Pharmacological modulation of GABA function in Autism Spectrum Disorders: a systematic review of human studies.

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Abstract

Autism Spectrum Disorders are an emerging health problem worldwide, but little is known about their pathogenesis. It has been hypothesized that autism may result from an imbalance between excitatory glutamatergic and inhibitory GABAergic pathways. Commonly used medications such as valproate, acamprosate, and arbaclofen may act on the GABAergic system and be a potential treatment for people with ASD. The present systematic review aimed at evaluating the state-of-the-art of clinical trials of GABA modulators in autism. To date there is insufficient evidence to suggest the use of these drugs in autistic subjects, even if data are promising. Of note, short-term use of all the reviewed medications appears to be safe. Future well designed trials are needed to elucidate these preliminary findings.

Key words:

Autism spectrum disorder; GABA; clinical trials; systematic review

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Autism Spectrum Disorders are an emerging health problem worldwide, but little is known about their pathogenesis. It has been hypothesized that autism may result from an imbalance between excitatory glutamatergic and inhibitory GABAergic pathways. Commonly used medications such as valproate, acamprosate, and arbaclofen may act on the GABAergic system and be a potential treatment for people with ASD. The present systematic review aimed at evaluating the state-of-the-art of clinical trials of GABA modulators in autism. To date there is insufficient evidence to suggest the use of these drugs in autistic subjects, even if data are promising. Of note, short-term use of all the reviewed medications appears to be safe. Future well designed trials are needed to elucidate these preliminary findings.

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Introduction

Autism Spectrum Disorders (ASD) are chronic neurodevelopmental conditions characterized by impaired reciprocal social interaction and restricted, repetitive and stereotyped patterns of behaviors or interests. These conditions may vary in severity, and could be burdened by numerous medical comorbidities which appear to be more frequent in ASD such as epilepsy, mental retardation, asthma, eczema, and gastrointestinal dysfunctions (Berg et al. 2011). ASD occurs more frequently in boys, with a 4:1 male-to-female ratio (Fombonne et al. 2006). It was originally thought to be quite rare, but the prevalence rates of ASD have been raising worldwide over the past few decades, from approximately 4 per 10 000 to 1 per 68 children (Chakrabarti and Fombonne 2005; Abrahams and Geschwind 2008; CDC 2014). Several reasons have been proposed to explain this dramatic increase, such as higher public awareness, broadening of the spectrum to include even milder forms, and improved clinical detection (Levy et al. 2009). As a result, autism has recently emerged as a major public health problem in most countries of the world as, additionally, ASD are lifelong chronic conditions. To date, there is an increasing interest in the pathophysiology of ASD, but, unfortunately, few data are available on the molecular mechanisms of these conditions and there are not validated and reliable diagnostic biomarkers.

Recently, several authors have started to investigate dysfunctions in the Gamma-Aminobutyric Acid (GABA) system as a potential pathophysiological pathway in autism. GABA is generally regarded as the main inhibitory neurotransmitter of the entire central nervous system. It is mainly formed from glutamate by means of the glutamic acid decarboxylase 65 and 67 kDa proteins (GAD65/67) (Bu et al. 1992; Rimvall and Martin 1994) and acts on two different classes of receptors: $GABA_A$, and $GABA_B$. The $GABA_A$ receptors are ionotropic channels while the $GABA_B$ are metabotropic channels coupled with a G-protein and they are all widely expressed in the brain. When GABA binds its receptors, an inhibitory signal is transduced. $GABA_A$ receptors determine the fast component of the inhibitory post-synaptic potentials (IPSP) by opening chloride ion-selective pores

(Sieghart and Sperk 2002). $GABA_A$ receptors are expressed post-synaptically in the brain and both pre-synaptically and post-synaptically in the spinal cord (Stell et al. 2003). Of note, $GABA_A$ receptors are sites of action for benzodiazepines, barbiturates and anesthetics (Nutt and Malizia 2001). Focusing on $GABA_B$, pre-synaptic $GABA_B$ receptors act by inhibiting the voltage-gated calcium channels. The resulting reduced intracellular calcium concentration will decrease the release of excitatory glutamate and inhibitory GABA (Mott and Lewis 1994). On the other hand, post-synaptic $GABA_B$ receptors are responsible for the slow, long-lasting component of IPSP by activating an inward rectifying potassium current (Kaupmann et al. 1998; Kuriyama et al. 2000). $GABA_B$ receptors are sites of action for baclofen and gamma hydroxybutyrate (GHB) and have less affinity for GABA than $GABA_A$ receptors (Mott and Lewis 1994).

In 2001, Hussman (2001) firstly proposed a theory which implicated GABAergic dysfunction in the pathogenesis of autism. Specifically, he hypothesized that ASD may result from a disruption of the equilibrium between excitatory glutamatergic and inhibitory GABAergic pathways. In particular, milder GABA dysfunction may determine an hyper-stimulation of neurons and, subsequently, an impairment in filtering stimuli from the external environment as well as internal cues (Casanova et al. 2006) whereas severe GABA impairment could result in seizures. In fact, it is known that epilepsy is a common comorbility in autism (Tuchman and Rapin 2002) and nearly seven out of ten patients display paroxystic activity in the electroencephalogram (EEG) (Kim et al. 2006). As the GABA system is involved in modulating neuron activation and neuronal synchrony, an imbalance of either $GABA_A$ and $GABA_B$ receptor pathways may result in increased postsynaptic neuronal excitability and altered glutamate release.

Several evidences, from animal models to human *in vivo* and *in vitro* studies, seem to support this hypothesis (Coghlan et al. 2012; Cellot and Cherubini 2014). From a preclinical perspective, animal models of ASD frequently display GABAergic impairment. For instance mice carrying a mutation in the Neuroligin 3 (NL3) gene (NL3R451C knock-in mice) showed GABA alterations and behavioral problems similar to autistic children: in particular, mice had an increase in number of

miniature inhibitory post-synaptic currents in the somatosensory cortex and in the CA3 region of the hippocampus (Pizzarelli and Cherubini 2013) and a decrease in GABA release at the synapsis between parvalbumin-positive basket cells and principal cells (Földy et al. 2013). Another ASD model, the knock out mouse for the homeobox-containing transcription factor engrailed-2 (EN2), showed a loss of GABAergic interneurons in the cortex and hippocampus (Sgadò et al. 2013). Additionally, mouse pups who are exposed to valproic acid *in utero* constitute a reliable ASD animal model: in these mice, the presence of autistic behaviors have been associated with a reduction in GABAergic signaling, both pre- and post-synaptically (Banerjee et al. 2013).

