyer, M. (1999). Towards a cognitive phenotype for autism: Increased prevalence of executive dysfunction and superior spatial span amongst siblings of children with autism. *Journal of Child Psychology and Psychiatry and Allied Disciplines, 40*(5), 705-718.

Hus, V., Gotham, K., & Lord, C. (2014). Standardizing ados domain scores: Separating severity of social affect and restricted and repetitive behaviors. *Journal of Autism and Developmental Disorders, 44*(10), 2400-2412. doi:10.1007/s10803-012-1719-1

IBM Corp. (2011). *Ibm spss statistics for windows, version 20.0*. Armonk, NY: IBM Corp. .

Iida, Y., Miyazaki, M., & Uchida, S. (2010). Developmental changes in cognitive reaction time of children aged 6–12 years. *European Journal of Sport Science, 10*(3), 151-158. doi:10.1080/17461390903515162

Ingram, R. E., & Luxton, D. D. (2005). Vulnerability-stress models. In B. L. Hankin & J. R. Z. Abela (Eds.), *Development of psychopathology: A vulnerability-stress perspective*. Thousand Oaks, CA: Sage Publications.

Iossifov, I., Ronemus, M., Levy, D., Wang, Z., Hakker, I., Rosenbaum, J., Yamrom, B., Lee, Y.-h., Narzisi, G., Leotta, A., Kendall, J., Grabowska, E., Ma, B., Marks, S., Rodgers, L., Stepansky, A., Troge, J., Andrews, P., Bekritsky, M., Pradhan, K., Ghiban, E., Kramer, M., Parla, J., Demeter, R., Fulton, Lucinda L., Fulton, Robert S., Magrini, Vincent J., Ye, K., Darnell, Jennifer C., Darnell, Robert B., Mardis, Elaine R., Wilson, Richard K., Schatz, Michael C., McCombie, W. R., & Wigler, M. (2012). De novo gene disruptions in children on the autistic spectrum. *Neuron, 74*(2), 285-299. doi:10.1016/j.neuron.2012.04.009

Isomura, T., Ogawa, S., Shibasaki, M., & Masataka, N. (2015). Delayed disengagement of attention from snakes in children with autism. *Frontiers in Psychology, 6*(241), 1-5. doi:10.3389/fpsyg.2015.00241

James, A. C., James, G., Cowdrey, F. A., Soler, A., & Choke, A. (2013). Cognitive behavioural therapy for anxiety disorders in children and adolescents. *Cochrane Database Syst Rev*(6), Cd004690. doi:10.1002/14651858.CD004690.pub3

Jensen, S. A., Fabiano, G. A., Lopez-Williams, A., & Chacko, A. (2006). The reading grade level of common measures in child and adolescent clinical psychology. *Psychological Assessment, 18*(3), 346-352. doi:10.1037/1040-3590.18.3.346

Jeste, S. S., & Geschwind, D. H. (2014). Disentangling the heterogeneity of autism spectrum disorder through genetic findings. *Nature Reviews: Neurology, 10*(2), 74-81. doi:10.1038/nrneurol.2013.278

Jones, E. J., Gliga, T., Bedford, R., Charman, T., & Johnson, M. H. (2014). Developmental pathways to autism: A review of prospective studies of infants at risk. *Neuroscience and Biobehavioral Reviews, 39*, 1-33. doi:10.1016/j.neubiorev.2013.12.001

Joseph, R. M., Keehn, B., Connolly, C., Wolfe, J. M., & Horowitz, T. S. (2009). Why is visual search superior in autism spectrum disorder? *Dev Sci, 12*(6), 1083-1096. doi:10.1111/j.1467-7687.2009.00855.x

Joshi, G., Wozniak, J., Petty, C., Martelon, M. K., Fried, R., Bolfek, A., Kotte, A., Stevens, J., Furtak, S. L., Bourgeois, M., Caruso, J., Caron, A., & Biederman, J. (2013). Psychiatric comorbidity and functioning in a clinically referred population of adults with autism spectrum disorders: A comparative study. *Journal of Autism and Developmental Disorders, 43*(6), 1314-1325. doi:10.1007/s10803-012-1679-5

Kaat, A. J., & Lecavalier, L. (2015). Reliability and validity of parent- and child-rated anxiety measures in autism spectrum disorder. *Journal of Autism and Developmental Disorders, 45*(10), 3219-3231. doi:10.1007/s10803-015-2481-y

Kagan, J., Reznick, J. S., & Snidman, N. (1987). The physiology and psychology of behavioral inhibition in children. *Child Development, 58*(6), 1459-1473.

Kagan, J., Snidman, N., Zentner, M., & Peterson, E. (1999). Infant temperament and anxious symptoms in school age children. *Development and Psychopathology, 11*(2), 209-224.

Kaminsky, L., & Dewey, D. (2002). Psychosocial adjustment in siblings of children with autism. *Journal of Child Psychology and Psychiatry and Allied Disciplines, 43*(2), 225-232.

Kanner, L. (1943). Autistic disturbances of affective contact.

Karalunas, S. L., Geurts, H. M., Konrad, K., Bender, S., & Nigg, J. T. (2014). Annual research review: Reaction time variability in adhd and autism spectrum disorders: Measurement and mechanisms of a proposed trans-diagnostic phenotype. *Journal of Child Psychology and Psychiatry and Allied Disciplines, 55*(6), 685-710. doi:10.1111/jcpp.12217

Karevold, E., Roysamb, E., Ystrom, E., & Mathiesen, K. S. (2009). Predictors and pathways from infancy to symptoms of anxiety and depression in early adolescence. *Developmental Psychology, 45*(4), 1051-1060. doi:10.1037/a0016123

Kennedy, S. J., Rapee, R. M., & Edwards, S. L. (2009). A selective intervention program for inhibited preschool-aged children of parents with an anxiety disorder: Effects on current anxiety disorders and temperament. *Journal of the American Academy of Child and Adolescent Psychiatry, 48*(6), 602-609. doi:10.1097/CHI.0b013e31819f6fa9

Kerekes, N., Brandstrom, S., Lundstrom, S., Rastam, M., Nilsson, T., & Anckarsater, H. (2013). Adhd, autism spectrum disorder, temperament, and character: Phenotypical associations and etiology in a swedish childhood twin study. *Comprehensive Psychiatry, 54*(8), 1140-1147. doi:10.1016/j.comppsych.2013.05.009

Kerns, C. M., & Kendall, P. C. (2012). The presentation and classification of anxiety in autism spectrum disorder. *Clinical Psychology: Science and Practice, 19*(4), 323-347. doi:10.1111/cpsp.12009

Kerns, C. M., Kendall, P. C., Berry, L., Souders, M. C., Franklin, M. E., Schultz, R. T., Miller, J., & Herrington, J. (2014). Traditional and atypical presentations of anxiety in youth with autism spectrum disorder. *Journal of Autism and Developmental Disorders, 44*(11), 2851-2861. doi:10.1007/s10803-014-2141-7

Kessler, R. C., Berglund, P., Demler, O., Jin, R., Merikangas, K. R., & Walters, E. E. (2005). Lifetime prevalence and age-of-onset distributions of dsm-iv disorders in the national comorbidity survey replication. *Archives of General Psychiatry, 62*(6), 593-602. doi:10.1001/archpsyc.62.6.593

Kessler, R. C., Chiu, W., Demler, O., & Walters, E. E. (2005). Prevalence, severity, and comorbidity of 12-month dsm-iv disorders in the national comorbidity survey replication. *Archives of General Psychiatry, 62*(6), 617-627. doi:10.1001/archpsyc.62.6.617

Klein, R. G. (1991). Parent-child agreement in clinical assessment of anxiety and other psychopathology: A review. *Journal of Anxiety Disorders, 5*(2), 187-198. doi:10.1016/0887-6185(91)90028-R

Kleinhans, N. M., Reiter, M. A., Neuhaus, E., Pauley, G., Martin, N., Dager, S., & Estes, A. (2016). Subregional differences in intrinsic amygdala hyperconnectivity and hypoconnectivity in autism spectrum disorder. *Autism Research, 9*(7), 760-772. doi:10.1002/aur.1589

Kleinhans, N. M., Richards, T., Weaver, K., Johnson, L. C., Greenson, J., Dawson, G., & Aylward, E. (2010). Association between amygdala response to emotional faces and social anxiety in autism spectrum disorders. *Neuropsychologia, 48*(12), 3665-3670. doi:10.1016/j.neuropsychologia.2010.07.022

Kochanska, G., Murray, K. T., & Harlan, E. T. (2000). Effortful control in early childhood: Continuity and change, antecedents, and implications for social development. *Developmental Psychology, 36*(2), 220-232.

