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The role of glutamate and its receptors in autism and the use of glutamate receptor antagonists in treatment

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Abstract

Glutamate is the major excitatory neurotransmitter in the brain and may be a key neurotransmitter involved in autism. Literature pertaining to glutamate and autism or related disorders (e.g., Fragile X syndrome) is reviewed in this article. Interest in glutamatergic dysfunction in autism is high due to increasing convergent evidence implicating the system in the disorder from peripheral biomarkers, neuroimaging, protein expression, genetics and animal models. Currently, there are no pharmaceutical interventions approved for autism that address glutamate deficits in the disorder. New treatments related to glutamatergic neurotransmission, however, are emerging. In addition, older glutamate-modulating medications with approved indications for use in other disorders are being investigated for re-tasking as treatments for autism. This review presents evidence in support of glutamate abnormalities in autism and the potential for translation into new treatments for the disorder.

Keywords

autism; mGluR; AMPA; NMDA; Kainate; proton spectroscopy; serum glutamate

Autism spectrum disorders

Autism spectrum disorders (ASD) are a complex set of behaviorally defined disorders, characterized by impairments in social interaction, communication and restricted or stereotyped behaviors (American Psychiatric Association 2013). During the DSM-IV era of autism diagnosis in the United States, impairments in these three areas were codified and required for the diagnosis of Autistic Disorder, part of the pervasive developmental disorders category of diagnoses. Researchers have moved away from the strict definition and have commonly combined 3 related disorders (Autistic Disorder, Asperger's Disorder and Pervasive Developmental Disorder - Not Otherwise Specified, or PDD-NOS) in DSM-IV together into the broader category of ASD. The newly released DSM-V moves toward this conceptualization by dropping Asperger's and PDDNOS, keeping Autistic Disorder and adding ASD. ASD are relatively common, with a population prevalence around 1 percent (Kogan et al. 2009). Hereafter, in this review I will use the term autism to collectively refer to ASD broadly, unless otherwise noted.

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Although in up to 10 percent of autism cases, there is a reasonably well-defined etiology (e.g., Fragile X syndrome, see below), most cases are idiopathic (Herman et al. 2007). As molecular discovery advances continue, the number of idiopathic cases will undoubtedly decrease. Importantly, the large number of idiopathic cases today may be partly due to underutilization of genetic testing services in the diagnostic workup for children with autism (Vande Wydeven et al. 2012).

Glutamate dysfunction has been central to conceptualizations of neurotransmitter involvement in autism for years, so much so that Fatemi (2008) proposed a *hyperglutamate theory* of autism, referring to a set of findings of increased levels of the amino acid in blood samples of children and adults with the disorder. The reverse has also been proposed as well, based on studies of glutamate receptor dysfunction and pharmacological effects of glutamatergic agonists and antagonists — a *hypoglutamate theory* (Carlsson 1998). This review covers evidence in support of glutamate involvement in autism from molecular biology to neuroimaging, ending with a discussion of current interest in the development of pharmaceutical interventions targeting glutamate receptors in the disorder. For this review, a search of published articles in PubMed using the keywords “autism” or “autistic” and “glutamate” was conducted. Studies of human neuroimaging, post-mortem analysis, genetics and treatment studies are reviewed, along with animal models of autism relevant to those topics.

Elevated blood plasma and serum in autism

Peripheral markers of glutamate dysfunction have been described in autism patients. An early article describing increased serum glutamate in autism also had the largest sample size to date (N=60 patients: Moreno et al. 1992). This was replicated by Moreno-Fuenmayor et al. (1996) in a smaller sample of autism patients compared to patient reference data from within the local hospital. This normative sample referent approach was also taken by Aldred et al. (2003), who in addition to reporting elevated plasma glutamate in children with autism, also observed significant elevations in groups of parents and siblings of the children with autism. Several studies have since used case control designs. Shinohe et al. (2006) reported that serum glutamate levels were significantly higher in adult subjects with autism (N=18) than in healthy control subjects (N=19). Social subscale scores on the ADI-R were correlated with glutamate (I.e., higher serum glutamate associated with poorer social ability). Three more recent studies using case controls have also reported significantly increased plasma glutamate (Tirouvanziam et al. 2011; Shimmura et al. 2011; Hassan et al. 2013). Only a single early study of plasma glutamate levels has failed to report increases in autism - Rolf et al. (1993) reported reduced glutamate in platelet rich samples from 18 individuals with autism. As glutamate does not easily pass the blood-brain barrier, it is somewhat unclear whether the elevated plasma glutamate levels reflect CNS levels of the amino acid. Direct measurement from post-mortem brain tissue using high performance liquid chromatography has shown elevations in glutamate and glutamine from the anterior cingulate cortex in 7 individuals with autism (Shimmura et al. 2013). Attempts to measure in vivo brain glutamate levels non-invasively using magnetic resonance spectroscopy have resulted in slightly more variable findings, however, as discussed below.

In-vivo evidence of increased glutamate in autism

Glutamate levels can be assessed *in vivo* using proton magnetic resonance spectroscopy (1H-MRS). 1H-MRS can provide measures of various metabolite concentrations in defined regions of the brain by their characteristic resonance in a strong magnetic field. Glutamate has a single resonance at 2.35 ppm, although many 1H-MRS studies combine the resonances of glutamine, glutamate and GABA together into a combined measure called Glx. This is due to concern over low signal-to-noise for the isolated glutamate signal, particularly in lower field strength magnets (e.g., 1.5 T). As there may be differences in autism in the concentration of those constituents, Glx or glutamate is specifically identified for each study listed below. It is also worth noting that although there are techniques for multi-voxel, "whole-brain" analyses of glutamate, most studies that focus on glutamate signals rely on single voxel, region of interest approaches due to limitations in the amount of signal produced by smaller voxels. Therefore, there are often differences in regions reported between research studies and differences in results may reflect either replication problems or regional variation in glutamate concentration.

