# King's Research Portal

*Document Version*
Publisher's PDF, also known as Version of record

[Link to publication record in King's Research Portal](Link to publication record in King's Research Portal)

Download date: 14. Jan. 2025

# The contribution of [1H] magnetic resonance spectroscopy to the study of excitation-inhibition in autism

Laura A. Ajram[a,b,1], Andreia C. Pereira[a,b,c,1], Alice M.S. Durieux[a,b], Hester E. Velthius[a], Marija M. Petrinovic[a,b,*,2], Grainne M. McAlonan[a,b,*,2]

[a] Department of Forensic and Neurodevelopmental Sciences, Institute of Psychiatry, Psychology and Neuroscience, King's College London, 16 De Crespigny Park, London, SE5 8AF, UK
[b] Sackler Institute for Translational Neurodevelopment, Institute of Psychiatry, Psychology and Neuroscience, King's College London, 16 De Crespigny Park, London, SE5 8AF, UK
[c] CIBIT - Coimbra Institute for Biomedical Imaging and Translational Research, Faculty of Medicine, ICNAS - Institute of Nuclear Sciences Applied to Health, University of Coimbra, Polo 3, 3000-548 Coimbra, Portugal

ABSTRACT

Autism spectrum disorder (ASD) affects over 1:100 of the population and costs the UK more than £32bn and the USA more than $175bn (£104bn) annually. Its core symptoms are social and communication difficulties, repetitive behaviours and sensory hyper- or hypo-sensitivities. A highly diverse phenotypic presentation likely reflects its etiological heterogeneity and makes finding treatment targets for ASD challenging. In addition, there are no means to identify biologically responsive individuals who may benefit from specific interventions. There is hope however, and in this review we consolidate how findings from magnetic resonance spectroscopy (MRS) add to the evidence that differences in the brain's excitatory glutamate and inhibitory γ-aminobutyric acid (GABA) balance may be both a key biomarker and a tractable treatment target in ASD.

## 1. Introduction

Autism spectrum disorder (ASD) is a highly heterogeneous lifelong neurodevelopmental syndrome characterised by deficits in social reciprocity and communication, and by restricted interests and stereotyped behaviours (DSM-5; American Psychiatric Association, 2013). Its prevalence is growing (Lai et al., 2014) and, in the UK alone, adults with ASD cost the economy over £32 billion/year, i.e. more than heart disease, cancer and stroke combined (Buescher et al., 2014). Therefore, there is a pressing need to identify the underlying biological mechanisms that would provide a basis for the development of ASD stratification markers and treatment options. Progress is happening however, based on accumulating evidence that multiple genetic and environmental risk factors for ASD converge to disrupt the balance between glutamate-mediated excitatory and γ-aminobutyric acid (GABA)-mediated inhibitory neurotransmission; and this may inform treatment targets for the disorder (Nelson and Valakh, 2015; Rubenstein and Merzenich, 2003).

## 2. Evidence for excitation-inhibition imbalance in autism spectrum disorder

A balanced interaction between glutamate and GABA is essential for synaptic maturation, refinement of neuronal circuitry and subsequently the regulation of cognition, emotion and behaviour (Lujan et al., 2005). An imbalance in excitation-inhibition (E-I) caused by disruption to any component of the glutamate and GABA signalling systems could therefore have potentially far-reaching consequences. Indeed, based on observations such as an increased incidence of epilepsy – a disorder of E-I balance – in individuals with ASD (Bolton et al., 2011; Muhle et al., 2004), and post-mortem evidence of altered expression of proteins involved in glutamate and GABA biosynthesis and neurotransmission in the brains of individuals with ASD (Blatt and Fatemi, 2011; Fatemi et al., 2002, 2009, 2014; Oblak et al., 2009, 2010, 2011; Purcell et al., 2001; Shimmura et al., 2013; Yip et al., 2007), E-I imbalance has been suggested to be a 'common final pathway' in ASD (Nelson and Valakh, 2015; Rubenstein and Merzenich, 2003).

Genetic studies are in line with this proposal. For example, studies of ASD susceptibility genes have repeatedly identified chromosome 15q11-13 as a particular region of interest as 1–3% of all ASD cases were reported to be associated with duplications in this region (Moreno-De-Luca et al., 2013; Urraca et al., 2013). This chromosomal region contains genes for three GABA$_A$ receptor subtype genes (i.e. β3, γ3 and α5). Mutations in this region, especially in the β3 subunit, convey a high risk for ASD and have been linked to repetitive symptoms and insistence on sameness (Chaste et al., 2014; Isles et al., 2016; Shao et al., 2003). Direct evidence for the link between GABA$_A$-β3 and ASD has also been generated from a β3 knock-out mouse. These mice have behaviours analogous to the condition; they also have seizures, which are a frequent comorbid complication of ASD caused by E-I imbalance (DeLorey et al., 1998). In addition, we and others have reported that environmental risk factors for ASD, such as prenatal exposure to maternal inflammation or the anti-epilepsy medication valproate, disrupt gene and protein expression across GABA pathways and trigger behavioural features which mimic the human condition (Fatemi et al., 2008; Fukuchi et al., 2009; Giovanoli et al., 2014; Meyer et al., 2011; Wei et al., 2016).

There is also good evidence for genetic abnormalities in glutamatergic signalling pathways in ASD and related conditions. This has, in part, been built on the advances made in fragile X syndrome, the most common genetic form of ASD, which is caused by a single gene mutation affecting fragile X mental retardation protein (FMRP) (Verkerk et al., 1991). FMRP negatively regulates postsynaptic glutamate receptor synthesis (including metabotropic glutamate receptor 5; mGluR5) by binding to ribosomes and stalling translation of target mRNAs (Darnell et al., 2011). mRNA translation at the synapse is then increased (Bear et al., 2004), leading to excess excitatory activity, enhanced long term depression, and ASD-like pathology in animal models (Lozano et al., 2014; Yu and Berry-Kravis, 2014). Lower levels of FMRP are found in individuals with idiopathic ASD (i.e. ASD without a known cause) who have a concomitant increase in mGluR5 (Fatemi et al., 2011; Fatemi and Folsom, 2011). In addition to alterations to metabotropic receptors in ASD, genetic aberrations have also been linked to ionotropic glutamate receptors, namely N-methyl-ᴅ-aspartate (NMDA) receptors. However, the picture is complex, and both increases and decreases in NMDA receptor function have been associated with the ASD phenotype (Gandal et al., 2012).

GABA and glutamate signalling in ASD may also be indirectly derailed by mutations in genes coding for proteins that stabilise GABAergic and glutamatergic synapses. For example, alterations of neurexin and neuroligin cell adhesion molecules (Ching et al., 2010; Jamain et al., 2003), postsynaptic density protein 95 (PSD-95) and/or Shank scaffold proteins (Uchino and Waga, 2015; Xing et al., 2016) confer a high risk for ASD. These molecules anchor pre- and post-synaptic neuron terminals and disruptions of the molecular assembly of the synapse can potentially cause E-I imbalance (Futai et al., 2007; Südhof, 2008). Consistent with this, neurexin 1-α (*Nrxn1-α*) knock-out mice show a reduction in spontaneous and evoked neurotransmitter release (Kattenstroth et al., 2004; Missler et al., 2003; Zhang, 2005) accompanied by behavioural abnormalities that resemble some of the core ASD symptoms of social impairment and inflexibility/stereotypy (Grayton et al., 2013). Similarly, mice with mutations in the neuroligin 3 gene (*Nlgn3*) exhibit brain-region specific synaptic dysfunction (e.g. a shift of synaptic transmission towards inhibition in the somatosensory cortex and towards excitation in the hippocampus) and impairments in social behaviour that mimic ASD (Burrows et al., 2015; Etherton et al., 2011a, 2011b; Jaramillo et al., 2014; Radyushkin et al., 2009; Tabuchi et al., 2007). Such "multiple" E-I anomalies, that can be not only brain region- but even neural circuit-specific, as shown in multiple animal models of ASD (Horder et al., 2018; Lee et al., 2017; Nelson and Valakh, 2015) further exemplify the complexities of defining and measuring the E-I (im)balance in ASD.

Finally, the metabolism of glutamate and GABA is tightly inter-linked so an alteration in either metabolite may potentially affect the other. The enzyme glutaminase catalyses the glutamate to glutamine cycle, allowing glutamine to be stored in astrocytes or converted to glutamate in both glutamatergic and GABAergic neurons (Rowley et al., 2012). In excitatory neurons, glutamate is transported into vesicles via vesicular glutamate transporters, whereas in inhibitory neurons, glutamate is first converted to GABA by the glutamic acid decarboxylase and then transported to vesicles via vesicular GABA transporters. Upon release, the neurotransmitters are taken up by high affinity membrane transporters and returned into neurons and surrounding glia, where they are recycled. Thus, GABA, glutamate and glutamine are in constant flux. In ASD however, the levels of the multiple enzymes controlling glutamine-glutamate-GABA cycles are altered (Fatemi et al., 2002; Shimmura et al., 2013; Yip et al., 2007), and so glutamine-glutamate-GABA metabolism is likely to be atypical in the ASD brain.