Koster, E. H., Crombez, G., Verschuere, B., Van Damme, S., & Wiersema, J. R. (2006). Components of attentional bias to threat in high trait anxiety: Facilitated engagement, impaired disengagement, and attentional avoidance. *Behaviour Research and Therapy, 44*(12), 1757-1771. doi:10.1016/j.brat.2005.12.011

Koster, E. H., Verschuere, B., Crombez, G., & Van Damme, S. (2005). Time-course of attention for threatening pictures in high and low trait anxiety. *Behaviour Research and Therapy, 43*(8), 1087-1098. doi:10.1016/j.brat.2004.08.004

Lahat, A., Lamm, C., Chronis-Tuscano, A., Pine, D. S., Henderson, H. A., & Fox, N. A. (2014). Early behavioral inhibition and increased error monitoring predict later social phobia symptoms in childhood. *Journal of the American Academy of Child and Adolescent Psychiatry, 53*(4), 447-455. doi:10.1016/j.jaac.2013.12.019

Lai, M.-C., Lombardo, M. V., & Baron-Cohen, S. (2013). Autism. *The Lancet, 383*(9920), 896-910. doi:http://dx.doi.org/10.1016/S0140-6736(13)61539-1

Lai, M.-C., Lombardo, M. V., Chakrabarti, B., & Baron-Cohen, S. (2013). Subgrouping the autism “spectrum": Reflections on dsm-5. *PLoS Biology, 11*(4), e1001544. doi:10.1371/journal.pbio.1001544

Lai, M.-C., Lombardo, M. V., Pasco, G., Ruigrok, A. N., Wheelwright, S. J., Sadek, S. A., & Baron-Cohen, S. (2011). A behavioral comparison of male and female adults with high functioning autism spectrum conditions. *PloS One, 6*. doi:10.1371/journal.pone.0020835

Lai, M.-C., Lombardo, M. V., Pasco, G., Ruigrok, A. N. V., Wheelwright, S. J., Sadek, S. A., Chakrabarti, B., Baron-Cohen, S., & Consortium, M. A. (2011). A behavioral comparison of male and female adults with high functioning autism spectrum conditions. *PloS One, 6*(6), e20835. doi:10.1371/journal.pone.0020835

Lai, M. C., Lombardo, M. V., Auyeung, B., Chakrabarti, B., & Baron-Cohen, S. (2015). Sex/gender differences and autism: Setting the scene for future research. *Journal of the American Academy of Child and Adolescent Psychiatry, 54*(1), 11-24. doi:10.1016/j.jaac.2014.10.003

Lainhart, J. E. (2009). Psychiatric problems in individuals with autism, their parents and siblings. *International Review of Psychiatry, 11*(4), 278-298. doi:10.1080/09540269974177

Landry, O., & Parker, A. (2013). A meta-analysis of visual orienting in autism. *Frontiers in Human Neuroscience, 7*, 833. doi:10.3389/fnhum.2013.00833

Landry, R., & Bryson, S. E. (2004). Impaired disengagement of attention in young children with autism. *Journal of Child Psychology and Psychiatry and Allied Disciplines, 45*(6), 1115-1122. doi:10.1111/j.1469-7610.2004.00304.x

Lang, P., Bradley, M., & Cuthbert, B. N. (2008). *International affective picture system (iaps): Affective ratings of pictures and instruction manual. Technical report a-8.* University of Florida: Gainesville, FL.

Lange-Küttner, C. (2012). The importance of reaction times for developmental science: What a difference milliseconds make. *International Journal of Developmental Science, 6*, 51-55. doi:10.3233/dev-2012-11089

Langley, A. K., Bergman, R. L., McCracken, J., & Piacentini, J. C. (2004). Impairment in childhood anxiety disorders: Preliminary examination of the child anxiety impact scale-parent version. *Journal of Child and Adolescent Psychopharmacology, 14*(1), 105-114. doi:10.1089/104454604773840544

Last, C. G., Perrin, S., Hersen, M., & Kazdin, A. E. (1996). A prospective study of childhood anxiety disorders. *Journal of the American Academy of Child and Adolescent Psychiatry, 35*(11), 1502-1510. doi:10.1097/00004583-199611000-00019

Lau, J. Y. (2013). Cognitive bias modification of interpretations: A viable treatment for child and adolescent anxiety? *Behaviour Research and Therapy, 51*(10), 614-622. doi:10.1016/j.brat.2013.07.001

Lautenbacher, S., Spernal, J., & Krieg, J. C. (2002). Divided and selective attention in panic disorder. A comparative study of patients with panic disorder, major depression and healthy controls. *European Archives of Psychiatry and Clinical Neuroscience, 252*(5), 210-213. doi:10.1007/s00406-002-0382-5

Lecavalier, L., Wood, J. J., Halladay, A. K., Jones, N. E., Aman, M. G., Cook, E. H., Handen, B. L., King, B. H., Pearson, D. A., Hallett, V., Sullivan, K. A., Grondhuis, S., Bishop, S. L., Horrigan, J. P., Dawson, G., & Scahill, L. (2014). Measuring anxiety as a treatment endpoint in youth with autism spectrum disorder. *Journal of Autism and Developmental Disorders, 44*(5), 1128-1143. doi:10.1007/s10803-013-1974-9

LeDoux, J. E. (2000). Emotion circuits in the brain. *Annual Review of Neuroscience, 23*, 155-184. doi:10.1146/annurev.neuro.23.1.155

Leekam, S. R., Lopez, B., & Moore, C. (2000). Attention and joint attention in preschool children with autism. *Developmental Psychology, 36*(2), 261-273.

Leekam, S. R., Prior, M. R., & Uljarevic, M. (2011). Restricted and repetitive behaviors in autism spectrum disorders: A review of research in the last decade. *Psychological Bulletin, 137*(4), 562-593. doi:10.1037/a0023341

Leyfer, O. T., Folstein, S. E., Bacalman, S., Davis, N. O., Dinh, E., Morgan, J., Tager-Flusberg, H., & Lainhart, J. E. (2006). Comorbid psychiatric disorders in children with autism: Interview development and rates of disorders. *Journal of Autism and Developmental Disorders, 36*(7), 849-861. doi:10.1007/s10803-006-0123-0

Liang, K.-Y., & Zeger, S. L. (1986). Longitudinal data analysis using generalized linear models. *Biometrika, 73*(1), 13-22.

Lidstone, J., Uljarević, M., Sullivan, J., Rodgers, J., McConachie, H., Freeston, M., Le Couteur, A., Prior, M., & Leekam, S. (2014). Relations among restricted and repetitive behaviors, anxiety and sensory features in children with autism spectrum disorders. *Research in Autism Spectrum Disorders, 8*(2), 82-92. doi:10.1016/j.rasd.2013.10.001

Liss, M., Saulnier, C., Fein, D., & Kinsbourne, M. (2006). Sensory and attention abnormalities in autistic spectrum disorders. *Autism, 10*(2), 155-172. doi:10.1177/1362361306062021

LoBue, V., & DeLoache, J. S. (2008). Detecting the snake in the grass: Attention to fear-relevant stimuli by adults and young children. *Psychological Science, 19*(3), 284-289.

LoBue, V., & Perez-Edgar, K. (2014). Sensitivity to social and non-social threats in temperamentally shy children at-risk for anxiety. *Dev Sci, 17*(2), 239-247. doi:10.1111/desc.12110

Lonigan, C. J., & Vasey, M. W. (2009). Negative affectivity, effortful control, and attention to threat-relevant stimuli. *Journal of Abnormal Child Psychology, 37*(3), 387-399. doi:10.1007/s10802-008-9284-y

Lonigan, C. J., Vasey, M. W., Phillips, B. M., & Hazen, R. A. (2004). Temperament, anxiety, and the processing of threat-relevant stimuli. *Journal of Clinical Child and Adolescent Psychology, 33*(1), 8-20. doi:10.1207/S15374424JCCP3301_2

Lord, C., Risi, S., Lambrecht, L., Cook, E. H., Jr., Leventhal, B. L., DiLavore, P. C., Pickles, A., & Rutter, M. (2000). The autism diagnostic observation schedule-generic: A standard measure of social and communication deficits associated with the spectrum of autism. *Journal of Autism and Developmental Disorders, 30*(3), 205-223. doi:10.1177/1362361310379241

Lord, C., Rutter, M., & Couteur, A. (1994). Autism diagnostic interview-revised: A revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. *Journal of Autism and Developmental Disorders, 24*. doi:10.1007/BF02172145

Lord, C., Rutter, M., DiLavore, P. C., Risi, S., Gotham, K., & Bishop, S. L. (2012). *Autism diagnostic observation schedule: Ados-2*. Los Angeles, CA: Western Psychological Services.