Focusing on genetic studies, mutations in genes encoding for GABA receptor subunits have been associated to autism (Wolpert et al. 2000; Buxbaum et al. 2002; Ma et al. 2005; Kim et al. 2006; Hogart et al. 2007) and animal models lacking different GABA receptor subunits display autistic behaviors (Cheh et al. 2006; Tabuchi et al. 2007; DeLorey et al. 2008).

According to post-mortem studies, subjects with ASD display a paucity of GABAergic Purkinje cells in the cerebellum (Ritvo et al. 1986; Bauman and Kemper 2005), an increased cell packing density of GABAergic interneurons in the hippocampus (Lawrence et al. 2010), small neuron size in the hippocampal pyramidal cell laminae and a decrease of neurons in the lateral amygdala and fusiform gyrus (Amaral et al. 2008; van Kooten et al. 2008). Additionally, a reduction of $GABA_A$ (Blatt et al. 2001; Guptill et al. 2007; Fatemi et al. 2009a; Oblak et al. 2009; Fatemi et al. 2014) and $GABA_B$ (Fatemi et al. 2009b; Fatemi et al. 2010) receptor levels have been reported in several brain tissue regions (i.e. hippocampus, superior frontal cortex) of individuals with ASD. Furthermore, some studies have shown decreased levels of GAD65/67 (which in turn are highly associated with intraneural GABA levels) in brain tissue from subjects with autism (Fatemi et al. 2002; Yip et al. 2007; Yip et al. 2009).

Focusing on *in vivo* studies, findings on GABA as a peripheral biomarker in autism have generally reported higher GABA plasma levels in patients with ASD compared to controls (Dhossche et al.

2002; El-Ansary et al. 2011; Abu Shmais et al. 2012; Russo 2013; Alabdali et al. 2014; El-Ansary and Al-Ayadhi 2014); only one small study has observed decreased levels of GABA in platelets of autistic patients (Rolf et al. 1993). Direct detection of GABA in the brain of autistic subjects has been hampered by technical difficulty of the measurement itself: however, magnetic resonance spectroscopy found a reduction of GABA levels in the frontal lobe (Harada et al. 2011) and in the left perisylvian region (Rojas et al. 2014) of autistic children compared to controls. Additionally, SPECT (Single Photon Emission Computed Tomography) technique (Mori et al. 2012) was capable of detecting a reduction in $GABA_A$ receptors in the frontal cortex and a PET (Positron Emission Tomography) study (Mendez et al. 2013) reported a deficit of α5 $GABA_A$ receptor subunits in the amygdala and nucleus accumbens of patient with ASD.

Despite the huge amount of data regarding the pathogenesis of ASD, currently, there is no treatment for the core symptoms of autism. As the excitatory-inhibitory imbalance theory of autism suggests a GABAergic deficit in ASD, animal studies have been designed in order to test this hypothesis: of note, pharmacological enhancement of GABA neurotransmission in a mouse model of autism has shown promising results (Han et al. 2014). In humans, different types of medications (i.e. acamprosate, arbaclofen, valproate) yield the capacity for modulating GABAergic function, but their use in ASD has been subject to a limited evidence based practice. The aim of the present systematic review is to evaluate the efficacy and safety of pharmacological GABAergic modulation in ASD.

Material and methods

In August 2015, we searched the following databases: MEDLINE, Psychology and Behavioral Sciences Collection, CINAHL, and the Cochrane Database of Systematic Reviews. To ensure a more inclusive research strategy the search terms were: (autis* OR Kanner OR Asperger OR PDD OR pervasive develop*) AND gaba*. All search terms were searched individually in each database

and combined together. The search strategy had no past time restriction and it was extended till the 31st of July 2015. It was limited to article in English, Italian, French, Spanish, and German. Additionally, all recovered papers were reviewed for further relevant references. Clinical trial registers were searched in order to find ongoing trials on the selected medications.

We selected clinical trials, yielding primary results on the effects of the administration of GABA modulators in patients with autism spectrum disorders. ASD was defined according to internationally valid diagnostic criteria such as the International Classification of Diseases (ICD) or the Diagnostic and Statistical Manual of Mental Disorders (DSM). In the present review, the following drugs were evaluated: acamprosate, arbaclofen, bumetanide, carnosine, flumazenil, riluzole and valproate. We included randomized clinical trials as well as open-label trials. We excluded case reports.

Two researchers (NB and LF) independently reviewed all information about the articles provided by the databases. Any discrepancies were solved by consensus. Agreement between reviewers was high (k=0.94). We assessed the methodological quality of the included studies according to the criteria developed by the Cochrane Collaboration (Higgins and Green 2011). We extracted data using a format which included study design, sample size, follow-up duration, drug dosage, adverse events and main findings.

Results

Our search strategy yielded 471 citations. After screening of title and abstract, only 50 were retained for full-text examination. Four additional articles were found after reviewing for further relevant references the recovered papers The studies which fulfilled the inclusion criteria are summarized in Table 1. Overall quality of the included studies is depicted in Figure 1 and Figure 2.

Acamprosate

Acamprosate is a homologous of GABA. Although its mechanism of action in the brain is not yet fully elucidated, acamprosate seems to have an indirect effect on $GABA_A$ receptors via inhibition of $GABA_B$ receptors (Kalk et al. 2014). Additionally, it binds N-Methyl-D-aspartic acid (NMDA) receptors but with low affinity, thus its effect on glutamate and NMDA receptors appears to be mediated by allosteric interaction rather than by direct competition (Kalk et al. 2014). In 2013, Erickson and coworkers (Erickson et al. 2013) conducted an 10-week open-label trial in 12 subjects with Fragile X syndrome and concomitant ASD. The authors administered acamprosate at the initial dosage of 333 mg/die, titrating it to a maximum of 1998 mg/day (weight>50 kg) or 1332 mg/day (weight<50 kg) over the first 6 weeks of the study. Mean acamprosate dosage was 1054 mg/day (range: 666–1998 mg/day). Patients were allowed to continue non-glutamatergic psychotropic medications. The main outcome measure was the Clinical Global Impressions-Improvement (CGI-I) score change between the baseline and the follow-up assessment. Secondary outcome measures were changes at the Aberrant Behavior Checklist (ABC) score, the Social Responsiveness Scale (SRS), the Compulsion Subscale of the Children's Yale-Brown Obsessive Compulsive Scale Modified for Pervasive Developmental Disorders (CY-BOCS-PDD) and the ADHD Rating Scale. Vital signs and laboratory tests were monitored during the study. The authors observed a significant improvement in nine subjects (75% of the sample, effect size: 2.00). Additionally, acamprosate ameliorated social behaviors as measured by means of the ABC- Social Withdrawal and Social Avoidance subscale (effect size: 0.81 and 0.64 respectively). Of note, the authors observed a significant reduction in hyperactivity and an improvement in communication skills. No serious side effect was reported. The most frequent mild adverse events were irritability (n=4), increased repetitive behavior (n=2) and gastrointestinal symptoms (n=2). These findings were in line with a previous report by the same researchers involving three adults with Fragile X syndrome and comorbid ASD (Erickson et al. 2010).

