Searching for Cross-diagnostic Convergence: Neural Mechanisms Governing Excitation and Inhibition Balance in Schizophrenia and Autism Spectrum Disorders

Supplemental Information

Operationalizing E/I balance, ways it might become disrupted, and hypothetical consequences of its disruption in relation to autism spectrum disorder (ASD) and schizophrenia (SCZ)

Precise E/I balance is critical for microcircuit function and long-range tuning of neural networks. However, achieving E/I balance is a complex computational challenge. This review raises critical questions about how the term "E/I balance" is operationalized, and what the mechanisms and consequences of its disruption may be. Here we define E/I balance as the ratio of excitatory and inhibitory neural activity within cortical microcircuits (i.e., spatially proximal, organized groups of neurons as in cortical columns). E/I ratio is "balanced" when the relative strength of co-occurring excitatory and inhibitory input enables adaptive neuronal responses under a range of circumstances that is optimal for information processing. However, achieving E/I balance is inherently complex. Feed-forward and feed-back connections, excitatory synapses on inhibitory neurons, inhibitory synapses on excitatory neurons, and recursive excitation (and inhibition) within individual neurons and local microcircuits(1) all contribute to balanced computation. Post-synaptic glutamatergic *N*-methyl-D-aspartate (NMDA) receptors exist on both excitatory pyramidal cells and inhibitory, GABAergic parvalbumin-positive interneurons(2,3). The latter exhibit tight control on local circuit excitation, highlighting the complexity of E/I interactions. As a result, we are faced with a one-to-many - or even many-to-many - mapping problem when attempting to link mechanisms to observed neuroimaging results. E/I imbalance can take several forms, varying in impact on brain functioning across resting and task states(4) (**Fig.1C,D**). **Table 1** depicts the predicted consequences when E/I ratio is either elevated or reduced. These scenarios represent those typically predicted for SCZ

(elevated) and ASD (both elevated and reduced). States where both excitation and inhibition are either increased or decreased but E/I ratio is maintained are also possible, but are less relevant here. However, the *degree* of E/I imbalance is likely significant. For instance, seizures occur past a critical threshold of hyper-excitability, but neural abnormalities may exist at lower levels of hyper-excitability as well.

Timing of E/I ratio disruption is a critical issue in considering its putative role in ASD and SCZ. Patterns of E/I imbalance can vary across development, based on timing of gene expession (**Fig.1A,B**), excitatory versus inhibitory GABA function(5), and neural circuit formation during critical periods(6). Not surprisingly, ASD and SCZ both emerge during periods of high brain plasticity and change (i.e., infancy for ASD, adolescence for SCZ). The nature of E/I imbalance during critical periods, and the extent to which it interacts with existing microcircuitry, may contribute to variability in timing of onset and differential symptom patterns across ASD and SCZ(7) (**Fig.1B,D,E**). For example, a brain with elevated E/I ratio since birth (or prenatally) would establish poorly differentiated circuits with more random formation of connections. Circuitry in such a regime would be, at its structural core, imprecisely tuned for functional response to input. An alternate scenario exists where E/I ratio is stable during early development, then tipped toward cortical disinhibition at a critical later moment. In this regime, core circuitry would be intact, but excited too often at rest and too infrequently and imprecisely during functional tasks. It may be the case that, by adulthood, E/I ratio is similarly tipped in both SCZ and ASD. In adulthood, then, both disorders would reflect symptoms associated with high baseline activity and circuitry difficult to excite in a coherent, efficient, and effective fashion. In ASD, however, symptom onset occurs much earlier in development. This suggests that more overt versus insidious brain pathology arises earlier as well. As a result, the initial formation and tuning of neural circuitry is likely more disrupted in ASD. The functional consequence of this scenario would be more diffuse symptoms and a clinical profile that is not episodic and does not

remit. Such a neural regime would also be less clearly responsive to pharmacological agents that can bring neurotransmitter levels at a given snapshot in time back into balance.

With this backdrop in mind, *how could E/I balance break?* Among many other possibilities, there could be a deficit in pyramidal cell structure and dendrite formation and density, a deficit in GABA synapses onto pyramidal cells, a deficit in feed-back and feed-forward thalamic gain, a deficit in the architectural features of distinct classes of interneurons, a problem in protein expression leading to a reduction of NMDA subunit integration into specific cell subtypes, a regionally specific deficit in the reticular thalamic nucleus, or a disruption in proteins involved in glutamate synthesis, to name a few putative upstream causal factors. Put simply, the ways in which microcircuit E/I balance could become disrupted are remarkably complex. Moreover, the *reason* for these alterations could also be many, as many genes involved in coding for neuron formation, proliferation, migration, organization, and function may be at play here. In other words, disrupting E/I balance could occur in N ways due to P genetic mechanisms, which could in turn lead to R distinct neural features (where R could be as many as N*P, or lower if multiple disruptions lead to convergent neural features). Furthermore, the N*P mechanisms may not be temporally constant. Rather, they may shift over the lifespan due to the time-dependent nature of cortical development and gene expression. With regard to the central question of the current review, this last feature could introduce delays between which mechanism is key - or even disrupted - in which disorder, and at which time point. Thus, while E/I imbalance is an intuitive hypothesis - and even likely - in both ASD and SCZ, its complexity presents a massive search space that moves over time. As a result, the nature, direction, cause, and timing of E/I disruption within and across disorders will require a major effort to pin down and in turn translate to neuroimaging.