Luby, J. L., Belden, A., Sullivan, J., & Spitznagel, E. (2007). Preschoolers' contribution to their diagnosis of depression and anxiety: Uses and limitations of young child self-report of symptoms. *Child Psychiatry and Human Development, 38*(4), 321-338. doi:10.1007/s10578-007-0063-8

MacLeod, C., & Mathews, A. (1988). Anxiety and the allocation of attention to threat. *The Quarterly Journal of Experimental Psychology Section A, 40*(4), 653-670. doi:10.1080/14640748808402292

MacLeod, C., & Mathews, A. (2012). Cognitive bias modification approaches to anxiety. *Annual Review of Clinical Psychology, 8*, 189-217. doi:10.1146/annurev-clinpsy-032511-143052

MacLeod, C., Mathews, A., & Tata, P. (1986). Attentional bias in emotional disorders. *Journal of Abnormal Psychology, 95*(1), 15-20.

Maes, J. H. R., Eling, P. A. T. M., Wezenberg, E., Vissers, C. T. W. M., & Kan, C. C. (2011). Attentional set shifting in autism spectrum disorder: Differentiating between the role of perseveration, learned irrelevance, and novelty processing. *Journal of Clinical and Experimental Neuropsychology, 33*(2), 210-217. doi:10.1080/13803395.2010.501327

Magiati, I., Chan, J. Y., Tan, W.-L. J., & Poon, K. K. (2014). Do non-referred young people with autism spectrum disorders and their caregivers agree when reporting anxiety symptoms? A preliminary investigation using the spence children's anxiety scale. *Research in Autism Spectrum Disorders, 8*(5), 546-558. doi:10.1016/j.rasd.2014.01.015

March, J. S., Parker, J. D. A., Sullivan, K., Stallings, P., & Conners, C. K. (1997). The multidimensional anxiety scale for children (masc): Factor structure, reliability, and validity. *Journal of the American Academy of Child and Adolescent Psychiatry, 36*(4), 554-565. doi:10.1097/00004583-199704000-00019

Mars, A. E., Mauk, J. E., & Dowrick, P. W. (1998). Symptoms of pervasive developmental disorders as observed in prediagnostic home videos of infants and toddlers. *Journal of Pediatrics, 132*(3 Pt 1), 500-504.

Matson, J. L., & Shoemaker, M. (2009). Intellectual disability and its relationship to autism spectrum disorders. *Research in Developmental Disabilities, 30*(6), 1107-1114. doi:10.1016/j.ridd.2009.06.003

Mattila, M. L., Hurtig, T., Haapsamo, H., Jussila, K., Kuusikko-Gauffin, S., Kielinen, M., Linna, S. L., Ebeling, H., Bloigu, R., Joskitt, L., Pauls, D. L., & Moilanen, I. (2010). Comorbid psychiatric disorders associated with asperger syndrome/high-functioning autism: A community- and clinic-based study. *Journal of Autism and Developmental Disorders, 40*(9), 1080-1093. doi:10.1007/s10803-010-0958-2

May, T., Cornish, K., & Rinehart, N. (2014). Does gender matter? A one year follow-up of autistic, attention and anxiety symptoms in high-functioning children with autism spectrum disorder. *Journal of Autism and Developmental Disorders, 44*(5), 1077-1086. doi:10.1007/s10803-013-1964-y

May, T., Cornish, K., & Rinehart, N. J. (2015). Mechanisms of anxiety related attentional biases in children with autism spectrum disorder. *Journal of Autism and Developmental Disorders, 45*(10), 3339-3350. doi:10.1007/s10803-015-2500-z

Mayes, S. D., Calhoun, S. L., Aggarwal, R., Baker, C., Mathapati, S., Molitoris, S., & Mayes, R. D. (2013). Unusual fears in children with autism. *Research in Autism Spectrum Disorders, 7*(1), 151-158. doi:10.1016/j.rasd.2012.08.002

Mazefsky, C. A., Folstein, S. E., & Lainhart, J. E. (2008). Overrepresentation of mood and anxiety disorders in adults with autism and their first-degree relatives: What does it mean? *Autism Research, 1*(3), 193-197. doi:10.1002/aur.23

Mazefsky, C. A., Kao, J., & Oswald, D. P. (2011). Preliminary evidence suggesting caution in the use of psychiatric self-report measures with adolescents with

high-functioning autism spectrum disorders. *Research in Autism Spectrum Disorders, 5*(1), 164-174. doi:10.1016/j.rasd.2010.03.006

Mazurek, M. O., Vasa, R. A., Kalb, L. G., Kanne, S. M., Rosenberg, D., Keefer, A., Murray, D. S., Freedman, B., & Lowery, L. A. (2013). Anxiety, sensory over-responsivity, and gastrointestinal problems in children with autism spectrum disorders. *Journal of Abnormal Child Psychology, 41*(1), 165-176. doi:10.1007/s10802-012-9668-x

McClowry, S. G., Rodriguez, E. T., & Koslowitz, R. (2008). Temperament-based intervention: Re-examining goodness of fit. *European journal of developmental science, 2*(1-2), 120-135.

McGrath, L. M., Oates, J. M., Dai, Y. G., Dodd, H. F., Waxler, J., Clements, C. C., Weill, S., Hoffnagle, A., Anderson, E., MacRae, R., Mullett, J., McDougle, C. J., Pober, B. R., & Smoller, J. W. (2016). Attention bias to emotional faces varies by iq and anxiety in williams syndrome. *Journal of Autism and Developmental Disorders, 46*(6), 2174-2185. doi:10.1007/s10803-016-2748-y

McLean, C. P., & Anderson, E. R. (2009). Brave men and timid women? A review of the gender differences in fear and anxiety. *Clinical Psychology Review, 29*(6), 496-505. doi:10.1016/j.cpr.2009.05.003

McLean, C. P., Asnaani, A., Litz, B. T., & Hofmann, S. G. (2011). Gender differences in anxiety disorders: Prevalence, course of illness, comorbidity and burden of illness. *Journal of Psychiatric Research, 45*(8), 1027-1035. doi:10.1016/j.jpsychires.2011.03.006

McManis, M. H., Bradley, M. M., Berg, W. K., Cuthbert, B. N., & Lang, P. J. (2001). Emotional reactions in children: Verbal, physiological, and behavioral responses to affective pictures. *Psychophysiology, 38*(2), 222-231. doi:10.1111/1469-8986.3820222

Messinger, D., Young, G. S., Ozonoff, S., Dobkins, K., Carter, A., Zwaigenbaum, L., Landa, R. J., Charman, T., Stone, W. L., Constantino, J. N., Hutman, T., Carver, L. J., Bryson, S., Iverson, J. M., Strauss, M. S., Rogers, S. J., & Sigman, M. (2013). Beyond autism: A baby siblings research consortium study of high-risk children at three years of age. *Journal of the American Academy of Child and Adolescent Psychiatry, 52*(3), 300-308. doi:10.1016/j.jaac.2012.12.011

Messinger, D. S., Young, G. S., Webb, S. J., Ozonoff, S., Bryson, S. E., Carter, A., & Zwaigenbaum, L. (2015). Early sex differences are not autism-specific: A baby siblings research consortium (bsrc) study. *Molecular Autism, 6*(32). doi:10.1186/s13229-015-0027-y

Meyer, K. A., Ingersoll, B., & Hambrick, D. Z. (2011). Factors influencing adjustment in siblings of children with autism spectrum disorders. *Research in Autism Spectrum Disorders, 5*(4), 1413-1420. doi:10.1016/j.rasd.2011.01.027

Meyer-Lindenberg, A., Hariri, A. R., Munoz, K. E., Mervis, C. B., Mattay, V. S., Morris, C. A., & Berman, K. F. (2005). Neural correlates of genetically abnormal social cognition in williams syndrome.

Mian, N. D., Carter, A. S., Pine, D. S., Wakschlag, L. S., & Briggs-Gowan, M. J. (2015). Development of a novel observational measure for anxiety in young children: The anxiety dimensional observation scale. *Journal of Child*

Psychology and Psychiatry and Allied Disciplines, 56(9), 1017-1025. doi:10.1111/jcpp.12407

Miller, G. A., & Chapman, J. P. (2001). Misunderstanding analysis of covariance. *Journal of Abnormal Psychology, 110*(1), 40-48.

Miller, L. D., Martinez, Y. J., Shumka, E., & Baker, H. (2014). Multiple informant agreement of child, parent, and teacher ratings of child anxiety within community samples. *Canadian Journal of Psychiatry. Revue Canadienne de Psychiatrie, 59*(1), 34-39.