The same authors conducted two trials in cohorts of patients with idiopathic ASD. In the first open-label study (Erickson et al. 2011) involving six patients, acamprosate was titrated to a maximum of 1332 mg/day [mean dose: 1110 mg/day±172 mg/day (range: 999–1332 mg/day)]. Each patient was followed-up for at least four weeks (mean follow-up time 20±8 weeks). Social responsiveness significantly improved as well as hyperactivity. Only mild gastrointestinal side effects were recorded (i.e. mild nausea and reduced appetite). The more recent study (Erickson et al. 2014a) recruited 12 patients with ASD in a single-blind placebo lead-in trial. Each patient received 2 weeks of placebo followed by 10 weeks of acamprosate. Mean acamprosate dosage was 27.9±11.3 mg/kg/day (range 10.7–44.2 mg/kg/day); concomitant psychotropic medications were allowed. Of note, three subjects were excluded from the study after the placebo phase and three were subsequently lost to follow-up. Main findings showed amelioration in social behavior and hyperactivity. Acamprosate was well tolerated and only mild adverse events were observed (gastrointestinal symptoms, headache, and irritability- which appeared to be dose-dependent).

Arbaclofen

Arbaclofen is the active R-isomer of baclofen and acts as a $GABA_B$ receptor agonist, blocking the release of glutamate presinaptically. Compared to baclofen, its R-enantiomer is well absorbed throughout the gastrointestinal tract and has higher bioavailability (Lai et al. 2009). Given its efficacy in modulating the GABA neurotransmission, it was proposed for testing in ASD. In 2014, Erickson and colleagues (Erickson et al. 2014b) enrolled 32 children and adolescents with ASD in a 8-week open-label trial. Arbaclofen was administered at the initial dose of 2 mg/day, then gradually titrated to a maximum dose of 20 mg/day (ages 6–11 years) or 30 mg/day (ages 12–17 years). Primary outcome measure was the ABC-Irritability subscale, while secondary outcomes were changes in the ABC, the CGI-S, the CGI-I, the SRS, the CYBOCS-PDD, the Child and Adolescent Symptom Inventory-4 (CASI) Anxiety scale, the ADHD Rating Scale-IV, and the Vineland Adaptive Behavior Scales (VABS). Seven patients were lost to follow-up or withdrawn from the

study. The authors observed a significant difference in the ABC-Irritability subscale (as well as in all the other subscales except for the Inappropriate Speech subscales), the CGI-S, the CGI-I, the SRS, the CYBOCS-PDD, the SRS, the CYBOCS-PDD, the CASI Anxiety scale, the ADHD Rating Scale-IV scores. No serious adverse events were observed. The most common registered side effects were agitation, irritability, fatigue, psychomotor hyperactivity, insomnia and diarrhea. Of note, a previous randomized placebo controlled study involving patients with Fragile X syndrome and no concomitant ASD observed no statistical significant effect on the primary outcome (irritability) (Berry-Kravis et al. 2012). Subsequent post-hoc analysis revealed significant amelioration in secondary outcomes, such as the ABC-Social Avoidance subscale, especially in patients with more severe social symptoms. Finally, a randomized placebo controlled trial (NCT01288716) of arbaclofen in ASD has been recently completed (Veenstra-VanderWeele et al. 2013). The study recruited 150 patients with ASD and impaired social function (identified as having an ABC lethargy/social withdrawal subscale score ≥8). Arbaclofen dosage was titrated at a maximum of 10 mg thrice a day (children between 5–11 years of age) or 15 mg thrice a day (adolescents between 12–21 years of age). Only 20 patients did not complete the study. Primary outcome measure was change in ABC-Social Withdrawal score, while secondary outcomes included completing CGI Severity and Improvement, and the Socialization and Communication subscales of the VABS. No statistical significant difference between placebo and arbaclofen was observed in the primary outcome measure; among secondary outcomes, only CGI Severity significantly improved in the arbaclofen group compared to the placebo. A significant improvement in the VABS socialization subscale in the arbaclofen group was found only in a subsequent per protocol analysis.

Bumetanide

Bumetanide is a chloride co-transporter NKCC1 antagonist diuretic which can reduce intracellular concentration of chloride in neurons, thus potentiating GABA inhibition. It successfully reduced

seizures in patients with temporal lobe epilepsy (Eftekhari et al. 2013). In 2010, this molecule was tested in five children with ASD in an open-label trial (Lemonnier and Ben-Ari 2010). Bumetanide was administered at a dosage of 1 mg/day for three months. Study results showed a significant improvement of the total scores of Childhood Autism Rating Scale (CARS), the ABC, the Regulation Disorder Evaluation Grid (RDEG) and the Repetitive and Restricted Behaviour (RRB). No significant changes were reported in the CGI-I or CGI-S score. No adverse event was reported. A subsequent double-blind randomized clinical trial (Lemmonier et al. 2012) enrolled 60 children with ASD. Each patient received bumetanide (1 mg/day) for three months. The authors observed a significant amelioration in CARS score and in CGI-I score. Five children dropped out from the study, but only one for an adverse event (hypokalemia). The other drop-outs were removed because of enuresis (n=2) and hyperactivity (n=2) due to wash-out of their former therapy (methylphenidate and risperidone). Recently, Hadjikhani and colleagues (2013) conducted a 10-month open-label study in which seven ASD patients were treated with bumetanide (1mg/day); their findings showed a significant improvement in face emotion recognition and in the total score at the Toronto Alexithymia Scale. Moreover, fRMI data showed an increased activation in face coding brain regions, as the fusiform cortex. No serious adverse event was reported apart from a mild case of hypokalemia (which was resolved after oral potassium supplementation).

