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Excitatory/inhibitory imbalance in autism spectrum disorders: Implications for interventions and therapeutics

Genoveva Uzunova¹, Stefano Pallanti¹,²,³,⁴ and Eric Hollander¹

¹Albert Einstein College of Medicine and Montefiore Medical Center, Bronx, NY, USA, ²Psychiatry and Behavioural Sciences, UC Davis Health System, CA, USA, ³Department Psychiatry, University of Florence, Florence, Italy, and ⁴Icahn School of Medicine at Mount Sinai, New York, NY, USA

ABSTRACT

Objectives: Imbalance between excitation and inhibition and increased excitatory-inhibitory (E-I) ratio is a common mechanism in autism spectrum disorders (ASD) that is responsible for the learning and memory, cognitive, sensory, motor deficits, and seizures occurring in these disorders. ASD are very heterogeneous and better understanding of E-I imbalance in brain will lead to better diagnosis and treatments.

Methods: We perform a critical literature review of the causes and presentations of E-I imbalance in ASD. Results: E-I imbalance in ASD is due primarily to abnormal glutamatergic and GABAergic neurotransmission in key brain regions such as neocortex, hippocampus, amygdala, and cerebellum. Other causes are due to dysfunction of neuropeptides (oxytocin), synaptic proteins (neuroligins), and immune system molecules (cytokines). At the neuropathological level E-I imbalance in ASD is presented as a "minicolumnopathy". E-I imbalance alters the manner by which the brain processes information and regulates behaviour. New developments for investigating E-I imbalance such as optogenetics and transcranial magnetic stimulation (TMS) are presented. Non-invasive brain stimulation methods such as TMS for treatment of the core symptoms of ASD are discussed. Conclusions: Understanding E-I imbalance has important implications for developing better pharmacological and behavioural treatments for ASD, including TMS, new drugs, biomarkers and patient stratification.

I. Introduction. A heuristic model for development of interventions and therapeutics

A widely-accepted hypothesis on the aetiology of autism spectrum disorders (ASD) proposes that there is excitatory-inhibitory imbalance (E-I imbalance) in brain neural circuits (Rubenstein and Merzenich 2003). This imbalance underlies the social, behavioural, emotional, cognitive, sensory and motor control abnormalities. Increased E-I ratio in prefrontal cortex is shown to result in behavioural and social impairments characteristic of ASD using optogenetics (Yizhar et al. 2011). Decreased E-I ratio is found in Rett syndrome (RS), a form of ASD (Eichler and Meier 2008). We will refer to these mechanisms collectively as E-I imbalance. Several reviews highlight the significance of E-I imbalance in ASD (Rubenstein and Merzenich 2003; Polleux and Lauder 2004; Eichler and Meier 2008; Baroncelli et al. 2011; Ecker et al. 2013; Zikopoulos and Barbas 2013).

Studies show local hyperconnectivity and long-range hypoconnectivity and disconnection between neural circuits in ASD (Courchesne and Pierce 2005; Peters et al. 2013; Tye and Bolton 2013). The literature on ASD has greatly expanded and we consider it is necessary to write a review highlighting new advances on E-I imbalance in ASD such as the role of the immune system, new methods to study E-I, biomarkers, patient stratification and selection of appropriate treatments. We will focus on developments with significance for neuroscience-informed and research-based clinical therapeutic approaches.

Factors controlling the formation and functioning of excitatory and inhibitory synapses affect the E-I ratio. The earliest time point when E-I imbalance occurs in ASD is important to identify in order to develop early therapeutic interventions. There are challenges to studying E-I imbalance in human brain and much of our knowledge derives from animal models. Although this knowledge...
largely overlaps with humans, there are differences, and it is necessary to uncover the mechanisms in human ASD with techniques such as transcranial magnetic stimulation (TMS), electroencephalography (EEG), magnetoencephalography (MEG) and post-mortem brain studies.

II. Mechanisms and presentation of E-I imbalance

1. Mechanisms by which E-I imbalance impacts the clinical presentation of ASD

ASD are neurodevelopmental disorders with symptoms in two core domains – social-communication deficits and repetitive behaviours and restricted interests. Associated symptoms are irritability, hyperactivity, movement abnormalities, language delay, sensory issues, seizures, learning disabilities, developmental regression and gastrointestinal symptoms. Most ASD present without an apparent cause (i.e. idiopathic). One group, “syndromic forms”, result from single-gene defects such as Tuberous Sclerosis Complex 1 and 2 (TSC1 and 2), Fragile X syndrome (FXS), RS and Angelman syndrome. Toxins, infections, immunological, nutritional, epigenetic factors and stress may play roles in ASD.