How these diverse E-I anomalies might act mechanistically has been the subject of a number of recent comprehensive reviews (Foss-Feig et al., 2017; Gao and Penzes, 2015; Gatto and Broadie, 2010; Lee et al., 2017; Mullins et al., 2016; Nelson and Valakh, 2015; Uzunova et al., 2016). For example, Yizhar et al. (2011) have shown that increase of a cellular E-I balance in the mouse medial prefrontal cortex (mPFC) elicits elevated rhythmic high-frequency (gamma) activity (a disease correlate in clinical conditions; Orekhova et al., 2007) and social dysfunction. Furthermore, it was recently shown that reducing the E-I ratio by either reducing the excitability of pyramidal neurons or by increasing the excitability of inhibitory parvalbumin-positive neurons in the mPFC rescued social deficits in mice lacking the *CNTNAP2* gene, which has been implicated in ASD (Selimbeyoglu et al., 2017). Maintenance of E-I balance is thus essential to network function as not only functional, but also structural properties of neural circuits are defined by inter-neuronal communication.

## 3. [1H] Magnetic resonance spectroscopy evidence for E-I imbalance in ASD

The hypothesis that the glutamine-glutamate-GABA system is altered in ASD has also been addressed by using [1H] magnetic resonance spectroscopy (MRS) as a non-invasive method to measure tissue levels of E-I metabolites in the living human brain (Ford and Crewther, 2016; Schur et al., 2016). In brief, [1H]MRS uses the nuclear magnetic resonance properties of hydrogen atoms to generate a frequency spectrum (as opposed to the image in MR imaging) (Govindaraju et al., 2000; Juchem and Rothman, 2014; Stagg et al., 2011) in which different metabolites are identified by the position of their signal peak, i.e. chemical shift, along the frequency axis (in units of 'parts per million'; ppm). [1H]MRS metabolite levels can then be quantified by calculating the area under the corresponding peaks or by fitting the in vivo spectra to a basis set, i.e. linear combinations of measured or simulated model spectra of metabolites (Edden et al., 2014; Naressi et al., 2001; Provencher, 1993). [1H]MRS is not without limitations however. For an adequate signal-to-noise ratio, standard single voxel [1H]MRS data is acquired from a predefined and relatively large brain volume-unit (usually around 4–8 cm³) (Stagg et al., 2011; Zhu and Barker, 2011) compared with conventional MR image resolution (typically around 1–10 mm³) (Blüml, 2013). Therefore, both grey and white matter is usually sampled within the same voxel. To avoid errors when quantifying metabolites, these so-called 'partial volume' effects need to be corrected by segmenting spectroscopic voxel according to different tissue contents (Dager et al., 2008; Quadrelli et al., 2016). As shown in Table 1, most of the studies reported using tissue segmentation, however, exact approaches varied considerably between studies which might contribute to inconsistencies in the literature. Such partial volume effects are minimised in [1H]MRS imaging (MRSI), a less frequently used extension of [1H]MRS that allows data from multiple adjacent small (1 cm³ and below) voxels to be acquired simultaneously (Blüml, 2013; Oz et al., 2014; Schneider, 2016; Zhu and Barker, 2011).

**Table 1**
Summary of [1H]MRS studies reporting Glx, Glu and/or GABA + levels in ASD.

| Citation | ASD group N | Age (years) | [1H] MRS sequence (TR/TE/Av) | Quantification method (tissue segmentation) | Brain areas investigated (cm³) | Metabolite concentrations in ASD participants compared with controls | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Glx | Glu | GABA + |
| **Paediatric studies** | | Range | | | | | | |
| Friedman et al. (2006) | 45 | 3–4 | 1.5 T MRSI PEPSI (2000/20,272/na) | Estimated concentration (Y) | Grey matter (na) / White matter (na) | NSD / NSD | na | na |
| DeVito et al. (2007) | 26 | 6–17 | 3 T MRSI (1800/135/na) | Estimated concentration (Y) | Grey matter (na) / White matter (na) / Cerebellum (na) | Lower in ASD / NSD / Lower in ASD | na | na |
| Hardan et al. (2008) | 18 | 8–15 | 1.5 T MRSI STEAM (1600/20/4) | Estimated concentration (Y) | (L/R) Thalamus (5.8) | NSD | na | na |
| Harada et al. (2011) | 12 | 2–11 | 3 T STEAM (5000/15/48) / 3 T MEGA-PRESS (2500/68/256) | Estimated concentration (Y) | (L) Frontal lobe (27) / Lenticular nuclei (27) | na | NSD / NSD | Lower in ASD / NSD |
| Bejjani et al. (2012) | 8 / 26 | 7–15 / 6–17 | 1.5 T PRESS (1500/25/256) / 1.5 T MRSI PRESS (1500/30/8) | Estimated concentration (Y) | (L/R) Anterior cingulate cortex (2.4–3.6) / (L/R) Anterior cingulate cortex (1.1) | Higher in ASD / Higher in ASD (R) | na | na |
| Kubas et al. (2012) | 12 | 8–15 | 1.5 T PRESS (1500/35/192) | Creatine ratios (na) | (L/R) Frontal lobe (na) | Lower in ASD | na | Lower in ASD |
| Corrigan et al. (2013) | 45 | 3–4 | 1.5 MRSI | Estimated concentration (Y) | Grey matter (na) / White matter (na) | NSD / Lower in ASD | na | na |
| | 31 | 6–7 | PEPSI (2000/20/na) | | Grey matter (na) / White matter (na) | NSD / NSD | | |
| | 29 | 9–10 | | | Grey matter (na) / White matter (na) | NSD / NSD | | |
| Hassan et al. (2013) | 10 | 6–14 | 1.5 T PRESS (1500/30/3) | Estimated concentration (na) | Anterior cingulate cortex (8) / (L) Striatum (8) / (L) Cerebellum (8) / (L) Frontal lobe (8) | na | Higher in ASD / Higher in ASD / Higher in ASD / Higher in ASD | na |
| Joshi et al. (2013) | 7 | 12–17 | 4 T 2D-JPRESS (2000/30–250/16,32) | Estimated concentration (Y) | Anterior cingulate cortex (8) | na | Higher in ASD / NSD | na |
| Doyle-Thomas et al. (2014) | 20 | 7–18 | 3 T MRSI (2000/30/na) | Creatine ratios (na) | (L/R) Medial temporal lobe (3.38) / (L/R) Caudate (na) / (L/R) Putamen (na) / (L/R) Thalamus (na) | NSD / Higher in ASD / NSD | NSD | na |
| Gaetz et al. (2014) | 17 | 11 | 3 T MEGA-PRESS (1500/68/256) | Creatine ratios (Y) | (L) Motor cortex (27) / (L) Auditory cortex (24) / Visual cortex (27) | na | na | Lower in ASD / Lower in ASD / NSD |
| Rojas et al. (2014) | 17 | 14 | 3 T MEGA-PRESS (2500/70/512) | Creatine ratios (Y) | (L) Auditory cortex (36) | na | na | Lower in ASD |
| Brix et al. (2015) | 14 | 10 | 3 T PRESS (1500/35/128) / 3 T MEGA-PRESS (1500/68/256) | Estimated concentration / Creatine ratios (Y) | (L) Anterior cingulate cortex (27) | NSD | na | NSD |
| Cochran et al. (2015) | 13 | 13–17 | 3 T PRESS (2000/28/128) / 3 T MEGA-PRESS (2000/68/320) | Estimated concentration / Total creatine ratios (Y) | Anterior cingulate cortex (8 for PRESS, 18 for MEGA-PRESS) | na | NSD | NSD |
| Drenthen et al. (2016) | 15 | 14–18 | 3 T PRESS (2000/35/128) / 3 T MEGA-PRESS (2000/68/320) | Estimated concentration (na) | Occipital cortex (27) | na | NSD | NSD |
| Naaijen et al. (2016) | 51 | 8–13 | 3 T PRESS (3000/30/96) | Estimated concentration (Y) | Anterior cingulate cortex (8) / (L) Dorsal striatum (8) | na | Higher in ASD / NSD | na |
| Goji et al. (2017) | 34 | 2–12 | 3 T STEAM (5000/15/48) | Estimated concentration (na) | Anterior cingulate cortex (6 for STEAM, 27 for PRESS) | NSD | na | NSD |
| Hegarty et al. (2017) | 47 twin pairs | 4–9 / 10 | 3 T MRSI (2500/68/256) / 3 T MRSI PRESS (2300/35/na) | Total creatine ratios (Y) | (L) Cerebellum (6 for STEAM, 27 for PRESS) / (L/R) Thalamus (4.5) | NSD / Lower in ASD (L) | na | NSD / na |