Miller, M., Iosif, A. M., Young, G. S., Hill, M., Phelps Hanzel, E., Hutman, T., Johnson, S., & Ozonoff, S. (2015). School-age outcomes of infants at risk for autism spectrum disorder. *Autism Research*. doi:10.1002/aur.1572

Miller, M., Iosif, A. M., Young, G. S., Hill, M. M., & Ozonoff, S. (2016). Early detection of adhd: Insights from infant siblings of children with autism. *Journal of Clinical Child and Adolescent Psychology*, 1-8. doi:10.1080/15374416.2016.1220314

Mitte, K. (2008). Memory bias for threatening information in anxiety and anxiety disorders: A meta-analytic review. *Psychological Bulletin, 134*(6), 886-911. doi:10.1037/a0013343

Miyake, A., Friedman, N. P., Emerson, M. J., Witzki, A. H., Howerter, A., & Wager, T. D. (2000). The unity and diversity of executive functions and their contributions to complex "frontal lobe" tasks: A latent variable analysis. *Cognitive Psychology, 41*(1), 49-100. doi:10.1006/cogp.1999.0734

Moffitt, T. E. (2005). The new look of behavioral genetics in developmental psychopathology: Gene-environment interplay in antisocial behaviors. *Psychological Bulletin, 131*(4), 533-554. doi:10.1037/0033-2909.131.4.533

Mogg, K., Bradley, B. P., De Bono, J., & Painter, M. (1997). Time course of attentional bias for threat information in non-clinical anxiety. *Behaviour Research and Therapy, 35*(4), 297-303. doi:10.1016/S0005-7967(96)00109-X

Mogg, K., Bradley, B. P., & Hallowell, N. (1994). Attentional bias to threat: Roles of trait anxiety, stressful events, and awareness. *Quarterly Journal of Experimental Psychology. A: Human Experimental Psychology, 47*(4), 841-864.

Mogg, K., Bradley, B. P., Miles, F., & Dixon, R. (2004). Brief report time course of attentional bias for threat scenes: Testing the vigilance-avoidance hypothesis. *Cognition & Emotion, 18*(5), 689-700. doi:10.1080/02699930341000158

Monk, C. (2010). Neural circuitry of emotional face processing in autism spectrum disorders. *Journal of Psychiatry and Neuroscience, 35*(2), 105-114. doi:10.1503/jpn.090085

Monk, C. S., Nelson, E. E., McClure, E. B., Mogg, K., Bradley, B. P., Leibenluft, E., Blair, R. J., Chen, G., Charney, D. S., Ernst, M., & Pine, D. S. (2006). Ventrolateral prefrontal cortex activation and attentional bias in response to angry faces in adolescents with generalized anxiety disorder. *American Journal of Psychiatry, 163*(6), 1091-1097. doi:10.1176/ajp.2006.163.6.1091

Monk, C. S., Telzer, E. H., Mogg, K., & et al. (2008). Amygdala and ventrolateral prefrontal cortex activation to masked angry faces in children and adolescents

with generalized anxiety disorder. *Archives of General Psychiatry, 65*(5), 568-576. doi:10.1001/archpsyc.65.5.568

Mullen, E. M. (1995). *Mullen scales of early learning*. Circle Pines, MN: American Guidance Service.

Mundy, P. C., Henderson, H. A., Inge, A. P., & Coman, D. C. (2007). The modifier model of autism and social development in higher functioning children. *Res Pract Persons Severe Disabl, 32*(2), 124-139.

Muris, P., & Ollendick, T. H. (2005). The role of temperament in the etiology of child psychopathology. *Clinical Child and Family Psychology Review, 8*(4), 271-289. doi:10.1007/s10567-005-8809-y

Nakagawa, A., & Sukigara, M. (2012). Difficulty in disengaging from threat and temperamental negative affectivity in early life: A longitudinal study of infants aged 12–36 months. *Behavioral and Brain Functions, 8*(1), 1-8. doi:10.1186/1744-9081-8-40

Nakagawa, A., & Sukigara, M. (2013). Individual differences in disengagement of fixation and temperament: Longitudinal research on toddlers. *Infant Behavior & Development, 36*(4), 728-735. doi:10.1016/j.infbeh.2013.08.001

Nauta, M. H., Scholing, A., Rapee, R. M., Abbott, M., Spence, S. H., & Waters, A. (2004). A parent-report measure of children's anxiety: Psychometric properties and comparison with child-report in a clinic and normal sample. *Behaviour Research and Therapy, 42*(7), 813-839. doi:10.1016/s0005-7967(03)00200-6

Nigg, J. T. (2006). Temperament and developmental psychopathology. *Journal of Child Psychology and Psychiatry, 47*(3-4), 395-422. doi:10.1111/j.1469-7610.2006.01612.x

O'Toole, L. J., DeCicco, J. M., Berthod, S., & Dennis, T. A. (2013). The n170 to angry faces predicts anxiety in typically developing children over a two-year period. *Developmental Neuropsychology, 38*(5), 352-363. doi:10.1080/87565641.2013.802321

Oerlemans, A. M., Hartman, C. A., Franke, B., Buitelaar, J. K., & Rommelse, N. N. J. (2016). Does the cognitive architecture of simplex and multiplex asd families differ? *Journal of Autism and Developmental Disorders, 46*(2), 489-501. doi:10.1007/s10803-015-2572-9

Oerlemans, A. M., van der Meer, J. M., van Steijn, D. J., de Ruiter, S. W., de Bruijn, Y. G., de Sonneville, L. M., Buitelaar, J. K., & Rommelse, N. N. (2014). Recognition of facial emotion and affective prosody in children with asd (+adhd) and their unaffected siblings. *European Child and Adolescent Psychiatry, 23*(5), 257-271. doi:10.1007/s00787-013-0446-2

Olley, A., Malhi, G., & Sachdev, P. (2007). Memory and executive functioning in obsessive-compulsive disorder: A selective review. *Journal of Affective Disorders, 104*(1-3), 15-23. doi:10.1016/j.jad.2007.02.023

Ooi, J., Dodd, H. F., & Walsh, J. (2015). Shared cognition in childhood anxiety: Interpretation bias in preschool children and their parents. *Journal of Child and Family Studies, 24*(11), 3413-3422. doi:10.1007/s10826-015-0143-5

Orchard, F., Pass, L., & Reynolds, S. (2016). Associations between interpretation bias and depression in adolescents. *Cognitive Therapy and Research*. doi:10.1007/s10608-016-9760-6

Orsmond, G. I., & Seltzer, M. M. (2009). Adolescent siblings of individuals with an autism spectrum disorder: Testing a diathesis-stress model of sibling well-being. *Journal of Autism and Developmental Disorders, 39*(7), 1053-1065. doi:10.1007/s10803-009-0722-7

Osterling, J., & Dawson, G. (1994). Early recognition of children with autism: A study of first birthday home videotapes. *Journal of Autism and Developmental Disorders, 24*(3), 247-257.

Ozonoff, S., & Strayer, D. L. (1997). Inhibitory function in nonretarded children with autism. *Journal of Autism and Developmental Disorders, 27*(1), 59-77. doi:10.1023/A:1025821222046

Ozonoff, S., Young, G. S., Belding, A., Hill, M., Hill, A., Hutman, T., Johnson, S., Miller, M., Rogers, S. J., Schwichtenberg, A. J., Steinfeld, M., & Iosif, A.-M. (2014). The broader autism phenotype in infancy: When does it emerge? *Journal of the American Academy of Child and Adolescent Psychiatry, 53*(4), 398-407.e392. doi:10.1016/j.jaac.2013.12.020

Ozonoff, S., Young, G. S., Carter, A., Messinger, D., Yirmiya, N., Zwaigenbaum, L., Bryson, S., Carver, L. J., Constantino, J. N., Dobkins, K., Hutman, T., Iverson, J. M., Landa, R., Rogers, S. J., Sigman, M., & Stone, W. L. (2011). Recurrence risk for autism spectrum disorders: A baby siblings research consortium study. *Pediatrics, 128*(3), e488-e495. doi:10.1542/peds.2010-2825

Ozsivadjian, A., Hibberd, C., & Hollocks, M. J. (2014). Brief report: The use of self-report measures in young people with autism spectrum disorder to access symptoms of anxiety, depression and negative thoughts. *Journal of Autism and Developmental Disorders, 44*(4), 969-974. doi:10.1007/s10803-013-1937-1

Ozsivadjian, A., Hollocks, M. J., Southcott, J., Absoud, M., & Holmes, E. (2016). Anxious imagery in children with and without autism spectrum disorder: An investigation into occurrence, content, features and implications for therapy. *Journal of Autism and Developmental Disorders*. doi:10.1007/s10803-016-2840-3

Pahl, K. M., Barrett, P. M., & Gullo, M. J. (2012). Examining potential risk factors for anxiety in early childhood. *Journal of Anxiety Disorders, 26*(2), 311-320. doi:10.1016/j.janxdis.2011.12.013

Paulus, M. P. (2015). Cognitive control in depression and anxiety: Out of control? *Current Opinion in Behavioral Sciences, 1*, 113-120. doi:10.1016/j.cobeha.2014.12.003

Pellicano, E. (2012). The development of executive function in autism. *Autism Research and Treatment, 2012*, 8. doi:10.1155/2012/146132

Pelphrey, K. A., Shultz, S., Hudac, C. M., & Vander Wyk, B. C. (2011). Research review: Constraining heterogeneity: The social brain and its development in autism spectrum disorder. *Journal of Child Psychology and Psychiatry and Allied Disciplines, 52*(6), 631-644. doi:10.1111/j.1469-7610.2010.02349.x