There have been several studies of glutamate concentration in autism using 1HMRS. Page et al. (2006) were the first to examine Glx levels directly in autism, reporting higher concentrations in the right hippocampus in 20 individuals with autism compared to 13 healthy comparison subjects. A separate analysis of a region in the right parietal cortex did not exhibit significant differences in Glx between groups. Glx levels were not significantly correlated with IQ or autism symptom measures. Several other groups have reported significantly increased glutamate concentration since then in regions including the anterior cingulate gyrus (Joshi:2012ir; Bejjani et al. 2012) and auditory cortex (Brown et al. 2013). Others have reported reduced Glx in autism or no group differences in similar regions of interest (DeVito et al. 2007; Bernardi et al. 2011; Horder et al. 2013). Table 1 summarizes the findings to date from 1H-MRS studies of glutamate and Glx in autism.

No 1H-MRS studies to date have reported separate estimates of glutamine, glutamate's synthetic precursor. Additionally, since one of GABA's resonances is also confounded with the Glx measurement, and as GABA levels in autism have been reported to be reduced in 3 separate studies (Harada et al. 2010; Gaetz et al. 2013), future investigations should strongly consider reporting metabolites separately whenever possible. Another consideration for future efforts will be examination of co-morbid psychiatric and neurologic disorders. 1H-MRS technology has also been used to detect increased cortical glutamate concentration in patients with major depressive disorder (Hasler et al. 2007), obsessive-compulsive disorder (Whiteside et al. 2006), social anxiety disorder (Phan et al. 2005) and epilepsy (Doelken et al. 2010), all of which are common co-morbid conditions with autism.

1H-MRS studies of single gene disorders associated strongly with autism have yielded mixed findings. For example Bruno et al. (2013) observed *decreased* Glx in the caudate nucleus in 18 individuals with Fragile X syndrome. In contrast, Pan et al. (1999) observed *increased* gray matter concentration of glutamate in 6 girls with Rett syndrome. Additional larger studies of such disorders will be particularly valuable due to the known glutamatergic

neurotransmission deficits in several of the single gene conditions associated with autism (see below).

While 8 of the published MRS studies of Glx/Glu have reported increases in autism relative to controls, 4 have reported the reverse effect and a single study has reported no difference. In two studies reporting either negative effects or reductions in glutamate, subjects were sedated using drugs that are known to interact with GABA and glutamate transmission (DeVito et al. 2007; Corrigan et al. 2013), but such sedation is typically also used with lower functioning and/or younger children. Four studies to date have reported Glu levels separately or in addition to Glx (Harada et al. 2010; Joshi et al. 2012; Brown et al. 2013; Hassan et al. 2013). Of those, 3 reported increases in autism (Joshi et al. 2012; Brown et al. 2013; Hassan et al. 2013) and one reported no significant differences (Harada et al. 2010). Hassan et al. (2013) are the only group to date that have acquired both plasma and 1H-MRS measures of glutamate in the same sample, reporting significant increases in autism relative to controls with both measures, which were highly correlated.

Significant common limitations of the MRS technique are that it requires significant acquisition time, is therefore usually limited to a few regions of interest, and that those few regions of interest tend to be quite large. As such, the large variability in the MRS findings to date may reflect regional variation, sample specific characteristics (e.g., age, diet) or other methodological considerations. Indeed, for the 8 studies reporting from multiple regions of interest in Table 1 (not including L/R comparisons for same structure), 7 of the 8 report differing findings among those structures. None of them, however, report opposite findings for multiple structures (e.g., autism > control for one structure and autism < control for another). Further technological refinements allowing multi-voxel assessments within reasonable time-limits, as well as replication studies, will help resolve this problem, which ultimately may relate to poor statistical power to detect changes across multiple assessment sites.

To date, there have been no studies of the glutamate system using either positron emission tomography (PET) or single-photon emission computed tomography (SPECT) in autism. This is particularly unfortunate because only PET and SPECT can produce images of in vivo receptor binding, whereas 1H-MRS can only examine glutamate concentration and is not a receptor imaging technique. For a variety of reasons, ionotropic receptor radioligands have not been particularly successful for PET and SPECT (Majo et al. 2013). Recent developments in metabotropic glutamate receptor radioligands is encouraging, however, and this should be an exciting area of discovery for future autism studies (DeLorenzo et al. 2011).

Evidence of altered glutamate metabolism in autism

It is currently unclear why glutamate levels appear to be higher in brain and blood. Glutamate is synthesized from glutamine in the presynaptic terminals of neurons by the enzyme glutaminase. Much of the extracellular glutamate is reabsorbed by astrocytes and converted back into glutamine by glutamine synthetase. This recycling process is known as the glutamate-glutamine cycle. As we've seen from 1H-MRS studies, the published research

to date fails to separate the two molecules in analyses. Although levels of one of the molecules is highly predictive of levels of the other, it would still be of interest to separate them, particularly if there were alterations in the metabolic cycling between them. Shimmura et al. (2011) reported that while plasma levels of glutamate were significantly higher in children with autism, glutamine levels were significantly reduced compared to controls. A recent study by the same group of the anterior cingulate cortex in post-mortem tissue samples found that kidney-type glutaminase levels were reduced in the autism group, but not levels of liver-type glutaminase, glutamate synthetase or the glutamate dehydrogenases (Shimmura et al. 2013). This was interpreted to mean that the glutamate-glutamine cycle might be shifted in the direction of glutamine, but on the surface this would seem to be at odds with most of the plasma glutamate and 1H-MRS results for glutamate proper, which suggest elevated levels in autism. The authors point out, however, that glutaminase is also involved in the production of GABA and that alterations specific to GABAergic cells might be responsible for the results.