**Table 1** (*continued*)

| Citation | ASD group N | Age (years) | [1H] MRS sequence (TR/TE/Av) | Quantification method (tissue segmentation) | Brain areas investigated (cm³) | Glx | Glu | GABA + |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Metabolite concentrations in ASD participants compared with controls | | |
| Ito et al. (2017) | 112 | 4–14 | 3 T STEAM (5000/15/48) | Creatine ratios (na) | Anterior cingulate cortex (6 for STEAM, 27 for MEGA-PRESS) | NSD | NSD | Lower in ASD |
| | 114 | 3–14 | 3 T MEGA-PRESS (2500/68/256) | | (L) Cerebellum (6 for STEAM, 27 for MEGA-PRESS) | NSD | Higher in ASD | Lower in ASD |
| Port et al. (2017) | 16 | 6–14 | 3 T MEGA-PRESS (1500/68/256) | Creatine ratios (Y) | (L) Auditory cortex (24) | na | na | Lower in ASD |
| Puts et al. (2017) | 35 | 8–12 | 3 T MEGA-PRESS (2000/68/320) | Estimated concentration (Y) | (R) Sensorimotor cortex (27) | na | na | Lower in ASD |
| | | | | | Occipital cortex (27) | | | NSD |
| Cavalho Pereira et al. (2018) | 27 | 9–18 | 3 T MEGA-PRESS (1500/68/392) | Estimated concentration / Total creatine ratios (Y) | Medial prefrontal cortex (27) | NSD | na | NSD |
| **Adult studies** | | Mean (SD) | | | | | | |
| Page et al. (2006) | 25 | 35 (11) | 1.5 T PRESS (3000/35/160) | Estimated concentration / Total creatine ratios (Y) | (R) Amygdala – hippocampal complex (6) | Higher in ASD | na | na |
| | | | | | (R) Parietal lobe (8) | NSD | | |
| Bernardi et al. (2011) | 14 | 29 (6) | 3 T MRSI / PRESS (2000/30/na) | Estimated concentration (na) | (L/R) Anterior cingulate cortex (0.56) | Lower in ASD (R) | na | na |
| | | | | | (L/R) Thalamus (0.56) | NSD | | |
| | | | | | (L/R) Temporo-parietal junction (0.56) | NSD | | |
| | | | | | (L/R) Intraparietal sulcus (0.56) | NSD | | |
| Aoki et al. (2012) | 24 | 29 (6) | 3 T STEAM (3000/15/128) | Estimated concentration (Y) | Medial prefrontal cortex (8) | NSD | na | na |
| Brown et al. (2013) | 13 | 36 (6) | 3 T PRESS (2000/30/192) | Estimated concentration (na) | (L/R) Auditory cortex (7.5) | Higher in ASD | Higher in ASD | na |
| Horder et al. (2013) | 28 | 28 (6) | 1.5 T PRESS (3000/30/na) | Estimated concentration (Y) | (L) Basal ganglia (6) | Lower in ASD | na | na |
| | | | | | (L) Dorsolateral prefrontal cortex (7.68) | NSD | | |
| | | | | | (L) Medial parietal lobe (8) | NSD | | |
| Tebartz van Elst et al. (2014) | 29 | 35 (9) | 3 T PRESS (3000/30/na) | Estimated concentration (Y) | Anterior cingulate cortex (8) | Lower in ASD | Lower in ASD | na |
| | | | | | (L) Cerebellum (8) | NSD | NSD | |
| Libero et al. (2015) | 19 | 27 (1) | 3 T PRESS (2000/80/128) | Creatine ratios (Y) | Anterior cingulate cortex (5.4) | NSD | na | na |
| | | | | | Posterior cingulate cortex (10.8) | NSD | | |
| Robertson et al. (2015) | 20 | 29 (9) | 3 T MEGA-PRESS (1500/68/320) | Estimated concentration (Y) | Visual cortex (18.75) | NSD | na | NSD |
| | | | | | (L) Motor cortex (18.75) | NSD | | NSD |
| Libero et al. (2016) | 20 | 26 (1) | 3 T PRESS (2000/80/128) | Estimated concentration / Total creatine ratios (Y) | Anterior cingulate cortex (5.4) | NSD | na | na |
| | | | | | Posterior cingulate cortex (10.8) | NSD | | |
| Ajram et al. (2017) | 17 | 33 (2) | 3 T MEGA-PRESS (2000/68/na) | Estimated concentration (Y) | Dorsomedial prefrontal cortex (30) | NSD | na | NSD |
| Endres et al. (2017) | 24 | 40 (10) | 3 T PRESS (1500/30/256) | Estimated concentration (Y) | Anterior cingulate cortex (15) | NSD | na | na |
| | | | | | (L) Dorsal prefrontal cortex (15.63) | NSD | | |
| Port et al. (2017) | 15 | 21 (1) | 3 T MEGA-PRESS (1500/68/256) | Creatine ratios (Y) | (L) Auditory cortex (24) | na | na | NSD |
| Horder et al. (2018) | 25 | 30(1) | 3 T MEGA-PRESS (2000/68/368) | Estimated concentration (Y) | (L) Striatum (26.25) | Lower in ASD | Lower in ASD | NSD |
| | | | | | Medial prefrontal cortex (30) | NSD | NSD | NSD |
| Kirovski et al. (2018) | 12 | 28(6) | 3 T MEGAPRESS (1500/68/240) | Estimated concentration (Y) | (R) Dorsolateral prefrontal cortex (8) | na | na | NSD |
| | | | | | (R) Superior temporal sulcus (8) | | | NSD |

Estimated concentration refers to water- or external reference-scaled metabolites.

ASD, autism spectrum disorder; Av, number of averages; GABA +, γ-aminobutyric acid + macromolecules; Glx, glutamate + glutamine; Glu, glutamate; L, left; MRS, magnetic resonance spectroscopy; MRSI, magnetic resonance spectroscopy imaging; MEGA-PRESS, Meshcher-Garwood point-resolved spectroscopy; na, not applicable; NSD, not statistically different; PEPSI, proton echo planar spectroscopy imaging; PRESS, point-resolved spectroscopy; R, right; ROI, region of interest; STEAM, stimulated echo acquisition mode; TR, repetition time (ms); TE, echo time (ms); Y, reported.

However, MRSI is often associated with reduced spectral resolution, non-uniform water suppression and voxel bleeding artefact (i.e. signal contamination from neighbouring voxels into the voxel-of-interest), therefore single voxel [1H]MRS remains the technique of choice (Astrakas and Argyropoulou, 2016; Blüml, 2013; Duarte et al., 2012; Jansen et al., 2006).

This large volume also means that [1H]MRS samples tissue metabolites within both neurons and glia and in both intra-and extra-cellular space (Dager et al., 2008). Therefore, to account for possible variation in voxel tissue composition, some studies report metabolite concentrations as ratios to total creatine (creatine + phosphocreatine; tCr), which is assumed to be present at constant concentration in brain tissue and cerebrospinal fluid (Gussew et al., 2012). However, as an alternative to tCr ratios, water has been used as a reference because tCr itself can be altered in neurodevelopmental conditions (Alger, 2010). Finally, depending on technical factors, such as the magnetic field strength, it is not always possible to differentiate between glutamate and glutamine due to the overlap of their resonant peaks, and the combined signal termed Glx is commonly reported (Table 1), and thus is only a proxy measure of glutamate. Similarly, the GABA signal usually also has a contribution from 'macromolecules', and is denoted as GABA + (Edden et al., 2012; Henry et al., 2001; Mikkelsen et al., 2017; Near et al., 2011).

Despite these limitations, [1H]MRS is safe and therefore, in contrast to other in vivo imaging modalities such as positron emission tomography, it can be used repeatedly, and also in children. Indeed, many [1H]MRS studies of E-I metabolites in ASD have been conducted in paediatric populations. The initial studies focused on the glutamatergic system, reporting Glx and glutamate (Glu), with reduced levels found in the cortical grey matter, frontal lobes, thalamus and cerebellum in children with ASD (DeVito et al., 2007; Hegarty et al., 2017; Kubas et al., 2012) (Table 1). However, this was not replicated by others, with either no difference in Glx/Glu observed between ASD and control participants in cortical and subcortical areas (Brix et al., 2015; Cavalho Pereira et al., 2018; Cochran et al., 2015; Doyle-Thomas et al., 2014; Drenthen et al., 2016; Friedman et al., 2006; Goji et al., 2017; Harada et al., 2011; Hardan et al., 2008; Ito et al., 2017; Joshi et al., 2013; Naaijen et al., 2016) or higher levels reported in ASD – in the anterior cingulate cortex, frontal lobes, striatum (Bejjani et al., 2012; Doyle-Thomas et al., 2014; Joshi et al., 2013; Hassan et al., 2013; Naaijen et al., 2016) and cerebellum (Hassan et al., 2013; Ito et al., 2017) (Table 1).