Perez-Edgar, K., Bar-Haim, Y., McDermott, J. M., Chronis-Tuscano, A., Pine, D. S., & Fox, N. A. (2010). Attention biases to threat and behavioral inhibition in early childhood shape adolescent social withdrawal. *Emotion, 10*(3), 349-357. doi:10.1037/a0018486

Perez-Edgar, K., McDermott, J. N., Korelitz, K., Degnan, K. A., Curby, T. W., Pine, D. S., & Fox, N. A. (2010). Patterns of sustained attention in infancy shape the developmental trajectory of social behavior from toddlerhood through adolescence. *Developmental Psychology, 46*(6), 1723-1730. doi:10.1037/a0021064

Perez-Edgar, K., Reeb-Sutherland, B. C., McDermott, J. M., White, L. K., Henderson, H. A., Degnan, K. A., Hane, A. A., Pine, D. S., & Fox, N. A. (2011). Attention biases to threat link behavioral inhibition to social withdrawal over time in very young children. *Journal of Abnormal Child Psychology, 39*(6), 885-895. doi:10.1007/s10802-011-9495-5

Perez-Edgar, K., Roberson-Nay, R., Hardin, M. G., Poeth, K., Guyer, A. E., Nelson, E. E., McClure, E. B., Henderson, H. A., Fox, N. A., Pine, D. S., & Ernst, M. (2007). Attention alters neural responses to evocative faces in behaviorally inhibited adolescents. *Neuroimage, 35*(4), 1538-1546. doi:10.1016/j.neuroimage.2007.02.006

Pergamin-Hight, L., Naim, R., Bakermans-Kranenburg, M. J., van, I. M. H., & Bar-Haim, Y. (2015). Content specificity of attention bias to threat in anxiety disorders: A meta-analysis. *Clinical Psychology Review, 35*, 10-18. doi:10.1016/j.cpr.2014.10.005

Petalas, M. A., Hastings, R. P., Nash, S., Hall, L. M., Joannidi, H., & Dowey, A. (2012). Psychological adjustment and sibling relationships in siblings of children with autism spectrum disorders: Environmental stressors and the broad autism phenotype. *Research in Autism Spectrum Disorders, 6*(1), 546-555. doi:10.1016/j.rasd.2011.07.015

Pine, D. S., Cohen, P., Gurley, D., Brook, J., & Ma, Y. (1998). The risk for early-adulthood anxiety and depressive disorders in adolescents with anxiety and depressive disorders. *Archives of General Psychiatry, 55*(1), 56-64. doi:10.1001/archpsyc.55.1.56

Pine, D. S., Helfinstein, S. M., Bar-Haim, Y., Nelson, E., & Fox, N. A. (2009). Challenges in developing novel treatments for childhood disorders: Lessons from research on anxiety. *Neuropsychopharmacology, 34*(1), 213-228. doi:10.1038/npp.2008.113

Piven, J., Chase, G. A., Landa, R., Wzorek, M., Gayle, J., Cloud, D., & Folstein, S. (1991). Psychiatric disorders in the parents of autistic individuals. *Journal of the American Academy of Child and Adolescent Psychiatry, 30*(3), 471-478. doi:10.1097/00004583-199105000-00019

Podsakoff, P. M., MacKenzie, S. B., Lee, J. Y., & Podsakoff, N. P. (2003). Common method biases in behavioral research: A critical review of the literature and recommended remedies. *Journal of Applied Psychology, 88*(5), 879-903. doi:10.1037/0021-9010.88.5.879

Poljac, E., & Bekkering, H. (2012). A review of intentional and cognitive control in autism. *Frontiers in Psychology, 3*, 436. doi:10.3389/fpsyg.2012.00436

Posner, N., Snyder, C. R., & Davidson, B. J. (1980). Attention and the detection of signals. *Journal of Experimental Psychology, 109*(2), 160-174. doi:10.1080/00335558008248231

Psychology Software Tools Inc. (2012). *E-prime (version 2.0)*. Pittsburgh, PA: Psychology Software Tools, Inc.

Putnam, S. P., Gartstein, M. A., & Rothbart, M. K. (2006). Measurement of fine-grained aspects of toddler temperament: The early childhood behavior questionnaire. *Infant Behavior & Development, 29*(3), 386-401. doi:10.1016/j.infbeh.2006.01.004

Putnam, S. P., & Rothbart, M. K. (2006). Development of short and very short forms of the children's behavior questionnaire. *Journal of Personality Assessment, 87*(1), 102-112. doi:10.1207/s15327752jpa8701_09

Putnam, S. P., Rothbart, M. K., & Gartstein, M. A. (2008). Homotypic and heterotypic continuity of fine-grained temperament during infancy, toddlerhood, and early childhood. *Infant and Child Development, 17*(4), 387-405. doi:10.1002/icd.582

Quirk, G. J., & Mueller, D. (2008). Neural mechanisms of extinction learning and retrieval. *Neuropsychopharmacology, 33*(1), 56-72. doi:10.1038/sj.npp.1301555

Rapee, R. M. (2002). The development and modification of temperamental risk for anxiety disorders: Prevention of a lifetime of anxiety? *Biological Psychiatry, 52*(10), 947-957. doi:10.1016/S0006-3223(02)01572-X

Reed, P., & McCarthy, J. (2012). Cross-modal attention-switching is impaired in autism spectrum disorders. *Journal of Autism and Developmental Disorders, 42*(6), 947-953. doi:10.1007/s10803-011-1324-8

Rettew, D. C., & McKee, L. (2005). Temperament and its role in developmental psychopathology. *Harvard Review of Psychiatry, 13*(1), 14-27. doi:10.1080/10673220590923146

Ricketts, J., Jones, C. R., Happé, F., & Charman, T. (2013). Reading comprehension in autism spectrum disorders: The role of oral language and social functioning. *Journal of Autism and Developmental Disorders, 43*(4), 807-816. doi:10.1007/s10803-012-1619-4

Rinehart, N. J., Bradshaw, J. L., Brereton, A. V., & Tonge, B. J. (2001). Movement preparation in high-functioning autism and asperger disorder: A serial choice reaction time task involving motor reprogramming. *Journal of Autism and Developmental Disorders, 31*(1), 79-88. doi:10.1023/A:1005617831035

Ring, H., Woodbury-Smith, M., Watson, P., Wheelwright, S., & Baron-Cohen, S. (2008). Clinical heterogeneity among people with high functioning autism spectrum conditions: Evidence favouring a continuous severity gradient. *Behav Brain Funct, 4*, 11. doi:10.1186/1744-9081-4-11

Robinson, O. J., Charney, D. R., Overstreet, C., Vytal, K., & Grillon, C. (2012). The adaptive threat bias in anxiety: Amygdala-dorsomedial prefrontal cortex coupling and aversive amplification. *Neuroimage, 60*(1), 523-529. doi:10.1016/j.neuroimage.2011.11.096

Rodgers, J., Glod, M., Connolly, B., & McConachie, H. (2012). The relationship between anxiety and repetitive behaviours in autism spectrum disorder. *Journal of Autism and Developmental Disorders, 42*(11), 2404-2409. doi:10.1007/s10803-012-1531-y

Rodgers, J., Wigham, S., McConachie, H., Freeston, M., Honey, E., & Parr, J. R. (2016). Development of the anxiety scale for children with autism spectrum disorder (asc-asd). *Autism Research*. doi:10.1002/aur.1603

Rogers, S. J. (2000). Diagnosis of autism before the age of 3 *International review of research in mental retardation* (Vol. Volume 23, pp. 1-31): Academic Press.

Ronald, A., & Hoekstra, R. A. (2011). Autism spectrum disorders and autistic traits: A decade of new twin studies. *American Journal of Medical Genetics. Part B: Neuropsychiatric Genetics, 156B*(3), 255-274. doi:10.1002/ajmg.b.31159

Rothbart, M. K. (2004). Commentary: Differentiated measures of temperament and multiple pathways to childhood disorders. *Journal of Clinical Child and Adolescent Psychology, 33*(1), 82-87. doi:10.1207/S15374424JCCP3301_8

Rothbart, M. K. (2007). Temperament, development, and personality. *Current Directions in Psychological Science, 16*(4), 207-212.

Rothbart, M. K. (2011). *Becoming who we are: Temperament and personality in development*. New York: Guilford Press.

Rothbart, M. K., Ahadi, S. A., & Evans, D. E. (2000). Temperament and personality: Origins and outcomes. *Journal of Personality and Social Psychology, 78*(1), 122-135.

Rothbart, M. K., Ahadi, S. A., Hershey, K. L., & Fisher, P. (2001). Investigations of temperament at three to seven years: The children's behavior questionnaire. *Child Development, 72*(5), 1394-1408. doi:10.1111/1467-8624.00355

Rothbart, M. K., & Bates, J. E. (1998). Temperament *Handbook of child psychology (5th edition, volume 3)*. New York: Wiley.