In GABAergic inhibitory neurons, glutamate is further synthesized into GABA by glutamate decarboxlyase (a.k.a. glutamic acid decarboxylase, GAD). GAD, the synthesizing enzyme between glutamate and GABA, exists in two isoforms, GAD67 and GAD65 that synthesize GABA for different roles within the neuron. Several groups have reported that GAD expression is reduced in post-mortem tissue studies of individuals with autism. Fatemi et al. (2002) found that expression of both the 65 and 67 kDa isoforms was significantly reduced, between 48 and 61%, in cerebellar and parietal cortex samples from 5 subjects with autism compared with control samples. A larger subsequent study confirmed the reduction in the cerebellar cortex for GAD67 (Yip et al. 2007). Reductions in GAD expression are consistent with observations of increased glutamate concentration in autism. Importantly, this type of GAD deficit also predicts a concurrent decrease in the concentration of GABA, which seems to be the case from recent spectroscopic imaging studies (Harada et al. 2010; Gaetz et al. 2013). It is worth noting that reduced numbers of a major class of inhibitory interneuron, those staining positive for parvalbumin (PV), are observed in multiple animal models of autism (Gogolla et al. 2009), which could also explain the reduced concentration of GABA.

Protein expression relevant to glutamate neurotransmission in autism

Glutamate receptors can be classified into ionotropic and metabotropic subtypes, both of which have been implicated in autism. The three ionotropic subtypes are the kainate receptor, AMPA receptor and NMDA receptor, all named for specific agonists that selectively bind the receptor with high affinity. The eight metabotropic glutamate receptors (mGluR), however, are simply numbered 1–8, and are classified into three groups based on receptor structure, functional similarity and common agonists. (Niswender and Conn 2010). Group I mGluRs, consisting of mGlurR1 and mGluR5, are specifically of interest in autism and related disorders. Group I mGluRs interact with the ionotropic NMDA receptor via Shank and Homer protein interaction with the NMDA/PSD-95 signaling complex (Sheng 2001), and play a role in NMDA-receptor mediated long-term potentiation and depression (LTP and LTD: D'Antoni et al. 2014).

Glutamate receptor expression studies in autism are limited because of the low availability of post-mortem tissue. Changes have been observed in ionotropic and metabotropic glutamate receptors expression in autism, however. Purcell et al. (2001) observed decreased AMPA receptor density in the cerebellum in post-mortem tissue samples. Increased metabotropic glutamate receptor expression has also been observed. Fatemi et al. (2011) reported increases in mGluR5 in cerebellar tissue samples from 11 adults with autism. Lohith et al. (2013) recently reported a trend for up-regulation of mGluR5 in the prefrontal cortices of post-mortem samples from 17 individuals with Fragile X syndrome, a single-gene disorder strongly associated with autism (see below).

Two post-mortem studies have examined NMDA receptors. Blatt et al. (2001) did not find significant changes in NMDA receptor expression in an autoradiographic investigation of hippocampal tissue samples from 4 males who had autism. Purcell et al. (2001), however, did observe significant upregulation of NMDA receptor subunit 1 protein levels in the cerebellum from 9 autism samples. With limited available postmortem samples, it remains unclear whether these findings represent regional expression differences between the hippocampus and cerebellum or lack of power to detect effects. In an animal model of autism (prenatal valproic acid exposure - see below), overexpression of the NR2B and NR2B subunits of the NMDA receptor was observed (Rinaldi et al. 2007).

Increased expression of the glutamate transporters EAAT1 and EAAT2 in the cerebellum of post-mortem tissue from autism patients has also been reported (Purcell et al. 2001). Because EAAT expression is controlled in part by the extracellular concentration of glutamate (Levy et al. 1995), it is possible that the EAAT overexpression is due to the increased glutamate concentration seen in plasma and spectroscopic studies, as reviewed above.

Genetics and glutamatergic neurotransmission in autism

Autism twin studies report high heritability for the disorder, as high as 90% for broadly defined ASD (Bailey et al. 1995). Risk for autism in siblings of affected individuals is up to twenty-fold higher than in the general population (Rutter et al. 1999; Constantino et al. 2010; Ozonoff et al. 2011). Although the spectrum clearly involves a genetic component, the underlying etiology remains unclear in most cases. In about 10–20 percent of cases, a known genetic etiology is present (Betancur 2011), with the most common cause being a mutation in the FMR1 gene (i.e., fragile X syndrome). Copy number variations (CNVs) are present in an additional 10 percent of cases (Sebat et al. 2007). The majority of cases, however, remain classified as idiopathic (Miles 2011). Autism appears to be be a final common phenotypic pathway for over 100 different genetic etiologies rather than a single disease entity (Betancur 2011). It may be more appropriate, therefore, to consider not a single autism, but the presence of multiple “autisms,” some of which may share common neurobiological features despite their genetic heterogeneity (Geschwind and Levitt 2007).

Genetic evidence clearly implicates glutamate receptors and transporter systems autism. There have been reported associations with the NMDA receptor subunits GRIN2B and GRIN2B (Tarabeux et al. 2011; Yoo et al. 2012). The GluR6 subunit of the kainate receptor

has exhibited maternal linkage disequilibrium in a study of 51 affected families (Jamain et al. 2002). Shuang and colleagues (2004) also observed two GluR6 single nucleotide polymorphisms preferentially transmitted in autism in 174 parent-child trios in a separate study. GluR6 is an interesting candidate because mice that are deficient in the receptor are less susceptible to kainite induced seizures (Mulle et al. 1998). In contrast, over-expression of GluR6 in rodents results in increased spontaneous seizures (Telfeian et al. 2000).