E-I imbalance may be due to increase in glutamatergic or decrease in GABAergic signalling. The E-I ratio in neocortex is determined by the activity of pyramidal glutamatergic neurones and inhibitory GABAergic parvalbumin (PV)-positive interneurons, and is modulated by minicolumns. They consist of self-contained neuronal aggregations and their afferent, efferent and interneuronal connections, mediating the interactions of neuronal microcircuits, and are pathologically altered in ASD (Casanova 2007; Opris and Casanova 2014). In ASD there is activation of microglia, patches of disorganised cortex, focal cortical dysplasia (smaller pyramidal neurones, reduced numbers of interneurons), and altered radial migration of neurones resulting in heterotopias, i.e. normal cells in an abnormal location (Casanova et al. 2013). Subependymal and subcortical heterotopias may cause seizures, developmental delay, dyslexia, obsessive–compulsive disorder (OCD) and ASD. ASD is proposed to be a minicolumnopathy with increased numbers of minicolumns and decreased minicolumn width (Casanova 2006; Casanova et al. 2002, 2003a, 2003b, 2010). The sheath around the minicolumns provides inhibitory control and may be decreased due to dense packing (Casanova 2008). These alterations cause inhibitory deficit, sensory noise overload and lack of stimulus discrimination evidenced by evoked gamma EEG power at frontal cortical sites. Importantly, after repetitive TMS, ASD individuals show significant improvement in cortical discrimination of stimuli (Sokhadze et al. 2009).

The E-I ratio of synaptic inputs in developing and adult cortical neurones is dynamically regulated (Zhang et al. 2011). One hypothesis underscores the importance of altered connectivity of excitatory and inhibitory cortical circuits in ASD (Zikopoulos and Barbas 2013). E-I imbalance occurs due to altered neuronal migration, development and network formation. This may result from lack of the extracellular matrix protein reelin supporting GABAergic neuronal development (Folsom and Fatemi 2013).

Brain synapses may be modified by immune proteins such as Major Histocompatibility Class I (MHC-I) antigens that are present on axons and dendrites of cortical neurones during early development, negatively regulate cortical synapse density, establishment of neuronal connections, strength of excitatory but not inhibitory synapses, and control the E-I balance onto cortical neurones (Glynn et al. 2011). Through MHC-I the immune system may regulate the E-I balance in ASD. The immune system may modulate E-I balance through proinflammatory cytokines such as IL-1β, IL-6 and TNFα, which play roles in synaptic plasticity (Beattie et al. 2002), trafficking of AMPA (Beattie et al. 2010), GABA A receptors and learning (Miller and Fahey 1994; Lai et al. 2006; Boulanger 2009; Besedovsky and del Rey 2011; del Rey et al. 2013; Wei et al. 2011, 2012). Mice with elevated brain IL-6 display autistic features and E-I imbalance. TNFα modulates AMPA receptor trafficking in neurons and glia (Beattie et al. 2010; He et al. 2012).

The E-I ratio depends on levels of ionotropic and metabotropic glutamate, GABA receptors (Banerjee et al. 2013; Billingslea et al. 2014), extracellular glutamate and GABA concentrations (Bejjani et al. 2012), oxytocin, reelin, Arc, neuronal translation (Gkogas et al. 2013; Gkogas and Sonenberg 2013), signal transduction, cell adhesion molecules and PSD proteins such as neurolgins (Bourgeron 2007; Kohl et al. 2013). Neuroendocrine factors such as oestrogens may cause E-I imbalance, evidenced in reeler mice (Macri et al. 2010).