Carnosine

The dipeptide L-carnosine acts by reducing zinc and copper influx near GABA receptors, thus indirectly enhancing GABA function (Trombley et al. 1998), particularly in the frontal cortex. Its high penetration through the blood-brain barrier candidates L-carnosine as a potential treatment for neuropsychiatric conditions with GABAergic dysfunction such as autism spectrum disorders. A double-blind randomized controlled trial (Chez et al. 2002) evaluated carnosine treatment efficacy in 31 children with ASD. Carnosine was administered orally at the dosage of 800 mg/day. Unfortunately, the authors did not compare directly mean changes from baseline to follow-up in the

two groups. Basing the analysis on the follow-up data, after an 8-week follow-up, no significant difference could be detected in the CGI-change (p=0.19), age-adjusted expressive vocabulary (p=0.5), age-adjusted receptive vocabulary (p=0.5), CARS (p=0.12), and Gillian Autism Rating Scale (p=0.34) between the carnosine (n=14) and the placebo group (n=17). Carnosine supplementation was overall well tolerated.

Flumazenil

Flumazenil is a benzodiazepine antagonist, commonly used to counteract benzodiazepine action (Brodgen and Goa 1991). Flumazenil increases $GABA_A$-mediated currents evoked by GABA itself, while it antagonizes the action of benzodiazepine agonist on the $GABA_A$ response (Weiss et al. 2002). The only study on this drug (Wray et al. 2000) in ASD involved two patients in a randomized placebo controlled crossover manner. Each subject was administered 2 mg of flumazenil or placebo in several subsequent crossover trials. Flumazenil dose was incremented at every trials up to 11.5 mg. After injection, an one-hour videotape was recorded for each participant and behaviors were scored in a blinded fashion. No significant difference was observed between flumazenil or placebo. No adverse event was reported.

Riluzole

Riluzole is recently developed drug, originally used for the treatment of amyotrophic lateral sclerosis. Its action is composed by inhibition of voltage-dependent sodium channels and by reduction of glutamate release and augmentation of its reuptake (Frizzo et al. 2004; Martino et al. 1993). Its exact mechanism of action remains unclear: uncertain evidence suggests that it may be an antagonist of NMDA post-synaptic receptors, while, more recently, riluzole has shown to directly affect post-synaptic $GABA_A$ receptors, binding to a site independent of the barbiturate, neurosteroid, and benzodiazepine sites (He et al. 2002). Ghaleiha and coworkers (Ghaleiha et al. 2013) evaluated riluzole as an adjunctive therapy to risperidone in a randomized placebo controlled

trial enrolling 49 patients with ASD. Participants were randomly assigned to either riluzole plus risperidone (n=25) or placebo plus risperidone (n=24) for 10 weeks. Risperidone dosage was titrated to 2 mg/day if body weight was <40 kg or 3 mg/day if body weight≥40 kg. Riluzole was titrated to 25 mg twice daily or 50 mg twice daily according to the body weight as for risperidone. In total, nine patients dropped out (riluzole n=5; placebo n=4), resulting in a sample size smaller than the expected from power calculation analysis. Riluzole in adjunction to risperidone determined a better improvement in irritability, lethargy/social withdrawal, stereotypy and hyperactivity compared to risperidone alone: however, only a per protocol analysis was conducted. The riluzole group showed significant weight gain.

Valproate

Valproate is used as an anticonvulsant and mood-stabilizer. Its role in modulating GABAergic transmission, as well as its mechanism of action, is still controversial. It has been proposed that valproate may increase GABA inhibitory effects in specific brain areas (Löscher 2002) and increase GABA brain levels, even if this effect could be observed only with higher dosage (Sawaya et al. 1975; Löscher 1981). Valproate does not bind directly GABA receptors, but modulates GABA concentrations by increasing its synthesis and reducing its metabolism. Even if GABAergic signaling could not be regarded as the only target for valproate action, a potential role of this drug in GABA functioning could not be ruled out.

So far, only three studies have been conducted so far evaluating valproate use in ASD. The first (Hellings et al. 2005) was a randomized placebo controlled trial enrolling 30 patients with PDD (Pervasive Developmental Disorder) and significant aggressive behavior (at least three times per week). Participants were randomly assigned to either valproate (n=16) or placebo (n=14) for 8 weeks. After that point, patients in both groups were offered to enter an open-maintenance trial of valproate: overall six patients from the placebo group and ten patients from the active group continued the trial. Valproate dosage was titrated to 20 mg/kg/day, in order to obtain drug plasma

blood levels between 70 and 100 mcg/mL. No significant difference in aggressive behavior and irritability was observed between the two groups. Only mild side effects were reported: however, two patients discontinued treatment due to skin rash and hyperammonemia. The second study (Hollander et al. 2006) was a randomized placebo controlled trial which recruited 13 autistic patients. Nine patients were randomized to valproate and four to placebo. Valproate dose was initially 125 mg/day and subsequently increased by 125 mg every 4 days during the first two weeks of treatment. Valproate plasma levels were controlled in order to assure comparable concentrations between subjects. The outcome measure was the change in the Children's Yale–Brown Obsessive Compulsive Scale (C-YBOCS), Compulsion subscale. After 8-week follow-up, the authors observed a significant improvement in compulsive symptoms in the valproate group compared to placebo. Of note, no significant difference in frequency and type of side effects between the two groups was observed. Recently, Hollander et al. (2010) conducted a 12-week randomized double-blind placebo controlled trial in 27 individuals with ASD. Three patients withdrew from the study due to lack of efficacy or adverse events. Valproate dose was titrated according to body weight to a maximum of 500 mg/day (if weight<40 kg) or 1000 mg/day (if weight>40 kg). A significant improvement in irritability (measured with the ABC-Irritability subscale and the CGI for irritability) was observed. No significant difference between the two groups was reported in levels of aggression or compulsive symptoms. No serious adverse event was recorded.

Ongoing trials

Interestingly, there are some ongoing clinical trials evaluating the efficacy of GABA modulators in ASD. One study (NCT02094651) will soon start to recruit children with ASD and epileptiform abnormalities. The study aims at evaluating the potential beneficial effect of valproate on epileptiform abnormalities and, in turn, on behavior in these subjects. The trial will be a randomized placebo controlled study lasting for 26 weeks. Additionally, a pilot phase IIa study (NCT01813318) comparing acamprosate and placebo is recruiting youth with ASD. All participants

will be randomly assigned to active treatment or placebo for 10 weeks and then social withdrawal and CGI-Improvement will be evaluated. A larger open-label trial (NCT01706523) has been designed in order to test safety and efficacy of chronic treatment with arbaclofen in 165 subjects with ASD but it has been terminated due to the failure of the randomized trial on arbaclofen (NCT01288716) (Veenstra-VanderWeele et al. 2013). Recently, a phase IIa study (NCT01966679) has been designed to test a new medication (AZD7325), originally developed for treatment of anxiety and acting as a $GABA_A$ positive modulator. In particular, it does not act as an intrinsic agonist but facilitates diazepam effect at $GABA_A$ receptor subunits α2 and α3. Additionally, it is a zolpidem antagonist at the $GABA_A$ receptor subunits α1. According to this, it does not have sedative effect, and displays a high tolerability and safety. AZD7325 will be tested in a randomized double blind fashion in 40 ASD adults (18 - 35 years old) with intelligence in the normal range and the presence of specific EEG patterns which will be treated for 6 weeks.