Rothbart, M. K., & Deryberry, D. (1981). *Development of individual differences in temperament*. Hillsdale, NJ: Erlbaum.

Rothbart, M. K., Ellis, L. K., Rosario Rueda, M., & Posner, M. I. (2003). Developing mechanisms of temperamental effortful control. *Journal of Personality, 71*(6), 1113-1144. doi:10.1111/1467-6494.7106009

Rothbart, M. K., & Posner, M. I. (2006). Temperament, attention, and developmental psychopathology *Developmental psychopathology, vol 2: Developmental neuroscience, second edition.* . Hoboken: Wiley.

Roy, A. K., Vasa, R. A., Bruck, M., Mogg, K., Bradley, B. P., Sweeney, M., Bergman, R. L., McClure-Tone, E. B., Pine, D. S., & Team, C. (2008). Attention bias toward threat in pediatric anxiety disorders. *Journal of the American Academy of Child and Adolescent Psychiatry, 47*(10), 1189-1196. doi:10.1097/CHI.0b013e3181825ace

Rubin, K. H., Burgess, K. B., & Hastings, P. D. (2002). Stability and social–behavioral consequences of toddlers' inhibited temperament and parenting behaviors. *Child Development, 73*(2), 483-495. doi:10.1111/1467-8624.00419

Rutter, M. (1978). Diagnosis and definition of childhood autism. *Journal of Autism and Childhood Schizophrenia, 8*(2), 139-161. doi:10.1007/BF01537863

Rutter, M. (2005). Environmentally mediated risks for psychopathology: Research strategies and findings. *Journal of the American Academy of Child and Adolescent Psychiatry, 44*(1), 3-18. doi:10.1097/01.chi.0000145374.45992.c9

Rutter, M., Bailey, A., & Lord, C. (2003). *The social communication questionnaire: Manual*. Los Angeles, CA: Western Psychological Services.

Safford, S. M., Kendall, P. C., Flannery-Schroeder, E., Webb, A., & Sommer, H. (2005). A longitudinal look at parent–child diagnostic agreement in youth treated for anxiety disorders. *Journal of Clinical Child and Adolescent Psychology, 34*(4), 747-757. doi:10.1207/s15374424jccp3404_16

Salazar, F., Baird, G., Chandler, S., Tseng, E., O'Sullivan, T., Howlin, P., Pickles, A., & Simonoff, E. (2015). Co-occurring psychiatric disorders in preschool and elementary school-aged children with autism spectrum disorder. *Journal of Autism and Developmental Disorders, 45*(8), 2283-2294. doi:10.1007/s10803-015-2361-5

Salemink, E., van den Hout, M. A., & Kindt, M. (2007). Selective attention and threat: Quick orienting versus slow disengagement and two versions of the dot probe task. *Behaviour Research and Therapy, 45*(3), 607-615. doi:10.1016/j.brat.2006.04.004

Sanders, Stephan J., Ercan-Sencicek, A. G., Hus, V., Luo, R., Murtha, Michael T., Moreno-De-Luca, D., Chu, Su H., Moreau, Michael P., Gupta, Abha R.,

Thomson, Susanne A., Mason, Christopher E., Bilguvar, K., Celestino-Soper, Patricia B. S., Choi, M., Crawford, Emily L., Davis, L., Davis Wright, Nicole R., Dhodapkar, Rahul M., DiCola, M., DiLullo, Nicholas M., Fernandez, Thomas V., Fielding-Singh, V., Fishman, Daniel O., Frahm, S., Garagaloyan, R., Goh, Gerald S., Kammela, S., Klei, L., Lowe, Jennifer K., Lund, Sabata C., McGrew, Anna D., Meyer, Kyle A., Moffat, William J., Murdoch, John D., O'Roak, Brian J., Ober, Gordon T., Pottenger, Rebecca S., Raubeson, Melanie J., Song, Y., Wang, Q., Yaspan, Brian L., Yu, Timothy W., Yurkiewicz, Ilana R., Beaudet, Arthur L., Cantor, Rita M., Curland, M., Grice, Dorothy E., Günel, M., Lifton, Richard P., Mane, Shrikant M., Martin, Donna M., Shaw, Chad A., Sheldon, M., Tischfield, Jay A., Walsh, Christopher A., Morrow, Eric M., Ledbetter, David H., Fombonne, E., Lord, C., Martin, Christa L., Brooks, Andrew I., Sutcliffe, James S., Cook, Edwin H., Jr., Geschwind, D., Roeder, K., Devlin, B., & State, Matthew W. (2011). Multiple recurrent de novo cnvs, including duplications of the 7q11.23 williams syndrome region, are strongly associated with autism. *Neuron, 70*(5), 863-885. doi:10.1016/j.neuron.2011.05.002

Scheuffgen, K., Happé, F., Anderson, M., & Frith, U. (2000). High "intelligence," low "iq"? Speed of processing and measured iq in children with autism. *Development and Psychopathology, 12*(1), 83-90.

Schmitz, J., Kramer, M., Tuschen-Caffier, B., Heinrichs, N., & Blechert, J. (2011). Restricted autonomic flexibility in children with social phobia. *Journal of Child Psychology and Psychiatry and Allied Disciplines, 52*(11), 1203-1211. doi:10.1111/j.1469-7610.2011.02417.x

Schwartz, C. B., Henderson, H. A., Inge, A. P., Zahka, N. E., Coman, D. C., Kojkowski, N. M., Hileman, C. M., & Mundy, P. C. (2009). Temperament as a predictor of symptomotology and adaptive functioning in adolescents with high-functioning autism. *Journal of Autism and Developmental Disorders, 39*(6), 842-855. doi:10.1007/s10803-009-0690-y

Schwartz, C. E., Snidman, N., & Kagan, J. (1999). Adolescent social anxiety as an outcome of inhibited temperament in childhood. *Journal of the American Academy of Child and Adolescent Psychiatry, 38*(8), 1008-1015. doi:10.1097/00004583-199908000-00017

Schwartz, C. E., Wright, C. I., Shin, L. M., Kagan, J., & Rauch, S. L. (2003). Inhibited and uninhibited infants "grown up": Adult amygdalar response to novelty. *Science, 300*(5627), 1952-1953. doi:10.1126/science.1083703

Schwichtenberg, A. J., Young, G. S., Hutman, T., Iosif, A. M., Sigman, M., Rogers, S. J., & Ozonoff, S. (2013). Behavior and sleep problems in children with a family history of autism. *Autism Research, 6*(3), 169-176. doi:10.1002/aur.1278

Seltzer, M. M., Abbeduto, L., Krauss, M. W., Greenberg, J., & Swe, A. (2004). Comparison groups in autism family research: Down syndrome, fragile x syndrome, and schizophrenia. *Journal of Autism and Developmental Disorders, 34*(1), 41-48. doi:10.1023/B:JADD.0000018073.92982.64

Semel, E., Wiig, E., & Secord, W. (2006). *Clinical evaluation of language fundamentals - 4th edition*. San Antonio: Harcourt Assessment, Inc.

Sharma, S., Woolfson, L. M., & Hunter, S. C. (2014). Maladaptive cognitive appraisals in children with high-functioning autism: Associations with fear, anxiety and theory of mind. *Autism, 18*(3), 244-254. doi:10.1177/1362361312472556

Shechner, T., Rimon-Chakir, A., Britton, J. C., Lotan, D., Apter, A., Bliese, P. D., Pine, D. S., & Bar-Haim, Y. (2014). Attention bias modification treatment augmenting effects on cognitive behavioral therapy in children aith anxiety: Randomized controlled trial. *Journal of the American Academy of Child and Adolescent Psychiatry, 53*(1), 61-71. doi:10.1016/j.jaac.2013.09.016

Sheppard, L. D., & Vernon, P. A. (2008). Intelligence and speed of information-processing: A review of 50 years of research. *Personality and Individual Differences, 44*(3), 535-551. doi:10.1016/j.paid.2007.09.015

Shivers, C. M., Deisenroth, L. K., & Taylor, J. L. (2013). Patterns and predictors of anxiety among siblings of children with autism spectrum disorders. *Journal of Autism and Developmental Disorders, 43*(6), 1336-1346. doi:10.1007/s10803-012-1685-7

Silani, G., Bird, G., Brindley, R., Singer, T., Frith, C., & Frith, U. (2008). Levels of emotional awareness and autism: An fmri study. *Social Neuroscience, 3*(2), 97-112. doi:10.1080/17470910701577020

Silverman, W. K., & Albano, A. M. (1996). *Anxiety disorders interview schedule for dsm-iv*. Oxford, UK: Oxford University Press.