E-I imbalance in brain may give rise to:

(a) altered synaptic plasticity, learning and memory discussed in Sections II.2 (Bateup et al. 2013) and III.2.b (Oberman et al. 2010);

(b) seizures (Moavero et al. 2010) due to increase in glutamatergic or decrease in GABAergic neuronal activity, frequent in syndromic ASD such as TSC, RS and FXS;

(c) neural network oscillatory abnormalities such as abnormal gamma oscillations (Grice et al. 2001; Orekhova et al. 2008; Gandal et al. 2010). Gamma
oscillations are instrumental for the synchronisation of neuronal discharges in cortical networks and sensory processing. Research suggests that they may be linked to cognitive functions such as selective attention, short- and long-term memory and multisensory integration;

(d) visual system abnormalities such as atypical visual perception (sensitivity to bright light, atypical face perception), attributed to E-I imbalance in occipital, parietal and frontal cortex (Milne et al. 2009). Gamma oscillations (30–90 Hz) depend on E-I balance and can be recorded using EEG. Measurement of the gamma response to contextual modulation of visual stimuli in control and ASD subjects has shown that controls exhibit a gamma response in the 60-Hz range influenced by contextual modulation. ASD subjects show a smaller gamma response not influenced by contextual modulation (Snijders et al. 2013). These findings indicate that E-I imbalance in ASD is due to GABAergic deficit, extending earlier studies showing atypical gamma response to visual stimuli in ASD (Grice et al. 2001; Sun et al. 2012; Wright et al. 2012).

Gamma oscillations may be a biomarker to stratify patients and follow the responses to therapy.

Another measurement of E-I imbalance is binocular rivalry in which perception reflects the contrasting effects of excitation and inhibition in cortex (Laing and Chow 2002; Said et al. 2013). Adult high-functioning ASD subjects show a slower rate of binocular rivalry than controls, longer duration of mixed percepts and increased likelihood to revert to the previously perceived object when exiting a mixed percept (Robertson et al. 2013). The atypical dynamics of binocular rivalry in ASD predict the autistic symptoms measured with the Autism Diagnostic and Observation Schedule (ADOS), suggesting that binocular rivalry may be a clinical biomarker. Another study found normal binocular rivalry in ASD subjects in comparison with controls and no correspondence between binocular rivalry and ASD severity (Said et al. 2013), indicating minimal E-I imbalance in visual system.

Children with high-functioning ASD exhibit early-stage visual processing abnormalities manifested by augmented evoked potentials elicited by task-irrelevant stimuli which disrupt stimulus discrimination (Baruth et al. 2010a). These abnormalities result from E-I imbalance within parietal-occipital and frontal cortical regions and can be reversed using TMS.

(e) general dyspraxia (impaired performance of skilled gestures) due to abnormalities in frontal-parietal-subcortical neural circuits important for learning of sensory representations of movement and/or the motor sequences necessary to execute them (Mostofsky et al. 2006; Dowell et al. 2009; MacNeil and Mostofsky 2012). This reflects an E-I imbalance in neural circuits within parietal, premotor and motor cortices. Praxis is associated with social, communicative and behavioural impairments suggesting that dyspraxia may be a biomarker of neurological abnormalities in ASD (Dziuk et al. 2007);

(f) behaviour changes and social dysfunction such as repetitive and disruptive behaviours, irritability, social avoidance/withdrawal.

Notably, E-I imbalance is not ASD-specific and is found in other related neurodevelopmental conditions such as attention deficit hyperactivity disorder (ADHD) and OCD (Zimmermann et al. 2014). The specific changes and brain localisation in the E-I imbalance are likely characteristic for each disorder.