Discussion

Evidence in the literature supports the hypothesis of GABAergic dysfunction in autism. Unfortunately, to date, only few clinical trials evaluating potential GABA modulators (such as arbaclofen or acamprosate) have been conducted, yielding mixed and inconclusive results. Several reasons could be accounted for these conflicted findings. In general, from a methodological point of view, GABA modulators have been evaluated mostly by means of open-label trials which are subjected to important biases (i.e. absence of blinding, reporting bias) and, frequently, outcome measures relied on parent-report (which could have induced expectancy biases). Sample sizes in all the reviewed trials were generally small, thus reducing the scientific validity of the findings. Moreover, almost all the selected trials allowed concomitant psychoactive medications which could have hampered the possibility to detect a real effect of the studied drug.

Specifically, arbaclofen and acamprosate were the molecules more extensively studied. In particular, randomized trials on arbaclofen have yielded negative results (Berry-Kravis et al. 2012;

Veenstra-VanderWeele et al. 2013). These findings have raised important concerns in the scientific community as preliminary data on the potential efficacy of this drug in improving autistic core symptoms were strong: in fact, arbaclofen targets specific brain mechanisms that could be altered in autism and appeared to be efficacious in animal models of autism and related conditions (Henderson et al. 2012). Thus, the failure of arbaclofen in clinical trials and the interruption of its development program was a major disappointment in autism research, and it could have hampered the GABAergic theory of ASD. It is important to note that the largest study on arbaclofen (Veenstra-VanderWeele et al. 2013) failed to obtain significant difference in the primary outcome measure (ABC-Social Withdrawal score), disappointing all the expectations of scientific community; however, it showed significant improvement in secondary outcome measures (among which also social impairment assessed by means of Vineland). The arbaclofen study could represent a starting point to better design pharmacological trial in autism. In fact, potential explanations for the lack of efficacy of arbaclofen and/or other medications could be the result of the extreme heterogeneity of autism: as symptoms may vary significantly in quality and severity among patients, it may be difficult to measure significant change with a single outcome. Thus, a better characterization of study participants with more restrictive inclusion criteria should be recommended. In fact, several studies may have been hampered by the inclusion of patients with wide IQ range or with high level of aggressiveness or irritability. Additionally, as GABA dysfunctions may not be present in each ASD patient, it could be argued that a potential predictor of the efficacy of GABA modulators is the presence of a primitive GABA impairment: thus, for instance, EEG characterization of participants (as it is performed in recent ongoing trials) could represent a potential marker of treatment utility and success. In fact, to date, none of the reviewed studies used endpoints that assessed specifically compound-induced GABAergic effects. Another important point is the design of more robust and specific outcome measures that should better capture improvement in core symptoms: to date, most of the questionnaires are not at all specific to psychopathological characteristics of ASD and are subjected to rater variability.

Apart from arbaclofen, acamprosate, bumetanide and valproate have been more intensively studied. Acamprosate appears to be effective on autistic symptoms: however, apart from one single blind randomized trial (Erickson et al. 2014a), all the reviewed studies (Erickson et al. 2011; Erickson et al. 2013) were open-label, a design which implies several bias (in particular it could not be ruled out the placebo effect). Clinical impression of improvement was the main outcome measure in all experiments. Overall, bumetanide showed a positive effect. The only bumetanide double blind randomized trial (Lemmonier et al. 2012) evaluated the change in ASD symptoms using more accurate and specific scales than for acamprosate trials, in particular CARS and ADOS. In fact, CGI scales are not specifically constructed for ASD patients. The other trials on bumetanide were open-label and with very small sample sizes (Lemonnier and Ben-Ari 2010; Hadjikhani et al. 2013). Valproate trials were well designed and yielded promising findings (Hellings et al. 2005; Hollander et al. 2006; Hollander et al. 2010): in particular, only one study did not show a positive result (Hellings et al. 2005). In general, all the reviewed trials on valproate have small sample sizes and short study follow-ups; additionally, they frequently included patients with wide IQ ranges, high symptom severity and used scales not specific for ASD (as obsessive-compulsive behaviors).

Trials on other GABAergic compounds have been sparse. Particularly, the carnosine study (Chez et al. 2002) was well designed but hampered by small sample size, which could have contributed to the lack of efficacy observed in autistic patients. The flumazenil trial (Wray et al. 2000) included only two subjects, and this may have determined the negative findings. On the contrary, the riluzole trial (Ghaleiha et al. 2013) showed promising results: however, the authors recruited less participants than expected and used only two outcome measures (ABC-irritability and CGI).

In conclusion, to date there is insufficient evidence to suggest the use of GABA modulators in autistic patients. Of note, almost all the evidence focused on children or adolescents and no study has been specifically designed for adults. However, short-term use of the reviewed drugs appears to be safe: longer studies are needed to elucidate the potential presence of chronic toxicity in humans.

Hopefully, future findings from ongoing clinical trials will shade more light on the potential therapeutic efficacy of GABA modulation in autism spectrum conditions. Furthermore, future well-designed multicenter studies with longer follow-up will help to elucidate this still promising line of research

Conflict of interest

All authors declared that there is no conflict of interest

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Figure legend

Figure 1. Risk of bias graph of the included studies

Figure 2. Risk of bias summary of the included studies (green circle: low risk of bias; red circle: high risk of bias; blank: unclear risk of bias)