No specific associations with AMPA receptors have been identified to date. Linkage in a chromosome 4q for a region containing the AMPA subunit 2 gene GRIA2 has been reported (Yonan et al. 2003), and Ramanathan et al. (2004) reported a single case of autism involving a deletion of the region including this gene. Among the metabotropic glutamate receptors (mGluR), Serajee et al. (2003) found evidence for a partial duplication of the mGluR subunit 8 gene (GRM8) among families affected by autism. GRM8 is located at 7q31, a region previously identified as associated with autism in linkage analyses (International Molecular Genetic Study of Autism Consortium (IMGSAC) 2001a; International Molecular Genetic Study of Autism Consortium (IMGSAC) 2001b).

Glutamate transporter genes have also been implicated. Family-based association studies of the single nucleotide polymorphisms have found associations with autism for the glutamate transporter genes SLC1A1 and SLC1A2 (Autism Genome Project Consortium et al. 2007; Jacob et al. 2011). Ramoz et al. (2004) found association between autism and the mitochondrial glutamate carrier gene SLC25A12. Interestingly, however, despite initial linkage with autism of a region of chromosome 2 containing the GAD1 gene (International Molecular Genetic Study of Autism Consortium (IMGSAC) 2001b), which encodes glutamate decarboxylase, GAD1 has not emerged as a strong signal in follow up studies in the disorder (Bacchelli et al. 2003).

Further genetic evidence of excitatory neurotransmission dysfunction in autism comes from studies implicating genes coding for cell-adhesion proteins. Neurexins and neuroligins are pre- and post-synaptic proteins, respectively, that form connections between cells at the excitatory post-synaptic density. On the pre-synaptic side, deletions of Neurexins 1 (NRXN1: Ching et al. 2010) and 3 (NRXN3: Vaags et al. 2012) have been associated with the autism phenotype. Rare structural variations in NRXN1 have also been described in autism patients (Yan et al. 2008; Camacho-Garcia et al. 2012; Camacho-Garcia et al. 2013). On the post-synaptic side, mutations of Neuroligin 3 (NLGN3) and 4 (NLGN4) have been implicated (Jamain et al. 2003; Laumonnier et al. 2004; Talebizadeh et al. 2006), although these mutations are not widely replicated, are considered to be quite rare within autism, and likely account for a very small fraction of cases (Gauthier et al. 2005; Wermter et al. 2008; Avdjieva-Tzavella et al. 2012; Liu et al. 2013).

Single-gene conditions associated with autism and impaired glutamatergic neurotransmission

Several of the known genetic disorders associated with autism have important implications for glutamatergic deficits in the disorder. Additionally, some of the most exciting new emerging treatments for autism have been developed in consideration of these known

etiologies. These disorders include Fragile X syndrome, 22q13 deletion syndrome and tuberous sclerosis, each of which is briefly reviewed next.

Fragile X syndrome (FXS) is the most common form of inherited intellectual developmental disability. The prevalence of FXS is estimated at 1 in 2500–5000 individuals (Hagerman 2008; Coffee et al. 2009). FXS is a trinucleotide repeat disorder caused by expansion of a CGG triplet to over 200 repeats on the 5' untranslated region of the *Fragile X Mental Retardation 1* (FMR1) gene. In turn, this expansion results in methylation and silencing of the gene. FMR1 codes for *Fragile X Mental Retardation Protein* (FMRP), a protein involved in regulating the expression of other genes, many of which are expressed in the synapse. Across all cases of autism, approximately 1–3 percent have the FMR1 mutation (Miles 2011), making it one of the leading single gene causes of autism. Within FXS, about 30 percent meet gold-standard diagnostic criteria for Autistic Disorder, and an additional 30 percent meet criteria for ASD (Rogers et al. 2001; Harris et al. 2008).

FMRP regulates messenger RNA, particularly within dendrites, where it plays a key role in the suppression of targeted mRNA translation. A major theory in FXS proposes a significant role for metabotropic glutamate receptors (mGluR) in the regulation of FMRP's inhibition of dendritic mRNA (Bear et al. 2004). The mGluR theory is based on the role FMPR plays in post-synaptic Group I mGluR activation, which increases protein synthesis in the synapse, resulting in the internalization of AMPA receptors associated with long term depression (LTD). FMRP is also activated by mGluR5 stimulation and then serves as a braking mechanism on translation. In FXS, the absence of FMRP allows for protein over expression, increased AMPA receptor internalization and excessive LTD. Excess LTD is a key neuronal phenotype in the knock out (KO) mouse model of FXS (Huber et al. 2002).

Critically, the mGluR theory predicts that mGluR antagonism downshifts this glutamate-signaled protein over expression that otherwise would naturally occur in the presence of FMRP. This is indeed the case in the KO mouse. A key paper tested a variety of well-described deficits in the FXS KO mouse with an additional knockdown of GRM5, genetically suppressing the mGluR5 by 50% (Dölen et al. 2007). In this genetic rescue study, the KO mice exhibited normalization of LTD and correction of several other key FXS phenotypes, including abnormalities observed in dendritic spines and audiogenic seizures (Dölen et al. 2007). Critically, seizures and excessive LTD in the KO mouse can also be rescued pharmacologically by mGluR5 antagonists (Yan et al. 2005; Michalon et al. 2012). Pre-clinical observations such as these have led to recent clinical trials of several mGluR5 agents in FXS patients, including the antagonist STX107 (Seaside/Roche, Phase II) and negative allosteric modulator AFQ056 (Novartis, Phase II/III) - see below. There is also excitement that mGluR pharmaceuticals may have beneficial effects for patients with autism due to some shared components of the molecular pathway between FXS and autism (Gürkan and Hagerman 2012).