2. Syndromic forms of ASD with marked E-I imbalance

TSC is a genetic disorder due to inactivating mutations in the TSC1 or 2 genes. It is associated with learning abnormalities, intellectual disabilities, developmental delay, autism, tubers, and epilepsy (Jeste et al. 2008, 2014). The pathological processes result from loss of the TSC1 or TSC2 proteins regulating the mammalian target of rapamycin (mTOR) pathway: abnormal protein synthesis, synaptic plasticity, reduced neuronal connectivity and CNS myelination, and E-I imbalance (Han and Sahin 2011; Tsai and Sahin 2011; Peters et al. 2012; Jülich and Sahin 2014). Loss of Tsc1 in mice leads to upregulation of mTOR, abnormal protein synthesis and enhanced excitatory synaptic function that alter circuit information processing (Bateup et al. 2011). Loss of Tsc1 in mice is accompanied with weakened inhibition resulting from insensitivity of the glutamatergic neurons to signals from GABAergic neurons due to mTOR deficiency (Bateup et al. 2013), suggesting that drugs enhancing GABA functions may not be effective in TSC. Tsc2 heterozygous knockout mice show deficiency in CA1 hippocampal long-term potentiation (LTP) as result of mTOR dysregulation and learning and memory deficits that are corrected using the mTOR inhibitor rapamycin (Ehniger et al. 2008, 2009). TSC1/2 are important for axon specification, guidance and regeneration, and have effects on neuronal connectivity and E-I balance (Choi et al. 2008; Tsai and Sahin 2011). The deficits in mTOR, GABAergic neurotransmission and E-I imbalance in TSC may be overcome by the neuropeptide oxytocin or mTOR inhibitors (Jülich and Sahin 2014).
**FXS** is a genetic disorder associated with ASD affecting primarily boys. It results from loss-of-function of the X-linked gene FMR1 encoding the RNA-binding protein FMRP (Ronesi and Huber 2008). Associated symptoms are anxiety, sensory abnormalities, hyperactivity, irritability, seizures (Tranfaglia 2012). The E-I imbalance changes in FXS are brain region-specific. Major mechanism is enhanced synaptic protein synthesis due to overactivation of group I metabotropic glutamate receptors (gpl mGluRs; Dolen and Bear 2008; D’Antoni et al. 2014). The functions of the affected synaptic proteins are abnormal leading to changes in excitation and inhibition (Ronesi et al. 2012). Proteins with enhanced translation are PSD-95, Arc, GluA1 and GluA2 receptors (Muddashetty et al. 2007), microtubule-associated protein 1B, striatal-enriched protein tyrosine phosphatase, calcium-calmodulin kinase II (Lüscher and Doll and Broadie 2014) and learning. There is enhanced gpl mGluR-LTD expressed with AMPA receptor internalisation. Besides gpl mGluRs, M1 muscarinic acetylcholine Gq-coupled receptors trigger LTD expressed with AMPA receptor internalisation which is enhanced in FXS (Volk et al. 2007). There are GABAergic deficits in cortex, hippocampus, amygdala, striatum, and subiculum, decreased GABA_A receptors and alterations in GABA production and metabolism in the Fmr1 KO mouse (Paluskiewitz et al. 2011; Doll and Broadie 2014) and learning. There is enhanced gpl mGluR-LTD expressed with AMPA receptor internalisation. Besides gpl mGluRs, M1 muscarinic acetylcholine Gq-coupled receptors trigger LTD expressed with AMPA receptor internalisation which is enhanced in FXS (Volk et al. 2007). There are GABAergic deficits in cortex, hippocampus, amygdala, striatum, and subiculum, decreased GABA_A receptors and alterations in GABA production and metabolism in the Fmr1 KO mouse (Paluskiewitz et al. 2011). Human ASD post-mortem brain studies report decreased GABA_A receptors and GABA_B receptors in anterior cingulate cortex (Oblak et al. 2009, 2010). There is hyperexcitability in layer 4 of somatosensory neocortex of the Fmr1 KO mouse (Gibson et al. 2008). The expression of NMDA receptors is increased in PFC and hippocampus of Fmr1 KO mice (Krueger et al. 2011; Uzunova et al. 2014). These findings provided basis for development of drugs decreasing gpl mGluR activity such as MTEP, MPEP, CTEP (Michalon et al. 2012), AFQ056 (Pop et al. 2014; Jacquemont et al. 2011), increasing GABAnergic functions such as Arbaclofen (Berry-Kravis et al. 2012), and decreasing NMDA receptor functions such as memantine. They aim to restore the E-I imbalance and target the core symptoms of ASD. Clinical trials with these drugs have yielded mixed results. This may be due to the heterogeneity of E-I imbalance and lack of appropriate outcome measures.

**RS** is due to loss-of-function mutations in the X-linked gene for the methyl-CpG binding protein 2 (MeCP2), a transcriptional activator and repressor. RS is associated with autism, regression of language, cognitive functions, social and motor skills, stereotypies, seizures, breathing difficulties, affecting primarily girls. Similarly to TSC and FXS, RS is a disorder of synaptic protein synthesis. There is disrupted development and functions of neuronal circuits and E-I imbalance. Using transgenic mice it is established that MeCP2 is critical for normal function of GABA-releasing neurons (Chao et al. 2010). Human RS post-mortem brain studies show abnormal minicolumns (Casanova et al. 2003a, 2003b). Experimental therapies aiming to restore the E-I imbalance are Insulin-like growth factor 1 (Tropea et al. 2009) for correcting the synaptic plasticity deficits and the ampakine CX546 (Kwaja and Sahin 2011) for correcting the protein synthesis deficits and increasing Brain-derived neurotrophic factor.