Figure

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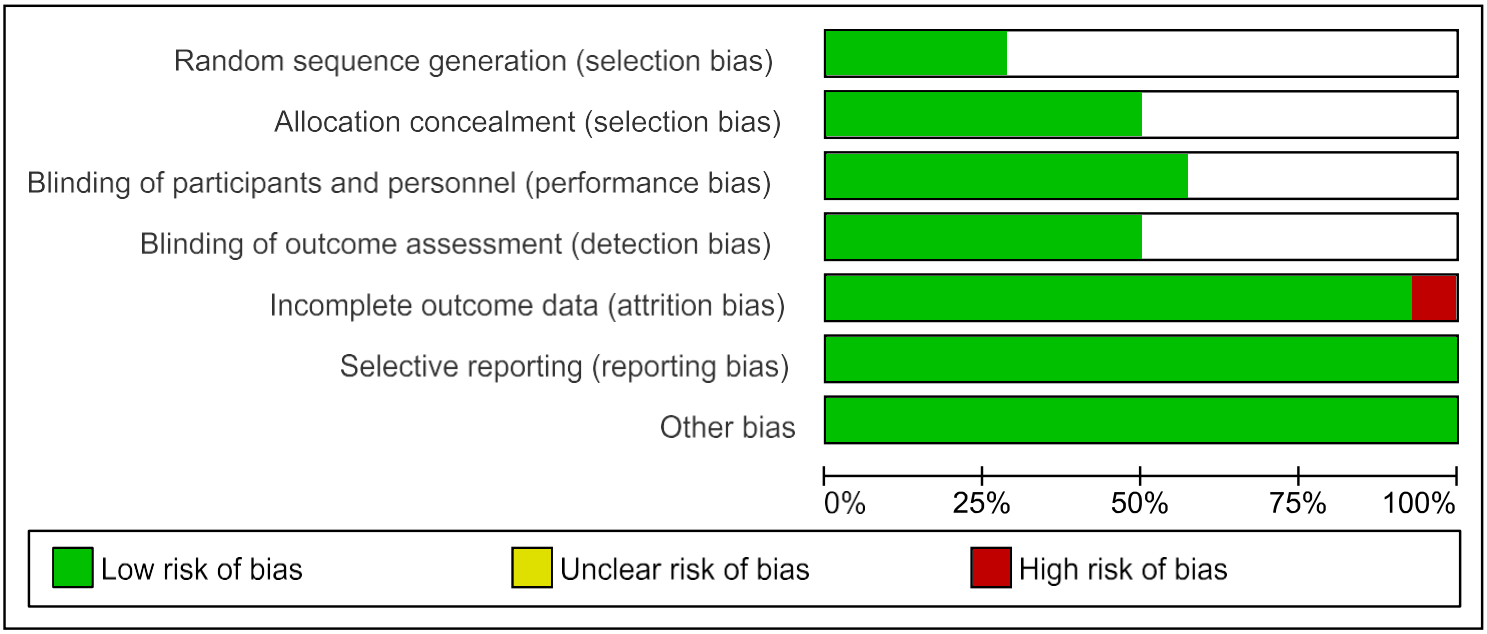

Figure

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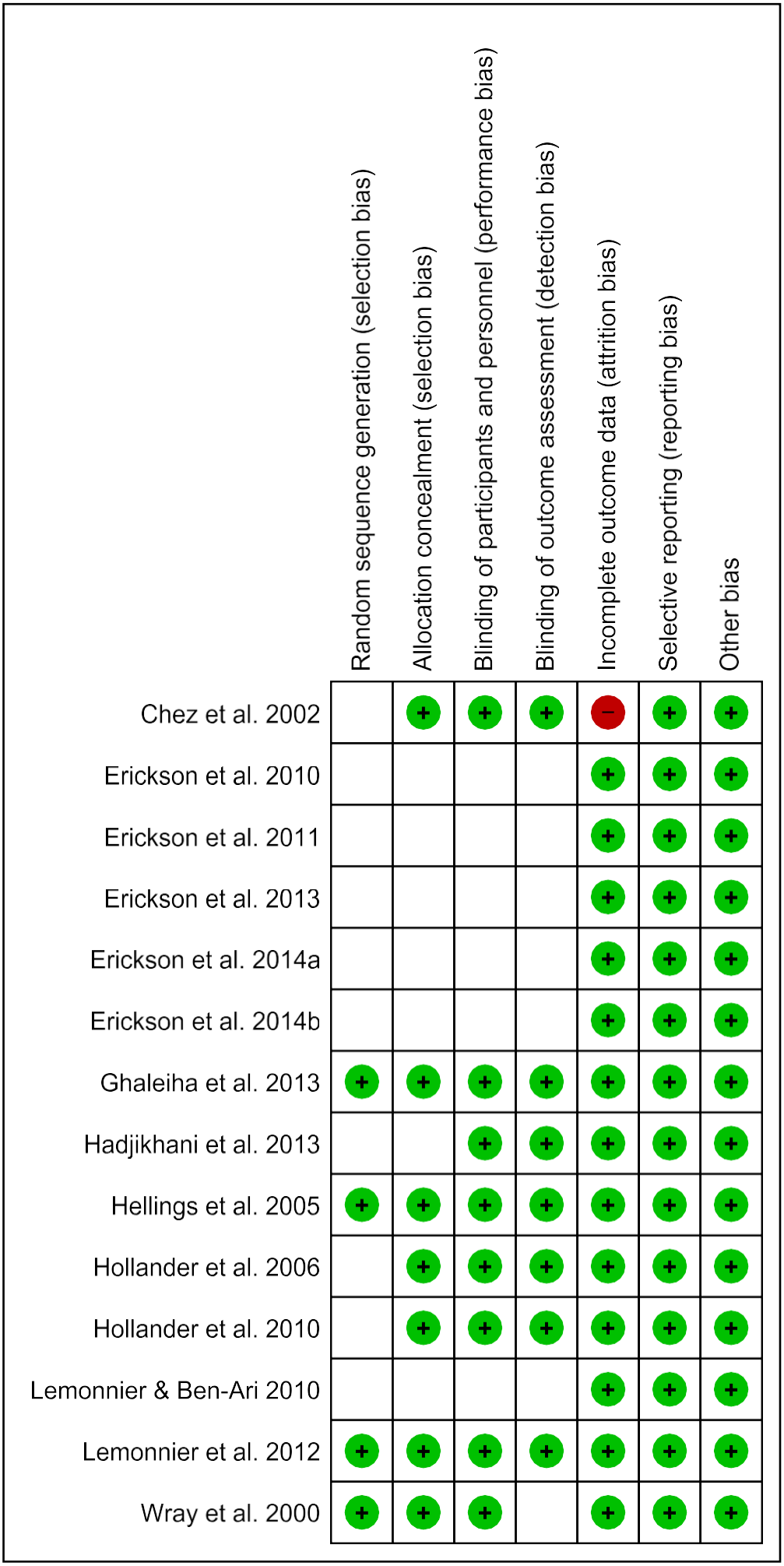