Understanding the specific nature and direction of E/I imbalance is nonetheless critical to understanding underlying disturbances and making predictions in clinical neuroscience (**Fig.1D**). For example, within visual circuits, increased E/I ratio may be associated with reduced

lateral inhibition and decreased filtering of non-relevant information (e.g., as with visual hallucinations in SCZ, sensory overload in ASD). Decreased E/I ratio may result in narrow microcircuit tuning and thereby inefficient integration of contextual details during visual perception (e.g., as in enhanced local perception in ASD). Consequently, distinct regimes of E/I imbalance will yield different outcomes when examined using non-invasive neuroimaging. Neuroimaging also offers important tools for understanding whether E/I imbalance is temporally (**Fig.1B**) or regionally (**Fig.1E**) specific, which likely contributes to divergence in ASD and SCZ neurobiological and clinical profiles.

In SCZ, the prevailing hypothesis implicates baseline cortical disinhibition(8,9). This view aligns with the glutamate hypothesis, wherein NMDA receptor hypo-function on interneurons may contribute to disease symptoms via hyper-excitable brain states(10-13). In ASD, evidence points less clearly toward a specific direction of E/I imbalance, perhaps due to significant clinical heterogeneity(14). Initial conceptualizations pointed toward cortical disinhibition, in part due to high seizures rates. However, mixed evidence for increased versus decreased E/I ratio has emerged(15). Whereas in SCZ studies implicate specific neurotransmitters, receptor types, and circuit dysfunction, no similarly clear and testable microcircuit model of E/I imbalance has emerged yet for ASD.

This review attempts to articulate findings from parallel SCZ and ASD literatures that help the field proactively address the challenge of defining specific profiles of E/I imbalance across networks and disease states. As described here and suggested by **Table 1** and **Figure 1**, different profiles of E/I imbalance ought to lead to predictable symptoms associated with distinct neural circuit disruptions. For example, seizures (as in ASD), hyper-excitability to sensory stimuli (as in some monogenic forms of ASD, such as Fragile X), and reduced sensory gating (as in SCZ) all may be clinical correlates of elevated E/I ratio. This relationship is directly testable by combining neuroimaging approaches with observable behavior. On the other hand, poor generalization of responses to similar stimuli (as in ASD) and hypo-responsiveness to

sensory input (as in other monogenic forms of ASD, such as Phelan-McDermid syndrome) may reflect clinical correlates of reduced E/I ratio. Thus, it is clear that district clinical features may arise as a function of the direction of E/I imbalance.

We posit that, though this issue is enormously complex, it is too soon to "throw the baby out with the bathwater." As highlighted in this review, there is emerging evidence for E/I imbalance across both ASD and SCZ. The field is positioned to more precisely test hypotheses related to E/I imbalance by combining multimodal neuroimaging, computational modeling, pharmacological challenges, studies in rare genetic disorders with known impacts on E or I function, cross-diagnostic explorations, and preclinical animal models that can reverse-engineer some of the imaging observations in humans.

Summary of recent MRS, EEG/MEG, and fMRI findings regarding E/I imbalance in ASD and SCZ

MRS. In SCZ, increased glutamine, glutamate, and Glx are seen in ACC and PFC in early-course and unmedicated patients, whereas glutamate and Glx either don't differ or are reduced in chronic and/or medicated patients. Metabolite levels map onto positive, negative, and cognitive symptoms, as well as to treatment responsiveness. In ASD, there is substantial regional variability in MRS findings, with glutamine, glutamate, Glx, and GABA being at times reduced, enhanced, or unchanged relative to controls dependent on brain region. More so than adults with ASD, children with ASD tend to exhibit increased glutamine, glutamate, and Glx, alongside decreased GABA.

EEG/MEG. ***Rest.*** SCZ is characterized by an increase in baseline (resting) EEG signals across most frequency bands, but particularly in the gamma band. In ASD, resting state activity is also elevated, with increases in alpha band power that may be disorder-specific. ***Task.***

Effects possibly consistent with cortical disinhibition are observed in the context of visual and auditory processing across the illness spectrum in SCZ. However, both event-related potentials (ERPs) and altered power, coupling, and phase-locking of neural activity are particularly altered in the gamma band. In ASD, there is mixed evidence for decreased (visual domain) and increased (tactile domain) inhibitory functioning, though task-based studies are limited. In rare genetic disorders associated with ASD, evidence is in line with possible cortical disinhibition, assessed during auditory and visual tasks.

fMRI. *Rest.* Resting state MRI has shown increased thalamic-sensory-motor and frontal-parietal connectivity, but reduced thalamic-prefrontal-cerebellar connectivity in SCZ, with concomitant elevations in BOLD signal variance. Whereas focused reductions in connectivity are more typically observed in chronic patients, hyper-connectivity is particularly salient in early-course SCZ patients and its attenuation corresponds to improvement with treatment (although choice of connectivity measures seems to matter; see(16,17)). In ASD, as is seen in MRS studies, there is a mixed pattern of hypo- and hyper-connectivity and null results at rest; when present, hypo-connectivity tends to characterize longer-range connections and hyper-connectivity more often is present in local circuits. In children with ASD, altered connectivity is associated with social symptom severity. ***Task.*** Few studies have used task-based MRI to examine E/I imbalance in either ASD or SCZ, but existing studies present a similar picture across the two disorders. In SCZ, evidence supports cortical disinhibition in primary auditory and visual cortices, while in ASD reduced inhibitory modulation was seen in higher visual regions and auditory cortex.

Supplemental References

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