By definition, ASD is a neurodevelopmental condition and how the brain matures is acknowledged to be quite different to even age matched controls (Amaral et al., 2008; Aoki et al., 2012; Redcay and Courchesne, 2005). Thus, results of [1H]MRS studies in ASD – as other MR modalities – may vary depending on the age group sampled. For example, Corrigan et al. (2013) have reported lower Glx in the cerebral white matter of 3–4-year-old children; however, there was no change in Glx when the same children were scanned again at age 6–7 and 9–10 years; and no difference in grey matter Glx at any age in childhood (Table 1). However, even in adulthood, when the brain may be expected to have fully matured, [1H]MRS studies of glutamate have generated conflicting findings. Lower Glx/Glu has been reported in the anterior cingulate cortex in ASD adults (Bernardi et al., 2011; Tebartz van Elst et al., 2014) (Table 1), but others have observed no significant difference in Glx between ASD adults and controls in this cortical region (Endres et al., 2017; Libero et al., 2015, 2016) (Table 1). Horder et al. (2013, 2018) reported lower Glx/Glu in the basal ganglia of adults with ASD, but unchanged cortical levels (Table 1).

In addition to differences in study samples and brain regions examined, different [1H]MRS sequences for quantification of Glx and/or Glu have been employed in the studies reviewed here (Table 1). While conventional [1H]MRS sequences (e.g. STEAM, PRESS) were used by most, unedited (equivalent to a PRESS) (Horder et al., 2018; Robertson et al., 2015) as well as the difference MEGA-PRESS spectra (edit ON-

edit OFF subtraction; please see below) (Ajram et al., 2017; Cavalho Pereira et al., 2018) were also used to quantify Glx and/or Glu (Table 1).

In order to sample GABA, customised spectral editing sequences, such as Meshcher-Garwood Point-resolved spectroscopy (MEGA-PRESS) carried out at 3 T or higher field strengths are required (Mescher et al., 1998; Mullins et al., 2014). In MEGA-PRESS, a GABA signal can be resolved from the more prominent Cr + PCr signal at 3 ppm by applying an editing pulse (edit ON) to the GABA signal at 1.9 ppm interleaved with edit OFF pulse (no frequency-selective editing pulses applied, or if applied, at a different frequency) (Cleve et al., 2015; Long et al., 2015; Maddock et al., 2016; Mescher et al., 1998; Mullins et al., 2014; Shungu et al., 2016). The application of this editing pulse results in the co-editing of other molecules present in the final spectrum, namely the Glx at 3.75 ppm and 'macromolecules' at 3 ppm (Cleve et al., 2015; O'Gorman et al., 2011). Thus, the GABA signal at 3 ppm is commonly reported as GABA + (Table 1). Furthermore, due to low tissue concentration of GABA, MEGA-PRESS requires larger ROI volumes (typically around 27 cm$^3$) and longer acquisition times ideally between 7 and 10 min (Mikkelsen et al., 2018). This was generally applied in all but two reviewed study (Kirovski et al., 2018; Kubas et al., 2012) (Table 1). In ASD, most studies of [1H]MRS GABA + have been conducted only in the last four years (Table 1). The majority have recruited children (Brix et al., 2015; Cavalho Pereira et al., 2018; Cochran et al., 2015; Drenthen et al., 2016; Gaetz et al., 2014; Goji et al., 2017; Harada et al., 2011; Ito et al., 2017; Kubas et al., 2012; Puts et al., 2017; Rojas et al., 2014), with three studies involving only adults (Ajram et al., 2017; Horder et al., 2018; Kirovski et al., 2018; Robertson et al., 2015), and one combining children and adults (Port et al., 2017). The pattern of GABA + differences in ASD compared to typically developing controls is a little more consistent than for Glx and Glu, with either lower measures or no difference in regional GABA + reported. Thus, lower GABA + has been found in the motor, auditory and frontal cortices and in the sensorimotor regions, anterior cingulate cortex and cerebellum (Gaetz et al., 2014; Harada et al., 2011; Ito et al., 2017; Kubas et al., 2012; Port et al., 2017; Puts et al., 2017; Rojas et al., 2014), whereas others have observed no differences in GABA + in the prefrontal and occipital cortices (Ajram et al., 2017; Brix et al., 2015; Cavalho Pereira et al., 2018; Cochran et al., 2015; Drenthen et al., 2016; Gaetz et al., 2014; Goji et al., 2017; Horder et al., 2018; Puts et al., 2017; Robertson et al., 2015), lenticular nuclei (Harada et al., 2011), motor cortex (Robertson et al., 2015), superior temporal sulcus (Kirovski et al., 2018), basal ganglia (Horder et al., 2018) and cerebellum (Goji et al., 2017) (Table 1).

Reduction in GABA in the somatosensory cortex has been associated with altered tactile function in children with ASD (Puts et al., 2017). Interestingly, developmental tactile perception abnormalities due to a loss of presynaptic inhibition of somatosensory neuron transmission have been shown to result in ASD-related social interaction deficits in mice (Orefice et al., 2016). However, even in the absence of group differences in GABA +, a role for GABA in ASD has still been argued based on findings that [1H]MRS GABA + levels in ASD are inversely correlated with scores on the Autism Spectrum Screening Questionnaire (Brix et al., 2015) and with the communication and delayed development scores of the Autism Diagnostic Interview – Revised (Cavalho Pereira et al., 2018). More difficult to disentangle are the results from Robertson et al. (2015). In their study, occipital GABA + measures were equivalent in ASD and control groups, but the ASD group had a deficit in the performance of a binocular rivalry test of visual function thought to rely upon visual cortical E-I balance. The authors went on to show that a positive correlation between GABA + and performance on a binocular rivalry task in control group was not evident in the ASD group, and suggested that the results supported an impairment in 'inhibitory signalling' in ASD (Robertson et al., 2015). This may well be the case, but because [1H]MRS captures the metabolites within a composite of cell types and does not capture active neurotransmission, it is

impossible to say with certainty.

## 4. E-I flux in ASD

One perspective on this constellation of [1H]MRS findings in ASD, is that their range may mirror the etiological and phenotypical heterogeneity of the spectrum as well as the variety in the typically developing control groups used for comparison. For example, [1H]MRS measures of glutamate and GABA+ differ even within the typically developing population (Bogner et al., 2010; Brix et al., 2017; Kaiser et al., 2005; O'Gorman et al., 2011; Sailasuta et al., 2008).

Another consideration for the field is that, in the main, indices of glutamate and GABA have been assessed separately in ASD; yet the results have been interpreted in terms of E-I balance (Dickinson et al., 2016). It could be argued that a true imbalance can only be uncovered if the level of each metabolite is reported. Thus, if a GABA decrease is accompanied by a glutamate decrease, then the system may remain balanced. Moreover, until recently, the logic of trying to capture metabolites within a glutamine-glutamate-GABA cycle that is constantly changing or in 'flux' in cohorts with and without ASD, has not been questioned. We now suggest that replication difficulties in cross-sectional studies of glutamate and GABA pathways in ASD arise, at least in part, because they sample only one part of an E-I system, which is a 'moving target'. As described earlier, the regulatory enzymes controlling this dynamic system are altered in ASD, and so E-I flux in ASD is therefore very likely to be atypical.

Supporting this hypothesis, there is now evidence that [1H]MRS is sensitive to changes in E-I flux elicited by pharmacological probes that challenge the system. This comes initially from studies in rats, which have established that [1H]MRS can reliably capture changes in glutamate and GABA levels following pharmacological interventions (Waschkies et al., 2014). Specifically, [1H]MRS was used to track the E-I response to pharmacologically relevant doses of compounds with a known mechanism of action on GABAergic and glutamatergic neurotransmission, namely vigabatrin, 3-mercaptopropionate, tiagabine, methionine sulfoximine, and riluzole. Clear drug dose-[1H]MRS effect relationships were demonstrated, with changes as low as 6% in glutamate and 12% in GABA reliably measured using [1H]MRS. The authors concluded that quantitative [1H]MRS of glutamate and GABA may usefully reveal E-I 'target engagement' – in other words, the interaction of a drug with its biological goal (Waschkies et al., 2014). We have recently extended this approach to humans. Using [1H]MRS, we found that the anti-glutamate and pro-GABA drug riluzole increased the proportion of GABA+ relative to the total level of GABA+ and Glx metabolites in the prefrontal cortex in ASD, but decreased it in controls, despite a comparable baseline between groups (Ajram et al., 2017).

This difference in E-I 'responsivity' in ASD has important implications for the development of pharmacological treatments for ASD, as it suggests that drugs may not act in the ASD brain the same way as in the typically developing brain. That is, selecting a compound for a trial/treatment in ASD based on its mechanism of action in the typical brain may not always be appropriate. Moreover, the response to pharmacological treatments in ASD may itself be heterogeneous. This may, for instance, explain why some – but not all – individuals with ASD can have paradoxical responses to GABA$_A$/benzodiazepine receptor agonists; usually these medications have a sedative effect, but 'excitation' has at times been reported in individuals with ASD (Lemonnier et al., 2012; Marrosu et al., 1987). It may also explain some of the trial disappointments in ASD. For example, lamotrigine, an anticonvulsant, was proposed to be a potential treatment for ASD because it inhibits synaptic glutamate release in excitatory neurons. Unfortunately, it did not improve clinical outcomes in children with ASD (Belsito et al., 2001). A similar failure followed the use of D-cycloserine, a partial agonist at the NMDA glutamate receptor (Belsito et al., 2001; Minshawi et al., 2016; Posey et al., 2004).