### III. Methods to interrogate E-I imbalance in autistic brain

#### 1. Preclinical (animal models)

(a) **Optogenetics** allows manipulating the activity of neurons and neuronal networks (Fenno et al. 2011). It is an invasive method in which light-sensitive ion channels (opsins) are introduced in neurons by viral (AAV or lentiviral) transfection or genetic means. Using light the neurons are activated or inhibited with temporal and spatial precision and the effects of these manipulations on behaviour of living animals is observed (Tye and Deisseroth 2012; Yizhar 2012). Optogenetic manipulation of principal excitatory or inhibitory parvalbumin-positive interneurons in the mouse medial prefrontal cortex (mPFC) shows that elevated (by activating the principal neurons) and not reduced (by activating the inhibitory neurons) E-I balance in the mPFC impairs social behaviour and conditioning (Yizhar et al. 2011). The elevation of the E-I ratio is accompanied by elevated baseline gamma oscillations, a clinical ASD biomarker (Sohal 2012). The social deficits can be reduced by increasing the interneuron inhibitory tone. This study provides support for E-I imbalance in ASD and indicates that the changes are reversible.

#### 2. Clinical

(a) **EEG and MEG** may be used to determine E-I imbalance in ASD alone, or together with TMS, and provide useful biomarkers and outcome measures. Characteristic EEG changes manifesting as excess oscillations in the high frequency range are found in ASD children (Cantor et al. 1986; Orekhova et al. 2007). The EEG findings alone in ASD can be non-specific and resemble those of toddlers indicating maturational lag (Cantor et al. 1986).
MEG studies find abnormal oscillations in ASD children and adolescents suggesting E-I imbalance (Wilson et al. 2007; Cornew et al. 2012). Children and adolescents with ASD exhibit reduced left hemispheric gamma oscillations (Wilson et al. 2007). ASD children exhibit elevations in delta (1–4 Hz), theta (4–8 Hz), alpha (8–12 Hz) and high frequency (20–120 Hz) power (Cornew et al. 2012). The increased alpha power in temporal and parietal regions correlates with the severity of ASD symptoms and is of particular interest because inhibitory interneurons play role in its maintenance. MEG studies report that gamma activation underlying face processing is abnormal in ASD (Wright et al. 2012). This is likely due to E-I imbalance in occipital regions. MEG oscillations may depend on medications (SSRIs, methylphenidate), state of arousal, anxiety, eyes open or closed condition and high- or low-functioning ASD. Nevertheless, the findings of abnormal brain activity using MEG and EEG partially correspond and may have clinical significance for biomarkers.

(b) TMS is a technique for non-invasive brain stimulation (NIBS) that can be applied to study and modulate cortical excitability and synaptic plasticity in vivo in humans (Pascual-Leone et al. 2011; Vicario and Nitsche 2013). TMS consists of application of rapidly changing magnetic fields to discrete brain regions using a coil positioned above the targeted region. The magnetic fields penetrate into the brain and induce electric currents depolarising groups of neurons. If applied to motor cortex, the depolarisation can produce contralateral muscle contraction, motor evoked potential (MEP), measured using electromyography (EMG). If applied to non-motor cortical regions, TMS may evoke field potentials which are recorded using EEG. In addition to effects in the targeted neurons, TMS may produce transsynaptic distributed network effects that can be detected using neuroimaging. TMS may be combined with EEG or functional magnetic resonance imaging (fMRI; Oberman et al. 2010). TMS is particularly useful because it detects changes in synaptic plasticity (Oberman et al. 2013). TMS is subdivided into single-pulse (spTMS), paired-pulse (ppTMS) or repetitive (rTMS). SpTMS is a measure of cortical excitability when applied together with EMG or EEG. ppTMS can be used to study the E-I ratio (Kobayashi and Pascual-Leone 2003) whereas theta burst stimulation (TBS) may be used to study LTP, LTD and metaplasticity (Huang et al. 2005). TBS can measure synaptic plasticity in cortex determined by GABA and glutamate receptors. Using ppTMS it is possible to measure intracortical inhibition mediated by GABA$_{(A)}$ or GABA$_{(B)}$ receptors (Oberman et al. 2010). TMS studies in ASD and FXS subjects and controls show that ASD patients have increased cortical excitability and that ASD and FXS patients have altered synaptic plasticity. The findings of hyperplasticity in ASD are consistent irrespective of the aetiology, suggesting that this may be a biomarker (Oberman et al. 2010). The findings of altered synaptic plasticity in ASD and FXS patients are consistent with animal models. Combination of TMS and EEG shows that in ASD there are robust patterns of over- and under connectivity in frontal and temporal cortical regions in the eyes closed resting state (Murias et al. 2007). In the theta frequency range (3–6 Hz) there is locally elevated coherence in frontal and temporal cortex within the left hemisphere, and in the lower alpha range (8–10 Hz) there is reduced coherence within frontal regions in adults with ASD (Murias et al. 2007). The TMS studies need to be expanded with different brain areas and larger patient populations.