Simonoff, E., Pickles, A., Charman, T., Chandler, S., Loucas, T., & Baird, G. (2008). Psychiatric disorders in children with autism spectrum disorders: Prevalence,

comorbidity, and associated factors in a population-derived sample. *Journal of the American Academy of Child and Adolescent Psychiatry, 47*(8), 921-929. doi:10.1097/CHI.0b013e318179964f

Solomon, B., DeCicco, J. M., & Dennis, T. A. (2012). Emotional picture processing in children: An erp study. *Developmental Cognitive Neuroscience, 2*(1), 110-119. doi:10.1016/j.dcn.2011.04.002

Solomon, M., Miller, M., Taylor, S. L., Hinshaw, S. P., & Carter, C. S. (2012). Autism symptoms and internalizing psychopathology in girls and boys with autism spectrum disorders. *Journal of Autism and Developmental Disorders, 42*(1), 48-59. doi:10.1007/s10803-011-1215-z

Solomon, M., Ozonoff, S. J., Cummings, N., & Carter, C. S. (2008). Cognitive control in autism spectrum disorders. *International Journal of Developmental Neuroscience, 26*(2), 239-247. doi:10.1016/j.ijdevneu.2007.11.001

Sparrow, S. S., Cicchetti, D. V., & Balla, D. A. (2005). *Vineland adaptive behavior scales, second edition (vineland-ii)*. Bloomington, NM: Pearson Assessments.

Spence, S. H. (1998). A measure of anxiety symptoms among children. *Behaviour Research and Therapy, 36*(5), 545-566. doi:10.1016/S0005-7967(98)00034-5

Spencer, M. D., Holt, R. J., Chura, L. R., Suckling, J., Calder, A. J., Bullmore, E. T., & Baron-Cohen, S. (2011). A novel functional brain imaging endophenotype of autism: The neural response to facial expression of emotion. *Transl Psychiatry, 1*, e19. doi:10.1038/tp.2011.18

Stone, W. L., Hoffman, E. L., Lewis, S. E., & Ousley, O. Y. (1994). Early recognition of autism: Parental reports vs clinical observation. *Archives of Pediatrics and Adolescent Medicine, 148*(2), 174-179. doi:10.1001/archpedi.1994.02170020060010

Stroop, J. R. (1935). Studies of interference in serial verbal reactions. *Journal of Experimental Psychology, 18*(6). doi:10.1037/h0054651

Sucksmith, E., Allison, C., Baron-Cohen, S., Chakrabarti, B., & Hoekstra, R. A. (2013). Empathy and emotion recognition in people with autism, first-degree relatives, and controls. *Neuropsychologia, 51*(1), 98-105. doi:10.1016/j.neuropsychologia.2012.11.013

Sukhodolsky, D. G., Scahill, L., Gadow, K. D., Arnold, L. E., Aman, M. G., McDougle, C. J., McCracken, J. T., Tierney, E., Williams White, S., Lecavalier, L., & Vitiello, B. (2008). Parent-rated anxiety symptoms in children with pervasive developmental disorders: Frequency and association with core autism symptoms and cognitive functioning. *Journal of Abnormal Child Psychology, 36*(1), 117-128. doi:10.1007/s10802-007-9165-9

Sullivan, M., Finelli, J., Marvin, A., Garrett-Mayer, E., Bauman, M., & Landa, R. (2007). Response to joint attention in toddlers at risk for autism spectrum disorder: A prospective study. *Journal of Autism and Developmental Disorders, 37*(1), 37-48. doi:10.1007/s10803-006-0335-3

Susa, G., Pitică, I., Benga, O., & Miclea, M. (2012). Anxiety-related attention biases in preschoolers: An investigation using the pictorial dot-probe task. *Procedia - Social and Behavioral Sciences, 33*, 637-641. doi:10.1016/j.sbspro.2012.01.199

Taylor, L. J., Maybery, M. T., Wray, J., Ravine, D., Hunt, A., & Whitehouse, A. J. O. (2015). Are there differences in the behavioural phenotypes of autism spectrum disorder probands from simplex and multiplex families? *Research in Autism Spectrum Disorders, 11*, 56-62. doi:10.1016/j.rasd.2014.12.003

The Research Units On Pediatric Psychopharmacology Anxiety Study, G. (2002). The pediatric anxiety rating scale (pars): Development and psychometric properties. *Journal of the American Academy of Child and Adolescent Psychiatry, 41*(9), 1061-1069. doi:10.1097/00004583-200209000-00006

Thomas, A., & Chess, S. (1977). *Temperament and development*. New York, NY: Brunner/Mazel.

Thomas, A., & Chess, S. (1984). Genesis and evolution of behavioral disorders: From infancy to early adult life. *American Journal of Psychiatry, 141*(1), 1-9. doi:10.1176/ajp.141.1.1

Tick, B., Colvert, E., McEwen, F., Stewart, C., Woodhouse, E., Gillan, N., Hallett, V., Lietz, S., Garnett, T., Simonoff, E., Ronald, A., Bolton, P., Happé, F., & Rijsdijk, F. (2015). Autism spectrum disorders and other mental health problems: Exploring etiological overlaps and phenotypic causal associations. *Journal of the American Academy of Child and Adolescent Psychiatry, 55*(2), 106-113.e104. doi:10.1016/j.jaac.2015.11.013

Tompkins, V., Guo, Y., & Justice, L. M. (2013). Inference generation, story comprehension, and language skills in the preschool years. *Reading and Writing, 26*(3), 403-429. doi:10.1007/s11145-012-9374-7

Tonnsen, B. L., Malone, P. S., Hatton, D. D., & Roberts, J. E. (2013). Early negative affect predicts anxiety, not autism, in preschool boys with fragile x syndrome. *Journal of Abnormal Child Psychology, 41*(2), 267-280. doi:10.1007/s10802-012-9671-2

Top, D. N., Stephenson, K. G., Doxey, C. R., Crowley, M. J., Kirwan, C. B., & South, M. (2016). Atypical amygdala response to fear conditioning in autism spectrum disorder. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging, 1*(4), 308-315. doi:10.1016/j.bpsc.2016.01.008

Trubanova, A., Donlon, K., Donlon, K., Kreiser, N. L., Kreiser, N. L., Ollendick, T. H., Ollendick, T. H., White, S. W., & White, S. W. (2014). Under-identification of asd in females: A case series illustrating the unique presentation of asd in young adult females. *Scandinavian Journal of Child and Adolescent Psychiatry and Psychology; Vol 2, No 2 (2014): Special Issue: Autism Spectrum Disorder*.

Uljarević, M., & Evans, D. W. (2016). Relationship between repetitive behaviour and fear across normative development, autism spectrum disorder, and down syndrome. *Autism Research*, n/a-n/a. doi:10.1002/aur.1674

Uljarević, M., & Hamilton, A. (2013). Recognition of emotions in autism: A formal meta-analysis. *Journal of Autism and Developmental Disorders, 43*(7), 1517-1526. doi:10.1007/s10803-012-1695-5

Ung, D., Selles, R., Small, B. J., & Storch, E. A. (2015). A systematic review and meta-analysis of cognitive-behavioral therapy for anxiety in youth with high-functioning autism spectrum disorders. *Child Psychiatry and Human Development, 46*(4), 533-547. doi:10.1007/s10578-014-0494-y

Uzefovsky, F., Allison, C., Smith, P., & Baron-Cohen, S. (2016). Brief report: The go/no-go task online: Inhibitory control deficits in autism in a large sample. *Journal of Autism and Developmental Disorders, 46*(8), 2774-2779. doi:10.1007/s10803-016-2788-3

Valicenti-McDermott, M., Hottinger, K., Seijo, R., & Shulman, L. (2012). Age at diagnosis of autism spectrum disorders. *The Journal of Pediatrics, 161*(3), 554-556. doi:10.1016/j.jpeds.2012.05.012

van Brakel, A. M. L., Muris, P., & Bögels, S. M. (2004). Relations between parent- and teacher-reported behavioral inhibition and behavioral observations of this temperamental trait. *Journal of Clinical Child and Adolescent Psychology, 33*(3), 579-589. doi:10.1207/s15374424jccp3303_15

van Steensel, F. J., Bogels, S. M., & Perrin, S. (2011). Anxiety disorders in children and adolescents with autistic spectrum disorders: A meta-analysis. *Clinical Child and Family Psychology Review, 14*(3), 302-317. doi:10.1007/s10567-011-0097-0

van Steensel, F. J., Bogels, S. M., & Wood, J. J. (2013). Autism spectrum traits in children with anxiety disorders. *Journal of Autism and Developmental Disorders, 43*(2), 361-370. doi:10.1007/s10803-012-1575-z

van Steensel, F. J., Deutschman, A. A., & Bogels, S. M. (2013). Examining the screen for child anxiety-related emotional disorder-71 as an assessment tool for anxiety in children with high-functioning autism spectrum disorders. *Autism, 17*(6), 681-692. doi:10.1177/1362361312455875

Visu-Petra, L., Ţincaş, I., Cheie, L., & Benga, O. (2010). Anxiety and visual-spatial memory updating in young children: An investigation using emotional facial expressions. *Cognition & Emotion, 24*(2), 223-240. doi:10.1080/02699930903387546

Wallace, G. L., Anderson, M., & Happé, F. (2009). Brief report: Information processing speed is intact in autism but not correlated with measured intelligence. *Journal of Autism and Developmental Disorders, 39*(5), 809-814. doi:10.1007/s10803-008-0684-1

Warren, S. L., & Sroufe, L. A. (2004). Developmental issues. In T. H. Ollendick & J. S. March (Eds.), *Phobic and anxiety disorders in children and adolescents: A clinican's guide to effective psychosocial and phramacological interventions* New York: Oxford University Press.