## 5. Conclusions

We conclude that disruption within E-I systems could serve as a biomarker for ASD. However, we extend this observation to suggest that E-I systems in the ASD brain are pharmacologically atypical. However, the picture is far from straight-forward. This means that we need better strategies, potentially including [1H]MRS, to examine the biological response or target engagement to proposed treatments in sub-groups of individuals with ASD. The identification of more pharmacologically homogeneous sub-groups within the spectrum may inform a new generation of biologically-driven clinical trials and avoid the time and expense (and failure) of un-stratified large-scale studies.

## Author contributions

L.A.A., M.M.P., and G.M.M. designed the study. L.A.A., A.M.S.D., A.C.P., H.E.V., M.M.P., and G.M.M. wrote the manuscript. All authors contributed to and approved the final manuscript.

## Role of the funding source

## Declarations of interest

None.

## References

Ajram, L.A., Horder, J., Mendez, M.A., Galanopoulos, A., Brennan, L.P., Wichers, R.H., et al., 2017. Shifting brain inhibitory balance and connectivity of the prefrontal cortex of adults with autism spectrum disorder. Transl. Psychiatry 7, e1137.

Alger, J.R., 2010. Quantitative proton magnetic resonance spectroscopy and spectroscopic imaging of the brain: a didactic review. Top. Magn. Reson. Imaging 21, 115–128.

Amaral, D.G., Schumann, C.M., Nordahl, C.W., 2008. Neuroanatomy of autism. Trends Neurosci. 31, 137–145.

American Psychiatric Association, 2013. Diagnostic and Statistical Manual of Mental Disorders, 5th ed. American Psychiatric Press, Arlington.

Aoki, Y., Abe, O., Yahata, N., Kuwabara, H., Natsubori, T., Iwashiro, N., et al., 2012. Absence of age-related prefrontal NAA change in adults with autism spectrum disorders. Transl. Psychiatry 2, e178.

Astrakas, L.G., Argyropoulou, M.I., 2016. Key concepts in MR spectroscopy and practical approaches to gaining biochemical information in children. Pediatr. Radiol. 46, 941–951.

Bear, M.F., Huber, K.M., Warren, S.T., 2004. The mGluR theory of fragile X mental retardation. Trends Neurosci. 27, 370–377.

Bejjani, A., O'Neill, J., Kim, J.A., Frew, J.F., Jee, V.W., Ly, R., et al., 2012. Elevated glutamatergic compounds in pregenual anterior cingulate in pediatric autism spectrum disorder demonstrated by1H MRS and 1H MRSI. PLoS One 7, e38786.

Belsito, K.M., Law, P.A., Kirk, K.S., Landa, R.J., Zimmerman, A.W., 2001. Lamotrigine therapy for autistic disorder: a randomized, double-blind, placebo-controlled trial. J. Autism Dev. Disord. 31, 175–181.

Bernardi, S., Anagnostou, E., Shen, J., Kolevzon, A., Buxbaum, J.D., Hollander, E., et al.,

2011. In vivo 1H-magnetic resonance spectroscopy study of the attentional networks in autism. Brain Res. 1380, 198–205.

Blatt, G.J., Fatemi, S.H., 2011. Alterations in GABAergic biomarkers in the autism brain: research findings and clinical implications. Anat. Rec. (Hoboken) 294, 1646–1652.

Blüml, S., 2013. Magnetic resonance spectroscopy: basics. In: Blüml, S., Panigrahy, A. (Eds.), MR Spectroscopy of Pediatric Brain Disorders. Springer, New York, pp. 11–23.

Bogner, W., Gruber, S., Doelken, M., Stadlbauer, A., Ganslandt, O., Boettcher, U., et al., 2010. In vivo quantification of intracerebral GABA by single-voxel 1H-MRS—how reproducible are the results? Eur. J. Radiol. 73, 526–531.

Bolton, P.F., Carcani-Rathwell, I., Hutton, J., Goode, S., Howlin, P., Rutter, M., 2011. Epilepsy in autism: features and correlates. Br. J. Psychiatry 198, 289–294.

Brix, M.K., Ersland, L., Hugdahl, K., Grüner, R., Posserud, M.B., Hammar, A., et al., 2015. Brain MR spectroscopy in autism spectrum disorder—the GABA excitatory/inhibitory imbalance theory revisited. Front. Hum. Neurosci. 9, 1–12.

Brix, M.K., Ersland, L., Hugdahl, K., Dwyer, G.E., Grüner, R., Noeske, R., et al., 2017. Within- and between-session reproducibility of GABA measurements with MR spectroscopy. J. Magn. Reson. Imaging 46, 421–430.

Brown, M.S., Singel, D., Hepburn, S., Rojas, D.C., 2013. Increased glutamate concentration in the auditory cortex of persons with autism and first-degree relatives: A (1)H-MRS study. Autism Res. 6, 1–10.

Buescher, A.V., Cidav, Z., Knapp, M., Mandell, D.S., 2014. Costs of autism spectrum disorders in the United Kingdom and the United States. JAMA Pediatr. 168, 721–728.

Burrows, E.L., Laskaris, L., Koyama, L., Churilov, L., Bornstein, J.C., Hill-Yardin, E.L., et al., 2015. A neuroligin-3 mutation implicated in autism causes abnormal aggression and increases repetitive behavior in mice. Mol. Autism 6, 62.

Cavalho Pereira, A., Violante, I.R., Mouga, S., Oliveria, G., Castelo-Branco, M., 2018. Medial frontal lobe neurochemistry in autism spectrum disorder is marked by reduced N-acetylaspartate and unchanged gamma-aminobutyric acid and glutamate + glutamine levels. J. Autism Dev. Discord. 48, 1467–1482.

Chaste, P., Sanders, S.J., Mohan, K.N., Klein, L., Song, Y., Murtha, M.T., et al., 2014. Modest impact on risk for autism spectrum disorder of rare copy number variants at 15q11.2, specifically breakpoints 1 to 2. Autism Res. 7, 355–362.

Ching, M.S.L., Shen, Y., Tan, W., Jeste, S.S., Shafali, S.J., Morrow, E.M., Chen, X., et al., 2010. Deletions of NRXN1 (Neurexin-1) predispose to a wide spectrum of developmental disorders. Am. J. Med. Genet. B. Neuropsychiatr. Genet. 153B, 937–947.

Cleve, M., Gussew, A., Reichenbach, J.R., 2015. In vivo detection of acute pain-induced changes of GABA+ and Glx in the human brain by using functional 1H MEGA-PRESS MR spectroscopy. NeuroImage 105, 67–75.

Cochran, D.M., Sikoglu, E.M., Hodge, S.M., Edden, R.A.E., Foley, A., Kennedy, D.N., et al., 2015. Relationship among glutamine, γ-aminobutyric acid, and social cognition in autism spectrum disorders. J. Child Adolesc. Psychopharmacol. 25, 314–322.

Corrigan, N.M., Shaw, D.W., Estes, A.M., Richards, T.L., Munson, J., Friedman, S.D., et al., 2013. Atypical developmental patterns of brain chemistry in children with autism spectrum disorder. JAMA Psychiat. 70, 964–974.

Dager, S.R., Corrigan, N.M., Richards, T.L., Posse, S., 2008. Research applications of magnetic resonance spectroscopy to investigate psychiatric disorders. Top. Magn. Reson. Imaging 19, 81–96.

Darnell, J.C., Van Driesche, S.J., Zhang, C., Hung, K.Y., Mele, A., Fraser, C.E., 2011. FMRP stalls ribosomal translocation on mRNAs linked to synaptic function and autism. Cell 146, 247–261.

Delorey, T.M., Handforth, A., Anagnostaras, S.G., Homanics, G.E., Minassian, B.A., Asatourian, A., Fanselow, M.S., et al., 1998. Mice lacking the beta3 subunit of the GABAA receptor have the epilepsy phenotype and many of the behavioral characteristics of angelman syndrome. J. Neurosci. 18, 8505–8514.

Devito, T.J., Drost, D.J., Neufeld, R.W., Rajakumar, N., Pavlosky, W., Williamson, P., et al., 2007. Evidence for cortical dysfunction in autism: a proton magnetic resonance spectroscopic imaging study. Biol. Psychiatry 61, 465–473.

Dickinson, A., Jones, M., Milne, E., 2016. Measuring neural excitation and inhibition in autism: different approaches, different findings and different interpretations. Brain Res. 1648, 277–289.

Doyle-Thomas, K.A.R., Card, D., Soorya, L.V., Wang, A.T., Fan, J., Anagnostou, E., 2014. Metabolic mapping of deep brain structures and associations with symptomatology in autism spectrum disorders. Res. Autism Spectr. Disord. 8, 44–51.

Drenthen, G.S., Barendse, E.M., Aldenkamp, A.P., van Veenendaal, T.M., Puts, N.A., Edden, R.A., et al., 2016. Altered neurotransmitter metabolism in adolescents with high-functioning autism. Psychiatry Res. 256, 44–49.