FMRP is known to regulate the activity of more than 800 proteins, some of which are thought to be important in autism, such as NLGN3, mGluR5 and SHANK3 (Darnell et al. 2011). In addition to regulation by FMRP, these proteins share in common that they mutations in them are associated with risk for autism and they are constituents of a

functionally linked network between mGluR, AMPA receptor and NMDA receptor systems at the excitatory synapse. SHANK3 in particular has been of significant interest among these proteins as there are a number of autism patients for whom it plays a critical role who have either deletions or mutations in this gene (Moessner et al. 2007).

Phelan-McDermid syndrome, also known as 22q13 deletion syndrome, results from a of chromosome 22 (Phelan and McDermid 2012). Although the size of the deleted region varies, the SHANK3 gene is nearly always included. In addition to hypotonia and other characteristic physical phenotypes, Phelan- McDermid is associated with autism in as many as 75 percent of cases (Soorya et al. 2013). Conversely, SHANK3 deletion or mutation is observed in approximately 0.5 percent of all autism cases (Moessner et al. 2007). SHANK3 encodes a scaffolding protein on the post-synaptic density (PSD) of excitatory synapses, where it forms a complex at glutamatergic synapses by binding with neuroligins. Mutations of all of these constituents have been identified in autism, which in turn strongly implicate glutamatergic neurotransmission abnormalities in the disorder (Jamain et al. 2002; Arking et al. 2008). Insulin-like growth factor 1 (IGF-1), which has been shown to increase the length of excitatory PSDs in aging rats (Shi et al. 2005), also reduces glutamate-mediated AMPA receptor signaling in a SHANK3 mouse model of autism (Bozdagi et al. 2013), making it an attractive candidate for clinical trials. Although no clinical trials have yet been published using IGF-1 in idiopathic autism or Phelan-McDermid syndrome, several are currently underway (ClinicalTrials.gov: NCT01970345, NCT01777542, NCT01525901).

Tuberous sclerosis (TS) is a genetically-mediated disorder caused by mutations in either the TSC1 and TSC2 tumor suppressor genes. TSC is characterized by mental retardation and seizures and autism spectrum disorders. Autism is present in approximately 20–40 percent of those affected (Hunt and Shepherd 1993). The molecular basis of the disorder is reasonably well understood. TSC1 and TSC2 proteins act cellularly to suppress mammalian target of rapamycin (mTOR). This protein is a constituent of the pathway for mGluR-mediated LTD and likely regulates the phosphorylation of FMRP (Santoro et al. 2012). In TS, hyperactive mTOR therefore increases the downstream effects of glutamate signaling (Weston et al. 2012). In a TSC1 knock-out mouse model, treatment with rapamycin, an mTOR inhibitor, suppresses seizures (Zeng et al. 2008). Recent work with both TSC1 and TSC2 mouse models has shown beneficial effects of rapamycin on social behavior (Sato et al. 2012). This suggests that mTOR modulation may be an attractive target for intervention in autism (Ehninger and Silva 2011) and there are several clinical trials underway for treating TS and TS+autism with rapamycin.

Animal models and glutamate dysfunction

Evidence for the involvement of glutamate neurotransmission in autism has motivated interest in examining glutamatergic deficits in animal models, where preclinical studies of pharmacological rescue can undergo proof of concept. Several animal models of autism have demonstrated glutamatergic neurotransmission deficits. The FXS mouse model has obviously been highly influential in the development of the mGluR theory of FXS, as discussed previously. Another model worth consideration is that of prenatal exposure to valproic acid (VPA). VPA, an anticonvulsant and moodstabilizing drug, has been tentatively

identified as a risk factor for autism in humans (Rasalam et al. 2005). Behaviorally, the animals exhibit reduced sensorimotor gating, repetititve/stereotyped movements, and abnormal social behaviors (Schneider and Przewł ocki 2004). VPA exposed rats exhibit increased NMDA receptor expression and corresponding increases in long-term potentiation (Rinaldi et al. 2007). Gandal et al. (2010) have shown that mice exposed prenatally to VPA also exhibit two electrophysiological phenotypes associated with autism in humans, delayed auditory evoked responses (Roberts et al. 2010) and abnormal gamma-band oscillations (Rojas et al. 2008). Administration of MPEP, an mGluR5 antagonist, showed a trend towards improvement of the gamma-band deficit and significantly improvement the evoked response latency in the VPA mice (Gandal et al. 2010). It is noteworthy that gamma-band oscillations are also modulated via the GluR6 kainate receptor subunit (Fisahn 2005), which has exhibited genetic linkage in autism patients (Jamain et al. 2002).

NMDA receptor dysfunction is a common feature of several models, including SHANK3 (Duffney et al. 2013), NLGN1 (Blundell et al. 2010), FXS knockout mouse (Eadie et al. 2010) and MeCP2 transgenic mice (Asaka et al. 2006). NLGN1 knockout mice do not exhibit many behavioral deficits characteristic of autism, although increased repetitive behaviors are observed (Blundell et al. 2010). Interestingly, although NLGN1 expression does not differ in prenatal VPA-exposed mice (Gandal et al. 2010), its expression levels appear to be associated with the electrophysiological intermediate phenotypes in the VPA model, which were rescued by an mGluR5 antagonist. In FXS knockout mice, NMDA receptor co-agonists such as D-serine have been shown to rescue NMDA-mediated deficits in long-term potentiation (Bostrom et al. 2013).

A comprehensive discussion of the many different animal models associated with autism features is beyond the scope of this review. Interested readers will find an excellent review of the status of animal models relevant to glutamate in autism by Carlson (Carlson 2012), which suggests that glutamate dysfunction is a common theme in mouse models. Because it is currently unclear what specific autism behaviors are mediated by glutamate dysfunction, Carlson (Carlson 2012) summarizes by advocating that the rigorous behavioral and neurobiological assessment of the FMR1 knockout mouse be applied to other rodent models to evaluate the role of glutamate and other neurotransmitter systems in autism.