Table 1. Clinical studies of GABA modulators in autism spectrum disorders

Study ID	Study design	Sample characteristics	Follow-up period	Drug tested and dose	Other medication	Main findings	Outcomes	Adverse events	Current status
Completed studies									
Erickson et al, 2010	Case series, open label	3 (age range: 18-23 years; all males; all with comorbid Fragile X syndrome; all with mild to moderate intellectual disability-no IQ values)	16-28 weeks	Acamprosate 333mg/day to 1998 mg/day	Antipsychotic (ziprasidone, aripiprazole)	Improved communicative language	CGI-I	No serious adverse event	Completed and published
Erickson et al, 2011	Open label	6 (age range: 6-12.5 years; no information on gender or IQ values; inclusion criteria: absence of medical or psychiatric comorbidity)	At least 4 weeks	Acamprosate, maximum dose 1332 mg/day	Previous medications (antipsychotic or psychostimulants or desmopressin) allowed; glutamatergic agents such as memantine, riluzole, and D-cycloserine not permitted	Improved social behavior and hyperactivity	CGI-I, CGI-S, ABC, SRS	No serious adverse event	Completed and published
Erickson et al, 2013	Open label	12 (age range: 6-17 years; F/M: 2/10; 10 with ASD and 2 with PDD-NOS; all with	10 weeks	Acamprosate, 1998 mg/day if weight>50 kg; 1332 mg/day if weight<50	Previous medications (antipsychotic or psychostimulants) allowed	Improved social behavior, hyperactivity and communicative skills	CGI-I, CGI-S, ABC, SRS, CY-BOCS-PDD, ADHD-RS, VABS,	No serious adverse event	Completed and published

		comorbid Fragile X syndrome; body weight>15 kg; IQ value range: 36-61; inclusion criteria: CGI≥4; mental age>18 months; absence of psychiatric or medical comorbidity)		kg.				PPVT, CELF				
Erickson et al, 2014a	Single-blind placebo lead-in	12 (age range: 5-15 years; F/M: 3/9; body weight>15 kg; IQ value range: 25-96; inclusion criteria: CGI≥4; mental age>18 months; absence of medical comorbidity)	2 weeks on placebo, 10 weeks on active treatment	Acamprosate, mean dose 27.9 ± 11.3 mg/kg/day		Previous medications (antipsychotic or psychostimulants) allowed	Improved social behavior and hyperactivity	CGI-I, CGI-S, ABC, SRS, CY-BOCS-PDD, ADHD-RS	No serious adverse event		Completed and published	
Erickson et al, 2014b	Open label	32 (age range: 6-17 years; F/M: 3/29; 27 with ASD and 5 with PDD-NOS; IQ value mean:	8 weeks	Arbaclofen maximum dose 20 mg/day (ages 6–11 years) or 30 mg/day (ages 12–		Up to two concurrent psychoactive medications were allowed if dosing had been stable for	Improved irritability, social behavior, compulsive symptoms	ABC, CGI-I, CGI-S, SRS, CY-BOCS-PDD, ADHD-RS, CASI-4 anxiety	One serious case of aggression		Completed and published	

		56±22; inclusion criteria: CGI≥4; ABC score>17; absence of known genetic comorbidity; no history of seizures or on stable antiepileptic regimen and free of seizures for at least 6 months)		17 years)		at least 4 weeks		scale, VABS, Leiter-R brief IQ scale, and visual analog scale for 3 behaviors nominated by parents or caregivers				
Lemonnier & Ben-Ari, 2010	Open label	5 (age range: 3-11 years; F/M: 1/4; no information on IQ; inclusion criteria: absence of neurological disorders)	3 months	Bumetanide 1 mg/day		None	Improved autism severity, no different in the clinical global impression	CARS, ABC, CGI, RDEG, RRB	No serious adverse event		Completed and published	
Lemonnier et al, 2012	Double blind randomized	60 (age range: 3-11 years; F/M: 16/44; no information on IQ; inclusion criteria: absence of karyotype abnormalities and/or neurological	3 months	Bumetanide 1 mg/day		None (except melatonin)	Improved autism severity	CARS, ADOS, CGI	A case of hypokalemia		Completed and published	

		antecedents, including epilepsy and febrile seizures)										
Hadjikhani et al, 2013	Open label	7 (mean age: 19.3±4.6 years; all males; performance IQ: 101.4±14.5; no inclusion criteria reported)	10 months	Bumetanide 1 mg/day		Not reported	Improved emotion recognition accuracy and score at the TAS	Emotion recognition; TAS; fRMI data	A case of mild hypokalemia		Completed and published	
Chez et al, 2002	Double blind randomized	31 (age range: 3.2-12.5 years; F/M: 10/21; no information on IQ; inclusion criteria: absence of known genetic comorbidity; no family history of seizures)	8 weeks	Carnosine 800 mg/day		Not reported	No difference between carnosine and placebo	CGI; EOWPVT; ROWPVT; CARS; GARS	No serious adverse event		Completed and published	
Wray et al, 2000	Double blind randomized crossover	2 (age range: 3.3-11 years; all males; no information on IQ; no inclusion criteria	1 hour after administration	Flumazenil 2 mg up to 11.5 mg		Not reported	No difference compared to placebo	GMDS; CARS	No serious adverse event		Completed and published	

		reported)										
Ghaleiha et al, 2013	Double blind randomized	40 (age range: 5-12 years; F/M 7/33; body weight mean: 31.95 kg; no information on IQ; inclusion criteria: ABC-C irritability≥12; absence of significant medical and psychiatric comorbidities, including severe mental retardation; no hypersensitivity to riluzole, drug or alcohol abuse, and tardive dyskinesia; no psychotropic medication within 6	10 weeks	Riluzole 25 up to 50 mg/day (if weight<40 kg) or 100 mg/day (if weight≥40 kg)		Risperidone up to 2 mg/day (if weight<40 kg) or 3 mg/day (if weight≥40 kg)	Improvement in irritability, lethargy/social withdrawal, stereotypic behavior, and hyperactivity/non-compliance	ABC-C irritability; CGI-I	Increased appetite; weight gain		Completed and published	

		weeks prior to enrollment)										
Hellings et al, 2005	Double blind randomized	30 (age range: 6-20 years; F/M: 10/20; 29 with ASD and 1 with PDD-NOS; IQ value range: 20-137; inclusion criteria: significant aggression to self, others, or property at least three times per week, no previous adequate VPA trial for any indication, no clinical seizures within the past year, no history of degenerative neurological changes or	8 weeks	Valproate maximum dose 20 mg/kg/day		None	No difference compared to placebo	ABC (Irritability); POAS; CGI-S; CGI-I	One case of skin rash and one case of hyperammonemia		Completed and published	

		metabolic disorders, Tourette's Disorder, no history of thrombocyto penia, hepatitis, pancreatitis, pregnancy, or polycystic ovarian syndrome)										
Hollander et al, 2006	Double blind randomized	13 (age range: 5-17 years- although one subject had 40 years; no data on gender; 12 ASD and one PDD; IQ value range: 30-104; inclusion criteria: absence of present or past medical or psychiatric comorbidities , no recent or current use of divalproex, terfenadine, or astemizole	8	Valproate mean dose 822.92 mg/day (range=500 –1500 mg/day)		Only one participant was on a stable dose of risperidone	Improved compulsiv e symptoms	CY-BOCS	No serious adverse event		Complete d and published	