Duarte, J.M., Lei, H., Mlynarik, V., Gruetter, R., 2012. The neurochemical profile quantified by in vivo 1H NMR spectroscopy. NeuroImage 61, 342–362.

Edden, R.A., Puts, N.A., Barker, P.B., 2012. Macromolecule-suppressed GABA-edited magnetic resonance spectroscopy at 3T. Magn. Reson. Med. 68, 657–661.

Edden, R.A., Puts, N.A., Harris, A.D., Barker, P.B., Evans, C.J., 2014. Gannet: a batch-processing tool for the quantitative analysis of gamma-aminobutyric acid-edited MR spectroscopy spectra. J. Magn. Reson. Imaging 40, 1445–1452.

Endres, D., Tebartz Van Elst, L., Meyer, S.A., Feige, B., Nickel, K., Bubl, A., et al., 2017. Glutathione metabolism in the prefrontal brain of adults with high-functioning autism spectrum disorder: an MRS study. Mol. Autism 8, 10.

Etherton, M., Földy, C., Sharma, S., Tabuchi, K., Liu, X., Shamloo, M., et al., 2011a. Autism-linked neuroligin-3 R451C mutation differentially alters hippocampal and cortical synaptic function. PNAS 108, 13764–13769.

Etherton, M.R., Tabuchi, K., Sharma, M., Ko, J., Südhof, T.C., 2011b. An autism-associated point mutation in the neuroligin cytoplasmic tail selectively impairs AMPA receptor-mediated synaptic transmission in hippocampus. EMBO J. 30, 2908–2919.

Fatemi, S.H., Folsom, T.D., 2011. Dysregulation of fragile × mental retardation protein and metabotropic glutamate receptor 5 in superior frontal cortex of individuals with autism: a postmortem brain study. Mol. Autism 2, 6.

Fatemi, S.H., Halt, A.R., Stary, J.M., Kanodia, R., Schulz, S.C., Realmuto, G.R., 2002.

Glutamic acid decarboxylase 65 and 67 kDa proteins are reduced in autistic parietal and cerebellar cortices. Biol. Psychiatry 52, 805–810.

Fatemi, S.H., Reutiman, T.J., Folsom, T.D., Huang, H., Oishi, K., Mori, S., et al., 2008. Maternal infection leads to abnormal gene regulation and brain atrophy in mouse offspring: implications for genesis of neurodevelopmental disorderss. Schizophr. Res. 99, 56–70.

Fatemi, S.H., Reutiman, T.J., Folsom, T.D., Thuras, P.D., 2009. GABA(A) receptor downregulation in brains of subjects with autism. J. Autism Dev. Disord. 39, 223–230.

Fatemi, S.H., Folsom, T.D., Kneeland, R.E., Liesch, S.B., 2011. Metabotropic glutamate receptor 5 upregulation in children with autism is associated with underexpression of both Fragile X Mental retardation protein and GABAA receptor beta 3 in adults with autism. Anat. Rec. (Hoboken) 294, 1635–1645.

Fatemi, S.H., Reutiman, T.J., Folsom, T.D., Rustan, O.G., Rooney, R.J., Thuras, P.D., 2014. Downregulation of GABAA receptor protein subunits alpha6, beta2, delta, epsilon, gamma2, theta, and rho2 in superior frontal cortex of subjects with autism. J. Autism Dev. Disord. 44, 1833–1845.

Ford, T.C., Crewther, D.P., 2016. A comprehensive review of the (1)H-MRS metabolite spectrum in autism spectrum disorder. Front. Mol. Neurosci. 9, 14.

Foss-Feig, J.H., Adkinson, B.D., Ji, J.L., Yang, G., Srihari, V.H., McPartland, J.C., et al., 2017. Searching for cross-diagnostic convergence: neural mechanisms governing excitation and inhibition balance in schizophrenia and autism spectrum disorders. Biol. Psychiatry 81, 848–861.

Friedman, S.D., Shaw, D.W., Artru, A.A., Dawson, G., Petropoulous, H., Dager, S.R., 2006. Gray and white matter brain chemistry in young children with autism. Arch. Gen. Psychiatry 63, 786.

Fukuchi, M., Nii, T., Ishimaru, N., Minamino, A., Hara, D., Takasaki, I., et al., 2009. Valproic acid induces up- or down-regulation of gene expression responsible for the neuronal excitation and inhibition in rat cortical neurons through its epigenetic actions. Neurosci. Res. 65, 35–43.

Futai, K., Kim, M.J., Hashikawa, T., Scheiffele, P., Sheng, M., Hayashi, Y., 2007. Retrograde modulation of presynaptic release probability through signaling mediated by PSD-95–neuroligin. Nat. Neurosci. 10, 186–195.

Gaetz, W., Bloy, L., Wang, D.J., Port, R.G., Blaskey, L., Levy, S.E., Roberts, T.P., 2014. GABA estimation in the brains of children on the autism spectrum: measurement precision and regional cortical variation. NeuroImage 86, 1–9.

Gandal, M.J., Anderson, R.L., Billingslea, E.N., Carlson, G.C., Roberts, T.P., Siegel, S.J., 2012. Mice with reduced NMDA receptor expression: more consistent with autism than schizophrenia? Genes Brain Behav. 11, 740–750.

Gao, R., Penzes, P., 2015. Common mechanisms of excitatory and inhibitory imbalance in schizophrenia and autism spectrum disorders. Curr. Mol. Med. 15, 146–167.

Gatto, C.L., Broadie, K., 2010. Genetic controls balancing excitatory and inhibitory synaptogenesis in neurodevelopmental disorder models. Front. Synaptic. Neurosci. 2, 4.

Giovanoli, S., Weber, L., Meyer, U., 2014. Single and combined effects of prenatal immune activation and peripubertal stress on parvalbumin and reelin expression in the hippocampal formation. Brain Behav. Immun. 40, 48–54.

Goji, A., Ito, H., Mori, K., Harada, M., Hisaoka, S., Toda, Y., et al., 2017. Assessment of anterior cingulate cortex (ACC) and left cerebellar metabolism in asperger's syndrome with proton magnetic resonance spectroscopy (MRS). PLoS One 12, e0169288.

Govindaraju, V., Young, K., Maudsley, A.A., 2000. Proton NMR chemical shifts and coupling constants for brain metabolites. NMR Biomed. 13 (3), 129–153.

Grayton, H.M., Missler, M., Collier, D.A., Fernandes, C., 2013. Altered social behaviours in neurexin 1 knockout mice resemble core symptoms in neurodevelopmental disorders. PLoS One 8, e67114.

Gussew, A., Erdtel, M., Hiepe, P., Rzanny, R., Reichenbach, J.R., 2012. Absolute quantitation of brain metabolites with respect to heterogeneous tissue compositions in (1)H-MR spectroscopic volumes. MAGMA 25, 321–333.

Harada, M., Taki, M.M., Nose, A., Kubo, H., Mori, K., Nishitani, H., et al., 2011. Non-invasive evaluation of the GABAergic/glutamatergic system in autistic patients observed by MEGA-editing proton MR spectroscopy using a clinical 3 tesla instrument. J. Autism Dev. Disord. 41, 447–454.

Hardan, A.Y., Minshew, N.J., Melhem, N.M., Srihari, S., Jo, B., Bansal, R., et al., 2008. An MRI and proton spectroscopy study of the thalamus in children with autism. Psychiatry Res. 163, 97–105.

Hassan, T.H., Abdelrahman, H.M., Abdel Fattah, N.R., El-Masry, N.M., Hashim, H.M., El-Gerby, K.M., et al., 2013. Blood and brain glutamate levels in children with autistic disorder. Res. Autism Spectr. Disord. 7, 541–548.

Hegarty, J.P., Gu, M., Spielman, D.M., Cleveland, S.C., Hallmayer, J.F., Lazzeroni, L.C., et al., 2017. A proton MR spectroscopy study of the thalamus in twins with autism spectrum disorder. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 81, 153–160.

Henry, P.G., Dautry, C., Hantraye, P., Bloch, G., 2001. Brain GABA editing without macromolecule contamination. Magn. Reson. Med. 45, 517–520.

Horder, J., Lavender, T., Mendez, M.A., O'Gorman, R., Daly, E., Craig, M.C., et al., 2013. Reduced subcortical glutamate/glutamine in adults with autism spectrum disorders: A ^{1}H MRS study. Transl. Psychiatry 3, e279.

Horder, J., Petrinovic, M.M., Mendez, M.A., Bruns, A., Takumi, T., Spooren, W., et al., 2018. Glutamate and GABA in autism spectrum disorder-a translational magnetic resonance spectroscopy study in man and rodent models. Transl. Psychiatry 8, 106.

Isles, A.R., Ingason, A., Lowther, C., Walters, J., Gawlick, M., Stöber, G., et al., 2016. Parental origin of interstitial duplications at 15q11.2-q13.3 in schizophrenia and neurodevelopmental disorders. PLoS Genet. 12, e1005993.

Ito, H., Mori, K., Harada, M., Hisaoka, S., Toda, Y., Mori, et al., 2017. A proton magnetic resonance spectroscopic study in autism spectrum disorder using a 3-tesla clinical magnetic resonance imaging (MRI) system: the anterior cingulate cortex and the left cerebellum. J. Child Neurol. 32, 731–739.