TMS may be used to assess physiological biomarkers of E-I imbalance in ASD. It is possible to use common measures of E-I imbalance such as somatosensory evoked potentials (SEP) at baseline to stratify patients and understand responses to therapy. TMS may be used in ASD to induce acute and long-lasting changes in brain cortical excitability and functions depending on the protocol. Examples of use of TMS in ASD are discussed below.

Induction of paired association (PAS) using TMS in high-functioning ASD patients is impaired implying presence an LTP-like deficit (Jung et al. 2013). PAS consists of peripheral electrical stimulation paired with TMS applied to the contralateral motor cortex and depends on intact sensorimotor integration. The short intracortical interval inhibition (SICI) dependent on GABAergic transmission was normal and did not correlate with the PAS effect. These findings suggest that in patients with high-functioning ASD there is reduced excitatory synaptic connectivity and deficits in sensory-motor integration (Jung et al. 2013). The motor learning deficits are likely due to decreased connectivity within basal ganglia, thalamus, primary motor cortex (M1) and SMA (Mostofsky et al. 2009).

Low-frequency rTMS has been applied to modulate the abnormal gamma oscillations resulting from sensory-perceptual deficits in ASD (Baruth et al. 2010b). ASD individuals show augmented gamma band activity indiscriminative of stimulus type during the early stages of visual processing, a consequence of cortical E-I imbalance. Twelve sessions of bilateral slow rTMS to the DLPFC in ASD individuals significantly improved the discriminatory gamma band activity between relevant
and irrelevant stimuli. This was associated with improvement on the responses in the behavioural questionnaires with respect to irritability and repetitive behaviour. Therefore, it is possible to use EEG measurement of stimulus discrimination in ASD as a physiological biomarker of E-I imbalance before and after therapy. This study shows that rTMS can be useful to treat the core symptoms of ASD without major side effects.

In another study low frequency rTMS is applied to the bilateral DLPFC in children with ASD to determine if this will improve the error monitoring and correction deficit (Sokhadze et al. 2012). There was beneficial effect of rTMS and the study showed that the ERPs associated with responses to errors and behavioural performance measures may be used as functional outcome measures.

The brain regions affected by E-I imbalance in human ASD determined in the studies discussed above are indicated in Figure 1.

IV. Interventions for restoration of E-I balance in ASD

1. Pharmacological therapeutics

(a) Glutamatergic drugs. Group I mGlu (mGlu5) antagonists have not proven very effective in human ASD. **Ampakines** (enhancing the activity of AMPA receptors) have shown partial effectiveness in FXS. **Memantine**, NMDA receptor antagonist, has been tested in ASD treatment trials (Ghaleiha et al. 2013; Uzunova et al. 2014). A multi-site double-blind clinical trial with memantine tested the safety, tolerability and efficacy in a paediatric ASD patient group (NCT01592747). In the memantine study, similarly to clinical trials with mGlu5 antagonists, results may be different if patients were stratified on the basis of E-I imbalance. This may be done using gamma oscillations or SEPs as biomarkers. Biomarkers can identify patients with greater E-I imbalance and select a subgroup likely to have a favourable treatment response. Gamma oscillations and SEPs can also be used as **outcome measures** assessing the effects of therapy. Lack of patient stratification may explain why single-site memantine studies in which the patient population is homogenous have shown positive results, not confirmed in ASD multi-site studies.