Hollander et al, 2010	Double blind randomized	27 (age range: 4.85-14.92 years; F/M: 4/23; IQ value range: 30-126; inclusion criteria: CGI≥4; OAS-M≥13; ABC-Irritability≥18, absence of medical or psychiatric comorbidities, no psychopharmacological treatment in the previous 30 days, no hypersensitiv to divalproex sodium or beginning of a new alternative or psychosocial	12 weeks	Valproate maximum of 500 mg/day (if weight<40 kg) or 1000 mg/day (if weight>40 kg).		None	Improved irritability, no difference in aggression and clinical global impression	CGI-I; ABC-Irritbility; OAS-M; CYBOCS; VABS; YMRS; electroencephalogram	No serious adverse event		Completed and published	

		therapy within the previous 3 months; they excluded sexually active and pregnant females and nursing mothers or if subjects have VABS<2 years or a premature birth;)										
					Ongoing trials							
NCT01706523	Open-label	165 (inclusion criteria: age between 5 and 21 years old, seizure disorder must be adequately well-controlled, negative pregnancy test, no comorbid conditions that might interfere with the	100 weeks	Arbaclofen, 2 mg/day to 30 mg/day		no more than 2 psychoactive medications allowed	-	Safety and tolerability (ABC as secondary outcome)	-		Terminated	

		conduct of the study or confound the interpretation of the study data, or endanger the subject, no current use of illicit drugs or alcohol abuse, no current use of another investigational drug, or of vigabatrin, tiagabine, or riluzole										
NCT01288716	Randomized, double-blind, placebo controlled	150 (inclusion criteria: age between 5 and 21 years old, seizures must currently be treated with antiepileptics the subject must be seizure free for 6 months, or for 3 years if not currently receiving antiepileptic	8 weeks	Arbaclofen, 2 mg/day to 30 mg/day		Every concurrent medications allowed if at a stable dosage for more than 4 weeks	-	ABC-Social withdrawal	-		Completed	

		s, if the subject was receiving stable non-pharmacologic educational, behavioral, and/or dietary interventions, these programs must have been continuous for 2 months prior to admission, no comorbid medical conditions that might interfere with the conduct of the study										
NCT01813318	Randomized, double-blind, placebo controlled	36 (inclusion criteria: 5-17 year-old outpatients, general good health, stable seizure disorder (no seizures in 6 months; on same anti-convulsant	10 weeks	Acamprosate, 1998 mg/die if weight>50 kg; 1332 mg/die if weight<50 kg.		Permitted up to two concomitant psychotropic drugs (stable dosing for >60 days) not impacting glutamate GABA neurotransmission	-	ABC-Social Withdrawal, CGI-I (secondary outcomes: ABC-Irritability, Stereotypy, Hyperacti	-		Still recruiting	

		dose for >60 days), CGI-S of 4, ABC-Social withdrawal score of 13, creatinine clearance > 50 mL/min, no previous trial with acamprosate						vity, and Inappropriate Speech				
NCT02094651	Randomized, double-blind, placebo controlled	30 (age range: 4-8 years, IQ range: 40-80; inclusion criteria: frequent epileptiform discharges on EEG-defined as spikes, spike wave, and sharp waves occurring at greater than 15 events per hour, weight ≥ 12.5 kg; no history of epilepsy, known genetic abnormalities with high rates of epilepsy, or structural	26 weeks	Sodium valproate 800 mg/day		Not allowed previous treatment with valproate greater than 6 months duration within the last 12 months that was associated with significant side effects leading to termination of treatment, no concomitant use of medication including topiramate, lamotrigine, and drugs that inhibit cytochrome p450 enzyme	-	Reduction in epileptiform EEG discharges Secondary outcomes: behavioural changes (CBCL)	-		Still recruiting	

		brain lesion, no general anesthesia within the six months or sedation within 2 weeks of study enrollment. No recent introdyction of new behavioral or psychotropic treatment, absence of medical condition, that would be a contraindication to valproate, no severe behavioral management issues (e.g. self-injury, aggressiveness)										
NCT01966679	Randomized, double-blind, placebo controlled	40 (age range: 18-35 years; IQ>80; inclusion criteria: ABC-Social Withdrawal Score >10 evidence of	6 weeks			Existing allowed concomitant medication treatment stable for the 8 weeks prior to study entry, and no anticipated	-	EEG	-		Recruiting	

		EEG biomarker deficit, absence of current or past drug or alcohol abuse or dependence, no history of seizure disorder (except febrile seizures), no clinically significant aggressive, disruptive, or suicidal behavior in the 3 months prior to study enrollment, absence of chornic medical comorbiditie s, no history of paradoxical reactions to benzodiazepi nes Fredericia-corrected QT (QTcF) interval<450 msec, for sexually				changes						

		active female and male subjects, agreement to use double-barrier birth control method during the study										

Legend. Aberrant Behavior Checklist: ABC; ADHD Rating Scale, Fourth Edition: ADHD-RS; Autism Diagnostic Observation Schedule: ADOS; Childhood Autism Rating Scale: CARS; Child and Adolescent Symptom Inventory: CASI; Clinical Evaluation of Language Fundamentals, Fourth Edition: Child Behavior Checklist: CBCL; CELF; Children's Yale–Brown Obsessive-Compulsive Scale: CY-BOCS; Children's Yale-Brown Obsessive Compulsive Scale Modified for Pervasive Developmental Disorders: CY-BOCS-PDD; Clinical Global Impression-Improvement: CGI-I; Clinical Global Impression-Severity: CGI-S; Expressive One-Word Picture Vocabulary test: EOWPVT; Gilliam Autism Rating Scale: GARS; Griffiths Mental Development Scales: GMDS; Neuropsychiatric Inventory Questionnaire: NPI-Q; Overt Aggression Scale-Modified: OAS-M; Parent Overt Aggression Scale: POAS; Peabody Picture Vocabulary Test: PPVT; Regulation Disorder Evaluation Grid: RDEG; Receptive One-Word Picture Vocabulary test: ROWPVT; Repetitive and Restricted Behaviour: RRB; Social Responsiveness Scale: SRS; Toronto Alexithymia Scale: TAS; Vineland Adaptive Behavior Scale: VABS; Young Mania Rating Scale: YMRS

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