Warren, Z. E., Foss-Feig, J. H., Malesa, E. E., Lee, E. B., Taylor, J. L., Newsom, C. R., Crittendon, J., & Stone, W. L. (2012). Neurocognitive and behavioral outcomes of younger siblings of children with autism spectrum disorder at age five. *Journal of Autism and Developmental Disorders, 42*(3), 409-418. doi:10.1007/s10803-011-1263-4

Waszczuk, M. A., Zavos, H. S., Gregory, A. M., & Eley, T. C. (2014). The phenotypic and genetic structure of depression and anxiety disorder symptoms in childhood, adolescence, and young adulthood. *JAMA Psychiatry, 71*(8), 905-916. doi:10.1001/jamapsychiatry.2014.655

Waters, A. M., & Kershaw, R. (2015). Direction of attention bias to threat relates to differences in fear acquisition and extinction in anxious children. *Behaviour Research and Therapy, 64*, 56-65. doi:10.1016/j.brat.2014.11.010

Waters, A. M., Kokkoris, L. L., Mogg, K., Bradley, B. P., & Pine, D. S. (2010). The time course of attentional bias for emotional faces in anxious children. *Cognition & Emotion, 24*(7), 1173-1181. doi:10.1080/02699930903274355

Waters, A. M., Mogg, K., & Bradley, B. P. (2012). Direction of threat attention bias predicts treatment outcome in anxious children receiving cognitive-behavioural therapy. *Behaviour Research and Therapy, 50*(6), 428-434. doi:10.1016/j.brat.2012.03.006

Waters, A. M., Mogg, K., Bradley, B. P., & Pine, D. S. (2008). Attentional bias for emotional faces in children with generalized anxiety disorder. *Journal of the American Academy of Child and Adolescent Psychiatry, 47*(4), 435-442. doi:10.1097/CHI.0b013e3181642992

Waters, A. M., Neumann, D. L., Henry, J., Craske, M. G., & Ornitz, E. M. (2008). Baseline and affective startle modulation by angry and neutral faces in 4-8-year-old anxious and non-anxious children. *Biological Psychology, 78*(1), 10-19. doi:10.1016/j.biopsycho.2007.12.005

Watson, D., & Clark, L. A. (1984). Negative affectivity: The disposition to experience aversive emotional states. *Psychological Bulletin, 96*(3), 465-490.

Watson, D., Clark, L. A., & Carey, G. (1988). Positive and negative affectivity and their relation to anxiety and depressive disorders. *Journal of Abnormal Psychology, 97*(3), 346-353.

Watson, L. R., Baranek, G. T., Crais, E. R., Steven Reznick, J., Dykstra, J., & Perryman, T. (2007). The first year inventory: Retrospective parent responses to a questionnaire designed to identify one-year-olds at risk for autism. *Journal of Autism and Developmental Disorders, 37*(1), 49-61. doi:10.1007/s10803-006-0334-4

Webster-Stratton, C., & Taylor, T. (2001). Nipping early risk factors in the bud: Preventing substance abuse, delinquency, and violence in adolescence through interventions targeted at young children (0–8 years). *Prevention Science, 2*(3), 165-192. doi:10.1023/A:1011510923900

Wechsler, D. (2011). *Wasi-ii: Wechsler abbreviated scales of intelligence* New York, NY: Psychological Coproration.

Weems, C. F., & Costa, N. M. (2005). Developmental differences in the expression of childhood anxiety symptoms and fears. *Journal of the American Academy of Child and Adolescent Psychiatry, 44*(7), 656-663. doi:10.1097/01.chi.0000162583.25829.4b

Werner, E., Dawson, G., Osterling, J., & Dinno, N. (2000). Brief report: Recognition of autism spectrum disorder before one year of age: A retrospective study based on home videotapes. *Journal of Autism and Developmental Disorders, 30*(2), 157-162.

Whelan, R. (2008). Effective analysis of reaction time data. *The Psychological Record, 58*(3), 475-482.

White, S. W., Maddox, B. B., & Panneton, R. K. (2015). Fear of negative evaluation influences eye gaze in adolescents with autism spectrum disorder: A pilot study. *Journal of Autism and Developmental Disorders, 45*(11), 3446-3457. doi:10.1007/s10803-014-2349-6

White, S. W., Oswald, D., Ollendick, T., & Scahill, L. (2009). Anxiety in children and adolescents with autism spectrum disorders. *Clinical Psychology Review, 29*(3), 216-229. doi:10.1016/j.cpr.2009.01.003

White, S. W., Schry, A. R., & Maddox, B. B. (2012). Brief report: The assessment of anxiety in high-functioning adolescents with autism spectrum disorder. *Journal of Autism and Developmental Disorders, 42*(6), 1138-1145. doi:10.1007/s10803-011-1353-3

Whittle, S., Allen, N. B., Lubman, D. I., & Yucel, M. (2006). The neurobiological basis of temperament: Towards a better understanding of psychopathology. *Neuroscience and Biobehavioral Reviews, 30*(4), 511-525. doi:10.1016/j.neubiorev.2005.09.003

Wigham, S., & McConachie, H. (2014). Systematic review of the properties of tools used to measure outcomes in anxiety intervention studies for children with autism spectrum disorders. *PloS One, 9*(1), e85268. doi:10.1371/journal.pone.0085268

Wigham, S., Rodgers, J., South, M., McConachie, H., & Freeston, M. (2014). The interplay between sensory processing abnormalities, intolerance of uncertainty, anxiety and restricted and repetitive behaviours in autism spectrum disorder. *Journal of Autism and Developmental Disorders, 45*(4), 943-952. doi:10.1007/s10803-014-2248-x

Williams, J. M. G., Barnhofer, T., Crane, C., Hermans, D., Raes, F., Watkins, E., & Dalgleish, T. (2007). Autobiographical memory specificity and emotional disorder. *Psychological Bulletin, 133*(1), 122-148. doi:10.1037/0033-2909.133.1.122

Woltering, S., Liu, Z., Rokeach, A., & Tannock, R. (2013). Neurophysiological differences in inhibitory control between adults with adhd and their peers. *Neuropsychologia, 51*(10), 1888-1895. doi:10.1016/j.neuropsychologia.2013.06.023

Wood, J. J., & Gadow, K. D. (2010). Exploring the nature and function of anxiety in youth with autism spectrum disorders. *Clinical Psychology: Science and Practice, 17*(4), 281-292. doi:10.1111/j.1468-2850.2010.01220.x

World Health Organisation. (1993). *Mental disorders: A glossary and guide to their classification in accordance with the 10th revision of the international classification of disease - research diagnostic criteria: Icd-10*. Geneva: World Health Organisation.

Yiend, J., & Mathews, A. (2001). Anxiety and attention to threatening pictures. *Quarterly Journal of Experimental Psychology. A: Human Experimental Psychology, 54*(3), 665-681. doi:10.1080/713755991

Yoder, P., Stone, W. L., Walden, T., & Malesa, E. (2009). Predicting social impairment and asd diagnosis in younger siblings of children with autism spectrum disorder. *Journal of Autism and Developmental Disorders, 39*(10), 1381-1391. doi:10.1007/s10803-009-0753-0

Zainal, H., Magiati, I., Tan, J. W., Sung, M., Fung, D. S., & Howlin, P. (2014). A preliminary investigation of the spence children's anxiety parent scale as a screening tool for anxiety in young people with autism spectrum disorders. *Journal of Autism and Developmental Disorders, 44*(8), 1982-1994. doi:10.1007/s10803-014-2075-0

Zwaigenbaum, L., Bryson, S., Rogers, T., Roberts, W., Brian, J., & Szatmari, P. (2005). Behavioral manifestations of autism in the first year of life. *International Journal of Developmental Neuroscience, 23*(2-3), 143-152. doi:10.1016/j.ijdevneu.2004.05.001

Zwaigenbaum, L., Thurm, A., Stone, W., Baranek, G., Bryson, S., Iverson, J., Kau, A., Klin, A., Lord, C., Landa, R., Rogers, S., & Sigman, M. (2007). Studying the emergence of autism spectrum disorders in high-risk infants: Methodological and practical issues. *Journal of Autism and Developmental Disorders, 37*(3), 466-480. doi:10.1007/s10803-006-0179-x