(b) **GABA agonists**. The drug AZD7325 (AstraZeneca) is a selective modulator of the GABAA2/3 receptor, is well-tolerated and without tendency to induce cognitive deficits. AZD7325 is tested in a Fast-Fail Trial (FAST) as ASD treatment by restoring the E-I imbalance. An EEG biomarker is used as patient selection tool and means to assess the drug action, and side effects, attention and learning are measured. **Arbaclofen**, a GABA(B) agonist, has shown promise in treatment of FXS and ASD (Berry-Kravis et al. 2012; Henderson et al. 2012). A clinical trial with STX209 (Arbaclofen) on the neurobehavioral functions of children and adults with FXS has not shown difference from
placebo on the primary endpoint measure – ABC-I (Aberrant Behaviour Checklist-Irritability) (Berry-Kravis et al. 2012). However, analysis using ABC – Social Avoidance Scale showed significant beneficial treatment effect. Subsequent study of STX209 in ASD subjects ages 5–21 (ClinicalTrials.gov Identifier: NCT01706523) was terminated due to absence of effects on the primary outcome measure – ABC. Future studies will benefit from patient stratification.

(c) **mTOR inhibitors.** Rapamycin and its analogue everolimus used for management of the tumours and epilepsy in TSC (Franz 2011, Franz et al. 2013) may be beneficial for ameliorating neurocognitive manifestations in TSC and ASD (de Vries 2010), based on the hypothesis that mTOR overactivation is responsible for neurocognitive symptoms. Rapamycin has improved measures of learning and memory (immediate and delayed recall) by 20% from baseline in 5 of 8 TSC subjects (de Vries 2010).

(d) **Oxytocin (OT)** has improved ASD symptoms in clinical trials (Soorya et al. 2008; Green and Hollander 2010; Anagnostou et al. 2012). OT has advantages over mTOR inhibitors such as lower toxicity, lack of immunosuppression, effects on the phosphatidylinositol 3-kinase (PI3K) pathway and enhancement of GABAergic neurotransmission. In humans OT modulates a variety of behaviours relevant to ASD including anxiety, stress, social memory, recognition, bonding (affiliation), sexual and aggressive behaviours. In CA1 neurons OT stimulates protein synthesis and enhances LTP (Lin et al. 2012). There is relationship between OT and GABAergic neurotransmission in brain. GABAergic abnormalities are established in ASD (Yip et al. 2007; Fu et al. 2012; Mori et al. 2013). OT activates hippocampal interneurons (Ogier et al. 2008) and increases inhibitory GABAergic synapses in brain (Theodosis et al. 2006). OT receptor knockout mice exhibit reduced hippocampal GABAergic synapses, increased ratio glutamatergic/GABAergic synapses, increased seizure susceptibility, impaired social behaviour, increased aggression, reduced cognitive flexibility, and are a neurobehavioral model of ASD (Sala et al. 2011). Intracerebral administration of OT and vasopressin rescues these deficits.

(e) **Implication of E-I imbalance for development of pharmacologic drugs for treatment of ASD.** It is difficult to predict before treatment which patients will respond favourably. To predict the treatment response it is possible to administer a test drug dose and monitor for early efficacy biomarker effect to demonstrate target engagement of the drug. If there is positive effect, the drug can be administered for a longer period of time. If there is no effect on early efficacy biomarker or if there are side effects, the drug will not be administered.

2. **Clinical somatic treatments**

TMS can be applied as a therapeutic modality in ASD to restore the imbalance of excitation and inhibition, modulate synaptic plasticity and gamma oscillations. In future it will be possible to target additional brain regions affected by E-I imbalance such as cerebellum (Demirtas-Tatlidede et al. 2013). Pharmacological, behavioural or neuromodulation treatments that reverse the altered E-I balance may be developed. Various TMS protocols in terms of location of stimulation (i.e. dorsolateral prefrontal cortex, DLPFC, right operculum, supplementary motor area, SMA), intensity of stimulation (high frequency to activate – 20 Hz, and low frequency to inhibit – 1 Hz) have been tested in ASD clinical trials. With respect to the optimal brain area to target, no consensus is reached. In summary, the results of TMS in ASD are promising but inconclusive. Knowledge of the underlying mechanisms will allow clinicians to design TMS protocols so that the pathological changes in excitation, inhibition and neural connectivity are reversed. TMS may be preferred in patients who cannot take medications, are not responsive to therapies, and may be combined with medications, neurofeedback or behavioral challenges.

Another NIBS technique that has potential for inducing changes in neuronal activity and management of ASD is transcranial direct current stimulation (tDCS; D’Urso et al. 2015). tDCS is still under development and not FDA-approved method.