Drug treatments in autism involving glutamatergic neurotransmission

In this section, an overview is given of treatment studies to date targeted to the suppression of some aspect of glutamatergic transmission in autism and related genetic conditions such as Fragile X syndrome. Athough glutamate antagonists have also been considered for treating disorders commonly comorbid with autism, consideration of those comorbidities and their treatment is beyond the scope of the current review. Interested readers are referred to other reviews of the role of glutamate in treating disorders such as epilepsy, depression and ADHD (Sanacora et al. 2008; Hashimoto 2009; Ghasemi and Schachter 2011; Russo et al. 2012; Hosenbocus and Chahal 2013).

NMDA receptor antagonists

The availability of several NMDA receptor antagonists on the market that are approved for other indications has resulted in their use in a number of trials for treating autism. These include agents such as amantadine and memantine and acamprosate.

Amantadine is a commonly used drug for parkinsonism and is both a dopamine re-uptake inhibitor and non-competitive antagonist for NMDA receptors. To date, there is only a single trial published on amantadine in autism. In a 4-week, single-blinded, placebo-controlled trial of amantadine, King et al. (2001) reported improvements in clinician-rated measures of inappropriate speech and hyperactivity in the 39 children and adolescents who completed the study. Despite the improvements compared to placebo in the clinician-rated measures, the authors reported no improvements compared to placebo for parent-rated measures of irritability and hyperactivity, possibly because of a high placebo effect on the parent rated measures.

Memantine is an NMDA receptor antagonist approved for use in Alzheimer's disease. Several studies, almost all of which are small open-label trials, have investigated its use in autism and in FXS. Erickson et al. (2006) conducted a retrospective review of medical records for 18 children with pervasive developmental disorders, reporting that 11 of the 18 were responders to memantine in terms of improvements on clinical global impression. Chez et al. (2007) conducted an open-label add-on trial of memantine in 151 patients with autism over a 21-month interval. Significant improvements were noted in language and social behaviors. Two other open-label trials observed significant effects for irritability and hyperactivity (Owley et al. 2006; Niederhofer 2007). In a recent study, memantine was examined as an add-on therapy to risperidone, an atypical antipsychotic approved for use in autism. Ghaleiha et al. (2013) compared memantine plus risperidone versus placebo plus risperidone in children with autism in a 10-week randomized trial. In this study, significant improvements in the memantine-treated group compared to the risperidone only group were observed in for irritability, stereotyped behaviors and for hyperactivity. Memantine has also been tried in a small, open-label study in children with FXS who had co-morbid diagnoses of pervasive developmental disorder. Erickson et al. (2009) found improvements on clinical global impression for 4 of the 6 patients in that study. A randomized, double-blind, placebo controlled study of memantine in adults with autism is currently under way (ClinicalTrials.gov: NCT01078844).

Acamprosate is a weak NMDA receptor antagonist approved for use in treating alcoholism. Of note, it has been proposed that acamprosate also acts as an antagonist for mGluR5 (Harris et al. 2002). Erickson and colleagues have conducted several small trials of acamprosate in both FXS and autism (Erickson et al. 2010; Erickson et al. 2011; Erickson et al. 2013b). In a small, 10-week, open-label pilot in 12 children and adolescents with FXS, Erickson et al. (2013b) observed improvements in clinical global impression in 9 of the 12 subjects. Additional improvements were noted in social behavior and hyperactivity measures. In another small open-label study, 5 of 6 children with autism were judged to be responsive to an 8-week trial of acamprosate, with positive impacts on verbalizations and social behavior (Erickson et al. 2011). To date, acamprosate has not been tested in larger,

double-blind placebo controlled trial, although such a trial is currently underway for autism (ClinicalTrials.gov: NCT01813318).

Metabotropic glutamate receptor antagonists

Due to the excitement concerning the mGluR theory of FXS, and regulation by FMRP of proteins implicated in autism, trials of mGluR antagonists are among the most anticipated among new treatments for autism spectrum conditions. In an early open-label trial of fenobam, a mGluR5 antagonist, was tried in 12 individuals with FXS (Berry-Kravis et al. 2009). Fenobam was granted orphan-drug status by the FDA in 2008 to facilitate potential new indications. In this first trial of an mGluR5 agent in FXS, improvements were noted in a laboratory measure of prepulse inhibition of the acoustic startle response in 50 percent of the patients. AFQ056, a negative allosteric modulator of mGluR5 developed by Novartis, has been examined in a single randomized, placebo-controlled study (Jacquemont et al. 2011). No significant effects of the treatment were observed on the primary outcome measure for the study. In a posthoc exploration of the data, however, it was noted that 7 of the 30 patients with a fully methylated FMRI promotor had significant improvements relative to placebo, suggesting that the benefits may be more pronounced in the more severely affected patients. There are several ongoing trials of AFQ056 in FXS (ClinicalTrials.gov: NCT01433354, NCT01348087, NCT01253629, NCT01482143). To date, there are no studies of mGluR5 antagonists in idiopathic autism.