Jamain, S., Quach, H., Betancur, C., Rastam, M., Colineaux, C., Gillberg, C., et al., 2003.

Mutations of the X-linked genes encoding neuroligins NLGN3 and NLGN4 are associated with autism. Nat. Genet. 34, 27–29.

Jansen, J.F., Backes, W.H., Nicolay, K., Kooi, M.E., 2006. 1H MR spectroscopy of the brain: absolute quantification of metabolites. Radiology 240, 318–332.

Jaramillo, T.C., Liu, S., Pettersen, A., Birnbaum, S.G., Powell, C.M., 2014. Autism-related neuroligin-3 mutation alters social behavior and spatial learning. Autism Res. 7, 264–272.

Joshi, G., Biederman, J., Wosniak, J., Goldin, R.L., Crowley, D., Furtak, S., et al., 2013. Magnetic resonance spectroscopy study of the glutamatergic system in adolescent males with high-functioning autistic disorder: a pilot study at 4T. Eur. Arch. Psychiatry Clin. Neurosci. 263, 379–384.

Juchem, C., Rothman, D.L., 2014. Basis of magnetic resonance. In: Stagg, C., Rothman, D.L. (Eds.), Magnetic Resonance Spectroscopy. Academic Press, San Diego, pp. 3–14.

Kaiser, L.G., Schuff, N., Cashdollar, N., Weiner, M.W., 2005. Age-related glutamate and glutamine concentration changes in normal human brain: 1H MR spectroscopy study at 4 T. Neurobiol. Aging 26, 665–672.

Kattenstroh, G., Tantalaki, E., Südhof, T.C., Gottmann, K., Missler, M., 2004. Postsynaptic N-methyl-D-aspartate receptor function requires alpha-neurexins. PNAS 101, 2607–2612.

Kirovski, M., Suo, C., Enticott, P.G., Yücel, M., Fitzgerald, P.B., 2018. Sex-linked differences in gamma-aminobutyric acid (GABA) are related to social functioning in autism spectrum disorder. Psychiatry Res. 274, 19–22.

Kubas, B., Kulak, W., Sobaniec, W., Tarasow, E., Lebkowska, U., Walecki, J., 2012. Metabolite alterations in autistic children: a 1H MR spectroscopy study. Adv. Med. Sci. 57, 152–156.

Lai, M.C., Lombardo, M.V., Baron-Cohen, S., 2014. Autism. Lancet 383, 896–910.

Lee, E., Lee, J., Kim, E., 2017. Excitation/inhibition imbalance in animal models of autism spectrum disorders. Biol. Psychiatry 81, 838–847.

Lemonnier, E., Degrez, C., Phelep, M., Tyzio, R., Josse, F., Grandgeorge, M., et al., 2012. A randomised controlled trial of bumetanide in the treatment of autism in children. Transl. Psychiatry 2, e202.

Libero, L.E., Deramus, T.P., Lahti, A.C., Deshpande, G., Kana, R.K., 2015. Multimodal neuroimaging based classification of autism spectrum disorder using anatomical, neurochemical, and white matter correlates. Cortex 66, 46–59.

Libero, L.E., Reid, M.A., White, D.M., Salibi, N., Lahti, A.C., Kana, R.K., 2016. Biochemistry of the cingulate cortex in autism: an MR spectroscopy study. Autism Res. 9, 643–657.

Long, Z., Dyke, J.P., Ma, R., Huang, C.C., Louis, E.D., Dydak, U., 2015. Reproducibility and effect of tissue composition on cerebellar γ-aminobutyric acid (GABA) MRS in an elderly population. NMR Biomed. 28, 1315–1323.

Lozano, R., Rosero, C.A., Hagerman, R.J., 2014. Fragile X spectrum disorders. Intractable Rare Dis. Res. 3, 134–146.

Lujan, R., Shigemoto, R., Lopez-Bendito, G., 2005. Glutamate and GABA receptor signalling in the developing brain. Neuroscience 130, 567–580.

Maddock, R.J., Casazza, G.A., Fernandez, D.H., Maddock, M.I., 2016. Acute modulation of cortical glutamate and GABA content by physical activity. J. Neurosci. 36, 2449–2457.

Marrosu, F., Marrosu, G., Rachel, M.G., Biggio, G., 1987. Paradoxical reactions elicited by diazepam in children with classic autism. Funct. Neurol. 2, 355–361.

Mescher, M., Merkle, H., Kirsch, J., Garwood, M., Gruetter, R., 1998. Simultaneous in vivo spectral editing and water suppression. NMR Biomed. 11, 266–272.

Meyer, U., Feldon, J., Dammann, O., 2011. Schizophrenia and autism: both shared and disorder-specific pathogenesis via perinatal inflammation? Pediatr. Res. 69, 26R–33R.

Mikkelsen, M., Barker, P.B., Bhattacharyya, P.K., Brix, M.K., Buur, P.F., Cecil, K.M., 2017. Big GABA: edited MR spectroscopy at 24 research sites. NeuroImage 159, 32–45.

Mikkelsen, M., Loo, R.S., Puts, N.A.J., Edden, R.A.E., Harris, A.D., 2018. Designing GABA-edited magnetic resonance spectroscopy studies: Considerations of scan duration, signal-to-noise ratio and sample size. J. Neurosci. Methods 303, 86–94.

Minshawi, N.F., Wink, L.K., Shaffer, R., Plawecki, M.H., Posey, D.J., Liu, H., et al., 2016. A randomized, placebo-controlled trial of D-cycloserine for the enhancement of social skills training in autism spectrum disorders. Mol. Autism 7, 2.

Missler, M., Zhang, W., Rohlmann, A., Kattenstroth, G., Hammer, R.E., Gottmann, K., et al., 2003. Alpha-neurexins couple Ca2+ channels to synaptic vesicle exocytosis. Nature 423, 939–948.

Moreno-De-Luca, D., Sanders, S.J., Willsey, A.J., Mulle, J.G., Lowe, J.K., Geschwind, D.H., et al., 2013. Using large clinical data sets to infer pathogenicity for rare copy number variants in autism cohorts. Mol. Psychiatry 18, 1090–1095.

Muhle, R., Trentacoste, S.V., Rapin, I., 2004. The genetics of autism. Pediatrics 113, e472–e486.

Mullins, P.G., Mcgonigle, D.J., O'Gorman, R.L., Puts, N.A., Vidyasagar, R., Evans, C.J., Cardiff Symposium On, M.R.S.O.G., Edden, R.A., 2014. Current practice in the use of MEGA-PRESS spectroscopy for the detection of GABA. NeuroImage 86, 43–52.

Mullins, C., Fishell, G., Tsien, R.W., 2016. Unifying views of autism spectrum disorders: a consideration of autoregulatory feedback loops. Neuron 89, 1131–1156.

Naaijen, J., Zwiers, M.P., Amiri, H., Williams, S.C., Durston, S., Oranje, B., et al., 2016. Fronto-striatal glutamate in autism spectrum disorder and obsessive compulsive disorder. Neuropsychopharmacology 42, 2466–2467.

Naressi, A., Couturier, C., Castang, I., De Beer, R., Graveron-Demilly, D., 2001. Java-based graphical user interface for MRUI, a software package for quantitation of in vivo/medical magnetic resonance spectroscopy signals. Comput. Biol. Med. 31, 269–286.

Near, J., Simpson, R., Cowen, P., Jezzard, P., 2011. Efficient gamma-aminobutyric acid editing at 3T without macromolecule contamination: MEGA-SPECIAL. NMR Biomed. 24, 1277–1285.

Nelson, S.B., Valakh, V., 2015. Excitatory/inhibitory balance and circuit homeostasis in autism spectrum disorders. Neuron 87, 684–698.

Oblak, A., Gibbs, T.T., Blatt, G.J., 2009. Decreased GABAA receptors and benzodiazepine binding sites in the anterior cingulate cortex in autism. Autism Res. 2, 205–219.

Oblak, A.L., Gibbs, T.T., Blatt, G.J., 2010. Decreased GABA(B) receptors in the cingulate cortex and fusiform gyrus in autism. J. Neurochem. 114, 1414–1423.

Oblak, A.L., Gibbs, T.T., Blatt, G.J., 2011. Reduced GABAA receptors and benzodiazepine binding sites in the posterior cingulate cortex and fusiform gyrus in autism. Brain Res. 1380, 218–228.

O'Gorman, R.L., Michels, L., Edden, R.A., Murdoch, J.B., Martin, E., 2011. In vivo detection of GABA and glutamate with MEGA-PRESS: reproducibility and gender effects. J. Magn. Reson. Imaging 33, 1262–1267.

Orefice, L.L., Zimmerman, A.L., Chirila, A.M., Sleboda, S.J., Head, J.P., Ginty, D.D., 2016. Peripheral mechanosensory neuron dysfunction underlies tactile and behavioral deficits in mouse models of ASDs. Cell 166, 299–313.