Evidence supports the gut–brain connection and roles of inflammation and immunity ASD (Hsiao et al. 2013). This is relevant to E-I imbalance because proinflammatory cytokines released during gut–brain pathological processes may affect trafficking of AMPA, GABA receptors and synaptic plasticity. Based on this knowledge novel treatments for ASD such as Trichuris suis ova are currently tested by Dr. Eric Hollander’s team at Montefiore Medical Centre in a clinical trial with promising effects on repetitive and disruptive behaviours in ASD (Hollander et al. 2013).

3. **Early detection using event-related potentials (ERPs)**

Clinical studies in the USA (Luyster et al. 2011) and UK (Elsabbagh et al. 2012) evaluate early detection of ASD by measurement of neural correlates of E-I imbalance in infant brain as at 6–12 months of age using sensor nets.
to detect ERPs. ERPs are changes in brain electrical activity resulting from simultaneous activation of a group of neurons that can be time-locked to a stimulus. They are often recorded in response to a face-processing stimulus and are useful to test in infants because they do not require verbal abilities. The ERP components N290 and P400 (occipito-temporal region components) are precursors of the adult N170 component, a marker of detection of faces. The Nc component (negative central, observed over frontal regions) is a marker of attention. The group of Luyster examined the N290, P400 and Nc components in response to familiar and unfamiliar faces in 12-month-old infants at high vs. low ASD risk. They found that the N290 and P400 did not differ among the high- and low-risk infants. However, there were differences in the Nc responses among groups. The group of Elsabbagh determined in infants 6–10 months of age the ERP components P1, N290 and P400 as measures of the neural responses to dynamic eye gaze. The infant's sensitivity to eye gaze is a precursor to social and communicative skills. The researchers hypothesised that infants at risk for ASD have abnormal neural responses to eye gaze correlating with the behavioural impairments observed later and can be used for early diagnosis. These studies found that face-related ERP distinguish infants at risk for ASD from the controls and that atypical brain function precedes the onset of behavioural signs and overt ASD symptoms. Other studies show that toddlers at risk for ASD, infants whose siblings have ASD and whose siblings do not have ASD exhibit variability in the early trajectories of development measured using the ADOS and the Autism Diagnostic Interview – Revised (ADI-R), suggesting that there is need for early identification, regular monitoring and standardised assessments of young children suspected of having ASD (Lord et al. 2012). Studies on development of toddlers with early and later diagnosis of ASD show that ASD may involve developmental arrest, slowing or even regression (Landa et al. 2007). By early detection (3–6 months) it is possible to achieve better therapeutic results. For early ASD management it is feasible to apply a neurofeedback approach to retrain the E-I imbalance (Pineda et al. 2012). The various mechanism and clinical diagnostic and treatment aspects of E-I imbalance in ASD discussed are schematically presented in Figure 2.

Figure 2. Mechanisms and clinical aspects of E-I imbalance in ASD. The figure summarises the various aspects of E-I imbalance as discussed in the review. Central to ASD is E-I imbalance in key neural circuits in brain. In the lower panel are shown in the left box the major mechanisms currently thought to lead to E-I imbalance (Causative Mechanisms). In turn, they may give rise to various functional changes shown in the lower right box such as altered synaptic plasticity, learning and memory, seizures, etc. (Functional Consequences). These functional changes can measured for research and diagnostic purposes with different techniques shown in the top left box such as optogenetics and clinical methods such as EEG, MEG and NIBS (Methods to Interrogate). These latter methods can also be suitable ASD biomarkers and outcome measures. Based on the knowledge from the three components (causative mechanisms, functional consequences and methods to interrogate E-I imbalance) new therapeutics may be developed that are summarised in the top right box (Interventions for Reversal).
V. Summary, conclusions and future directions
Can a single model of E-I imbalance consistent with the various studies be proposed in ASD? ASD are very heterogeneous (Hollander et al. 2011) and there is a need to stratify patients based on clinical features and biomarkers. Measurement of E-I imbalance is influenced by the experimental method, subtype of ASD (e.g. FXS, TSC, high-functioning vs. low-functioning) and developmental period. We propose that E-I imbalance is a “final common pathway” in ASD but the different patient subgroups may have different mechanisms of E-I imbalance and respond to different treatments.

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References