Minocycline

Minocycline is a broad-spectrum antibiotic that also acts to suppress glutamatergic transmission (González et al. 2007). It appears to operate indirectly as an antagonist NMDA receptors by its actions on inflammatory cytokine pathways and inhibition of microglia-mediated release of quinolinic acid (Soczynska et al. 2012). Additionally, it may have a dual mechanism at AMPA receptors, where it acts both as a channel blocker and also to reduce receptor desensitization (Jin et al. 2012). In patients with FXS, a promising open label trial of minocycline in twenty patients reported significant improvement in irritability after 8 weeks of treatment (Paribello et al. 2010). Leigh et al. (2013) recently reported the first double-blind placebo controlled trial of minocycline in FXS. These authors observed improvements relative to placebo in clinical global impression and in anxiety and mood-related symptoms in a 3-month trial of minocycline in 55 children and adolescents with FXS. A recent 6-month open-label pilot study in 11 patients with autism did not find improvements in any clinical symptom measures, however (Pardo et al. 2013). It is noteworthy that minocycline, as with other tetracycline antibiotics, has anticonvulsant properties (Wang et al. 2012), because seizure disorders are common in both FXS and autism patients (Tuchman et al. 2010b; Berry-Kravis et al. 2010). It is also of interest that minocycline may have positive effects on mood and anxiety symptoms (Soczynska et al. 2012; Leigh et al. 2013) since these symptoms, although not considered core deficits in autism, are common in people on the spectrum (Mazefsky et al. 2008). Additional, larger randomized clinical trials are needed examining how these significant co-morbidities respond to minocycline treatment in autism patients.

GABA-B agonists

It is important to note that there is a role for other neurotransmitters in the modulation of glutamate. In particular, metabotropic GABA-B receptors play an important role in down regulation of glutamate transmission. GABA-B receptors are primarily located pre-synaptically and agonists of GABA-B result in reduced release of glutamate into the synaptic cleft via second-messenger activation of K^+ channels and inactivation of Ca^{2+} channels (Chalifoux and Carter 2011). Thus, GABA-B agonists represent an indirect way of reducing glutamatergic transmission from the presynaptic side and are of some interest as therapeutic agents in autism. Baclofen, a GABA-B receptor agonist, has been shown to reduce audiogenic seizures in the FMR1 knockout mouse (Pacey et al. 2009). Abnormalities in gamma-band oscillations have been responsive to baclofen in an NMDA receptor dysfunction mouse model of autism (NR1−/−: Gandal et al. 2012).The R isomer of baclofen (arbaclofen) has been tried in recent trials involving patients with FXS and autism. In FXS, a randomized, placebo-controlled crossover trial of arbaclofen in 63 subjects did not show significant improvement on its primary endpoint, the irritability sub scale of the Abberant Behavior Checklist (Berry-Kravis et al. 2012). Patients in that trial, however, did exhibit improvements on secondary measures of social avoidance and socialization compared to placebo. Erikson et al. (2013a) recently reported results of an 8-week open label trial in 32 children with autism. These authors found significant improvements measures of irritability, social withdrawal, social responsivity, obsessive-compulsive symptoms and clinical global impression, suggesting that a follow-on placebo-controlled trial should be conducted.

Summary, caveats and future directions

Glutamate is clearly implicated in the pathophysiology of autism. What is not particularly clear, however, is how glutamate-related dysfunction leads to core symptom deficits in autism. Studies involving known etiologies for autism spectrum disorder are a particularly effective way to establish linkages between behavior and glutamate dysfunction, but it is also evident that glutamate may be impacted differently depending on the specific sub-type of autism (e.g., Fragile X syndrome, Phelan- McDermid syndrome). Further, it must be emphasized that autism is frequently comorbid with other conditions where glutamate dysfunction is considered a key component. For example, approximately 20–30 percent of individuals with autism have a seizure disorder (Tuchman et al. 2010a). Psychiatric comorbidities in autism are also elevated over general population prevalence, but are highly variable between studies. For example, attention-deficit/hyperactivity disorder (ADHD) has variable reported prevalence in autism, ranging from 14% to 78% in various studies (Gargaro et al. 2011). Anxiety disorders are also highly prevalent in autism, with reports ranging from 11% to 84% (White et al. 2009). A number of different anxiety disorders are seen in autism, including generalized anxiety disorder, panic disorder, social anxiety disorder, simple phobias and obsessive-compulsive disorder. The glutamate system has been implicated in all of these disorders. The understanding of comorbidity in autism is limited, such that most of the studies reviewed do not characterize it in their patient samples. The exception to this is seizure disorders, which is typically a frank exclusion criterion in clinical studies of autism. The limited understanding of inter subject variability in symptom expression represents an important limitation in our understanding of glutamatergic changes

in autism that future studies will need to address. Stepping back from the question of how glutamate dysfunction is related to core autism symptoms, one might also ask if there is any relationship at all, once important co-morbidities are accounted for.

In addition to comorbidity limitations on prior human subjects research on glutamate and autism, additional variables that may confound the interpretation of studies include gender differences, age and history of medication. For example, glutamate concentration has been reported to be higher in male than in female participants (O'Gorman et al. 2011) and increased age is also related to decreased concentration of glutamate (Kaiser et al. 2005). Antipsychotic medications, which are increasingly used to treat irritability in autism, have been associated with reduced glutamate concentration in at least one prior study in patients with schizophrenia (Szulc et al. 2011). Careful examination of these potential confounds in human neuroimaging work will be necessary as the field moves forward. With respect to human serum and plasma findings on glutamate, these factors and other confounds are discussed in more detail in a recent critical review by Ghanizadeh (2013).

Even among different single-gene etiologies associated with autism, however, there is evidence of common molecular pathways involving glutamate. Disorders such as Fragile X and Phelan-McDermid offer an important opportunity to study glutamate pathophysiology in well-controlled conditions using animal models, where results can then be compared to more problematic, less well-controlled studies in the human conditions themselves. The fact that research involving autism patients with unknown etiologies seem to support similar findings of glutamate dysfunction (e.g., serum glutamate and spectroscopic imaging studies of increased glutamate) provides some support for the potential of a final common pathway across different pathophysiological mechanisms. It is this last point that provides hope for those with idiopathic autism. Emerging treatments developed from rationale provided by glutamatergic studies of these known etiologies may be applicable to newly identified genetic sub-types of autism. Evaluation of glutamate-based pharmaceutical interventions in autism are in an early stage and the next few years, we will know more definitively whether the early promising results will yield new drug indications in the disorder. To date, there is no clear evidence from any ionotropic or metabotropic receptor antagonist for a therapeutic effect in autism or a related disorder. It is particularly imperative, therefore, that double-blind, placebo controlled studies of larger samples in autism be conducted to provide such evidence.