Orekhova, E.V., Stroganova, T.A., Nygren, G., Tsetlin, M.M., Posikera, I.N., Gillberg, C., et al., 2007. Excess of high frequency electroencephalogram oscillations in boys with autism. Biol. Psychiatry 62, 1022–1029.

Oz, G., Alger, J.R., Barker, P.B., Bartha, R., Bizzi, A., Boesch, C., 2014. Clinical proton MR spectroscopy in central nervous system disorders. Radiology 270, 658–679.

Page, L.A., Daly, E., Schmitz, N., Simmons, A., Toal, F., Deeley, Q., et al., 2006. In vivo 1H-magnetic resonance spectroscopy study of amygdala-hippocampal and parietal regions in autism. Am. J. Psychiatry 163, 2189–2192.

Port, R.G., Gaetz, W., Bloy, L., Wang, D., Blaskey, L., Kuschner, E.S., et al., 2017. Exploring the relationship between cortical GABA concentrations, auditory gamma-band responses and development in ASD: evidence for an altered maturational trajectory in ASD. Autism Res. 10, 593–607.

Posey, D.J., Kem, D.L., Swiezy, N.B., Sweeten, T.L., Wiegand, R.E., McDougle, C.J., 2004. A pilot study of D-cycloserine in subjects with autistic disorder. Am. J. Psychiatry 161, 2115–2117.

Provencher, S.W., 1993. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. Magn. Reson. Med. 30, 672–679.

Purcell, A.E., Jeon, O.H., Zimmerman, A.W., Blue, M.E., Pevsner, J., 2001. Postmortem brain abnormalities of the glutamate neurotransmitter system in autism. Neurology 57, 1618–1628.

Puts, N.A.J., Wodka, E.L., Harris, A.D., Crocetti, D., Tommerdahl, M., Mostofsky, S.H., et al., 2017. Reduced GABA and Altered Somatosensory Function in Children with Autism Spectrum Disorder. Autism Res. 10, 608–619.

Quadrelli, S., Mountford, C., Ramadan, S., 2016. Hitchhiker's guide to voxel segmentation for partial volume correction of in vivo magnetic resonance spectroscopy. Magn. Reson. Insights 9, 1–8.

Radyushkin, K., Hammerschmidt, K., Boretius, S., Varoqueaux, F., El-Kordi, A., Ronnenberg, A., et al., 2009. Neuroligin-3-deficient mice: model of a monogenic heritable form of autism with an olfactory deficit. Genes Brain Behav. 8, 416–425.

Redcay, E., Courchesne, E., 2005. When is the brain enlarged in autism? a meta-analysis of all brain size reports. Biol. Psychiatry 58, 1–9.

Robertson, C.E., Ratai, E., Kanwisher, N., 2015. Reduced GABAergic action in autistic brain. Curr. Biol. 26, 80–85.

Rojas, D.C., Singel, D., Steinmetz, S., Hepburn, S., Brown, M.S., 2014. Decreased left perisylvian GABA concentration in children with autism and unaffected siblings. NeuroImage 86, 28–34.

Rowley, N.M., Madsen, K.K., Schousboe, A., Steve White, H., 2012. Glutamate and GABA synthesis, release, transport and metabolism as targets for seizure control. Neurochem. Int. 61, 546–558.

Rubenstein, J.L.R., Merzenich, M.M., 2003. Model of autism: increased ratio of excitation/inhibition in key neural systems. Genes Brain Behav. 2, 255–267.

Sailasuta, N., Ernst, T., Chang, L., 2008. Regional variations and the effects of age and gender on glutamate concentrations in the human brain. Magn. Reson. Imaging 2, 667–675.

Schneider, J.F., 2016. MR spectroscopy in children: protocols and pitfalls in non-tumorous brain pathology. Pediatr. Radiol. 46, 963–982.

Schur, R.R., Draisma, L.W., Wijnen, J.P., Boks, M.P., Koevoets, M.G., Joels, M., 2016. Brain GABA levels across psychiatric disorders: a systematic literature review and meta-analysis of (1) H-MRS studies. Hum. Brain Mapp. 37, 3337–3352.

Selimbeyoglu, A., Kim, C.K., Inoue, M., Lee, S.Y., Hong, A.S.O., Kauvar, I., et al., 2017. Modulation of prefrontal cortex excitation/inhibition balance rescues social behavior in CNTNAP2-deficient mice. Sci. Transl. Med. 9.

Shao, Y., Cuccaro, M.L., Hauser, E.L., Raiford, K.L., Menold, M.M., Wolpert, C.M., et al., 2003. Fine mapping of autistic disorder to chromosome 15q11-q13 by use of phenotypic subtypes. Am. J. Hum. Genet. 72, 539–548.

Shimmura, C., Suzuki, K., Iwata, Y., Tsuchiya, K.L., Ohno, K., Matsuzaki, H., et al., 2013. Enzymes in the glutamate-glutamine cycle in the anterior cingulate cortex in postmortem brain of subjects with autism. Mol. Autism 4, 6.

Shungu, D.C., Mao, X., Donzales, R., Soones, T.R., Dyke, J.P., van der Veen, J.W., et al., 2016. Brain γ-aminobutyric acid (GABA) detection in vivo with the J-Editing 1 H MRS technique: a comprehensive methodological evaluation of sensitivity enhancement, macromolecule contamination and test-retest reliability. NMR Biomed. 29, 932–942.

Stagg, C.J., Bachtiar, V., Johansen-Berg, H., 2011. What are we measuring with GABA magnetic resonance spectroscopy? Commun. Integr. Biol. 4, 573–575.

Südhof, T.C., 2008. Neuroligins and neurexins link synaptic function to cognitive disease. Nature 455, 903–911.

Tabuchi, K., Blundell, J., Etherton, M.R., Hammer, R.E., Liu, X., Powell, C.M., et al., 2007. A neuroligin-3 mutation implicated in autism increases inhibitory synaptic transmission in mice. Science 318, 71–76.

Tebartz Van Elst, L., Maier, S., Fangmeier, T., Endres, D., Mueller, G.T., Nickel, K., et al., 2014. Disturbed cingulate glutamate metabolism in adults with high-functioning autism spectrum disorder: evidence in support of the excitatory/inhibitory imbalance

hypothesis. Mol. Psychiatry 19, 1314–1325.

Uchino, S., Waga, C., 2015. Novel therapeutic approach for autism spectrum disorder: focus on SHANK3. Curr. Neuropharmacol. 13, 786–792.

Urraca, N., Cleary, J., Brewer, V., Pivnick, E.K., McVicar, K., Thibert, R.L., et al., 2013. The interstitial duplication 15q11.2-q13 syndrome includes autism, mild facial anomalies and a characteristic EEG signature. Autism Res. 6, 268–279.

Uzunova, G., Pallanti, S., Hollander, E., 2016. Excitatory/inhibitory imbalance in autism spectrum disorders: implications for interventions and therapeutics. World J. Biol. Psychiatry 17, 174–186.

Verkerk, A.J., Pieretti, M., Sutcliffe, J.S., Fu, Y.H., Kuhl, D.P., Pizutti, A., et al., 1991. Identification of a gene (FMR-1) containing a CGG repeat coincident with a break-point cluster region exhibiting length variation in Fragile X syndrome. Cell 65, 905–914.

Waschkies, C.F., Bruns, A., Müller, S., Kapps, M., Borroni, E., von Kinelin, M., et al., 2014. Neuropharmacological and neurobiological relevance of in vivo (1)H-MRS of GABA and glutamate for preclinical drug discovery in mental disorders. Neuropsychopharmacology 39, 2331–2339.

Wei, R., Li, Q., Lam, S., Lueng, J., Cheung, C., Zhang, X., et al., 2016. A single low dose of valproic acid in late prenatal life alters postnatal behavior and glutamic acid decarboxylase levels in the mouse. Behav. Brain Res. 314, 190–198.

Xing, J., Kimura, H., Wang, C., Ishizuka, K., Kushima, I., Arioka, Y., et al., 2016. Resequencing and association analysis of six PSD-95-related genes as possible susceptibility genes for schizophrenia and autism spectrum disorders. Sci. Rep. 6, 27491.

Yip, J., Soghomonian, J., Blatt, G.J., 2007. Decreased GAD67 mRNA levels in cerebellar Purkinje cells in autism: pathophysiological implications. Acta Neuropathol. 113, 559–568.

Yizhar, O., Fenno, L.E., Prigge, M., Schneider, F., Davidson, T.J., O'Shea, D.J., et al., 2011. Neocortical excitation/inhibition balance in information processing and social dysfunction. Nature 477, 171–178.

Yu, T., Berry-Kravis, E., 2014. Autism and Fragile X syndrome. Semin. Neurol. 34, 258–265.

Zhang, W., 2005. Extracellular domains of-neurexins participate in regulating synaptic transmission by selectively affecting N- and P/Q-Type Ca2+ channels. J. Neurosci. 25, 4330–4342.

Zhu, H., Barker, P.B., 2011. MR spectroscopy and spectroscopic imaging of the brain. Methods Mol. Biol. 711, 203–226.