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Table 1

1H-MRS Studies of Glutamate

Study	N	Ages: Mean (SD)	Magnet and Technique	Regions	Measure	Findings	Notes
1	HC: 13/19* ASD: 20/17*	HC: 34.3 (9.3) ASD: 35.6 (11.5)	1.5 T, SV	R Hippocampus, R Parietal cortex	Glx	HC < ASD R Hippocampus, HC <> ASD R Parietal	Subjects matched on IQ
2	HC: 29 ASD: 26	HC: 11.1 (2.4) ASD: 9.8 (3.2)	3.0 T, MRSI	L/R Frontal lobe, L/R Temporal lobe, L/R Occipital lobe, L/R Cerebellum, L/R Cerebral WM	Glx	HC > ASD L/R Frontal, Occipital and Cerebellum, HC <> ASD L/R Temporal and WM	Matched on IQ
3	HC: 10 ASD: 8	HC: 13.2 (2.5) ASD: 11.2 (2.6)	1.5 T, SV	ACC	Glx	HC < ASD	HC > ASD on IQ
4	HC: 16 ASD: 26	HC: 11.8 (3.0) ASD: 10.2 (3.3)	1.5 T, MRSI	ACC	Glx	HC < ASD	Matched on IQ
5	HC: 10 ASD: 12	HC: 5.9 (3.2) ASD: 5.2 (3.0)	3 T, SV	Frontal Lobe and Lenticular Nucleus	Glu	HC <> ASD Frontal Lobe and Lenticular Nucleus	GABA/Glu ratio lower in ASD compared to HC, most subjects scanned under Triclofos sedation
6	HC: 14 ASD: 14	HC: 29.7 (8.3) ASD: 29.2 (6.1)	3.0 T, MRSI	Bilateral ACC, thalamus, IPS, TPJ	Glx	HC > ASD R ACC, HC <> ASD L ACC, L/R IPS and L/R TPJ	Matched on IQ, large age range (21 to 50 years)
7	HC: 7 ASD: 7	HC: n.r. ASD: 14 (1.8)	4.0 T, SV	ACC, L/R MTL	Glu	HC < ASD ACC, HC <> ASD L/R MTL	Age and IQ not reported separately for HC group, but were not significantly different from ASD
8	HC: 15 ASD: 13 pASD: 15	HC: 41.08 (6.77) ASD: 36.89 (6.80) pASD: 41.22 (6.87)	3.0 T, SV	L/R Heschl's gyrus	Glx, Glu	HC < ASD, HC <> pASD L/R Heschl's gyrus for Glx and Glu	Age and IQ matched, large age range (25 to 48 years)
9	HC: 14 nASD: 15 bASD: 13	HC: 29 (6.0) nASD: 27 (6.4) bASD: 34 (8.8)	1.5 T, SV	L Basal Ganglia, L Frontal Lobe and L Medial Parietal Lobe	Glx	HC > nASD, bASD, nASD <> bASD Basal Ganglia, HC <> nASD, bASD Frontal and Parietal Lobes	Matched on IQ, HC group slightly older, ADI communicatio n score negatively correlated with Basal Ganglia Glx
10	HC: 10,18,29 DD: 13,14,12 ASD: 45,31,29 ***	3–4 years, 6–7 years, 9–10 years ***	1.5 T, MRSI	Gray matter and White matter	Glx	HC > ASD in white matter, 3–4 year age range only, HC > DD in white matter, 3–4 year and 9–10 year age ranges	Longitudinal acquisition for ASD, cross-sectional for HC, ASD and DD groups scanned under propofol sedation
11	HC: 10 ASD: 10	HC: 11.3 (2.7) ASD: 11.4 (2.7)	1.5 T, SV	Bilateral ACC, L Cerebellum, L Striatum, L Frontal Lobe	Glu	HC < ASD, all regions tested	Age and gender matched. Also measured plasma glutamate, higher in ASD than HC
12	HC: 16 ASD: 20	HC: 12.9 (4.1) ASD: 11.5 (3.0)	3.0 T, MRSI	Caudate, Putamen, Thalamus	Glx	HC < ASD for putamen, HC <> ASD for caudate and thalamus	Glx positively correlated with age

Table notes: Studies: 1. Page et al. (2006) 2. DeVito et al. (2007) 3. Beijani et al. (2012) Experiment 1 4. Bejjani et al. (2012) Experiment 2 5. Harada et al. (2010) 6. Bernardi et al. (2011) 7. Joshi et al. (2012) 8. Brown et al. (2013) 9. Horder et al. (2013) 10. Corrigan et al. (2013) 11. Hassan et al. (2013) 12. Doyle-Thomas et al. (2014). HC = healthy control; ASD = autism spectrum disorder; nASD = narrowly defined ASD; bASD = broadly defined ASD; pASD = parents of children with ASD; SV = single voxel; MRSI = magnetic resonance spectroscopic imaging (multi-voxel, aka chemical shift imaging); IQ = intelligence quotient; L = left; R = right;

* first N is for hippocampal voxel, second N is for parietal cortex;

** first number is for SV study, Experiment 1, second number is for MRSI study, Experiment 2;

*** numbers correspond to N and age group for 3 age groups included in study; WM = white matter; TE = time to echo; Glx = Glutamate+Glutamine+GABA; Glu = Glutamate; ACC = Anterior Cingulate Cortex, IPS = Intraparietal sulcus, TPJ = temporoparietal junction, MTL = Medial Temporal Lobe.