Title
Autism in the Balance: Elevated E-I Ratio as a Homeostatic Stabilization of Synaptic Drive

Journal
Neuron, 101(4)

ISSN
0896-6273

Authors
Goel, Anubhuti
Portera-Cailliau, Carlos

Publication Date
2019-02-01

DOI
10.1016/j.neuron.2019.01.033

Peer reviewed

CellPress

Autism in the Balance: Elevated E-I Ratio as a Homeostatic Stabilization of Synaptic Drive

Anubhuti Goel[1,*] and Carlos Portera-Cailliau[1,2,*]
[1]Department of Neurology, David Geffen School of Medicine at UCLA, 710 Westwood Plaza, Los Angeles, CA 90095, USA
[2]Department of Neurobiology, David Geffen School of Medicine at UCLA, 710 Westwood Plaza, Los Angeles, CA 90095, USA
*Correspondence: anubhuthi@ucla.edu (A.G.), cpcailliau@mednet.ucla.edu (C.P.-C.)
https://doi.org/10.1016/j.neuron.2019.01.033

In this issue of *Neuron*, Antoine et al. (2019) find reduced feedforward inhibition in cortical neurons in four genetic mouse models of autism but without evidence of increased spontaneous or sensory-evoked activity.

Alterations in the balance of excitation and inhibition (E-I ratio) in the cerebral cortex have been proposed as an explanation for various neurological and psychiatric disorders, from schizophrenia to autism. In the case of autism, the argument for an elevated E-I ratio seems particularly convincing because it is easy to imagine how some common symptoms of the disorder, such as seizures, sensory hypersensitivity, or hyperarousal, could be a consequence of neuronal hyperexcitability leading to excessive neuronal activity. In many ways, the notion that E-I ratio is elevated in autism has influenced (if not prejudiced) much of the research in this field over the last two decades.

However, robust and incontrovertible evidence for increased firing of neurons in the cortex of animal models of autism (as predicted by the E-I imbalance) has been elusive. Even in the case of Fmr1 knockout mice, a model of fragile X syndrome, where there is broad consensus for hyperexcitability (reviewed in Contractor et al., 2015), the data on neuronal spiking are mixed with studies that recorded spontaneous or sensory-evoked neural activity showing either elevated, normal, or even slightly reduced firing. There are several reasons that could explain such variability, including the fact that different laboratories used different techniques (calcium imaging versus electrophysiology), different preparations (*in vivo* versus acute slices), or mice of different ages and of different background strains. It is also possible that different genetic mutations associated with autism might affect E-I balance in different ways. Unfortunately, major conceptual advances in the field of autism—not to mention the discovery of novel treatments—may be hampered by the fact that we do not yet know the answer to several important questions on this issue: is E-I ratio elevated across different types of autism (a common mechanism of sorts)? If E-I ratio is indeed higher in a given disorder, does it actually result in a hyperexcitable state in which cortical neurons fire excessively? Critically, what will be the implications of identifying the exact E-I ratio in autism vis-à-vis helping us understand how symptoms come about or discover new treatments to alleviate them?

In this issue of *Neuron*, Antoine et al. (2019) address the debate on E-I balance in autism by using a disciplined and systematic approach in four different genetic mouse models of autism: Tsc2, Fmr1$^{-/y}$, 16p11.2$^{del/+}$, and Cntnap2$^{-/-}$. These mutant lines are used by many investigators as models of the human disorders tuberous sclerosis, fragile X syndrome, autism associated with 16p11.2 deletion, and cortical dysplasia-focal epilepsy syndrome, respectively.

Antoine et al. (2019) begin by using *in vitro* patch-clamp recordings of layer 2/3 (L2/3) pyramidal neurons in acute slices from primary somatosensory cortex (S1). There are a couple of compelling reasons for why they choose to focus on S1. First, atypical sensory processing is common in autism disorders and may be associated with sensory over-reactivity and tactile defensiveness, which is the maladaptive behavioral response of avoiding a sensory stimulus that is perceived as aversive (but is innocuous to neurotypical controls). Second, Antoine et al. (2019) have been studying the circuits responsible for typical sensory processing in mice for many years and have significant expertise in how S1 processes tactile inputs from the whiskers on the snout. Remarkably, across the four genotypes, Antoine et al. (2019) found a robust decrease in inhibitory postsynaptic currents (IPSCs) evoked by stimulation of L4, which is the primary recipient of sensory inputs from the thalamus and then relays those inputs to L2/3. They also observed a more modest decrease in excitatory currents, but overall, it is an increase in E-I conductance ratio that emerges as a common synaptic deficit in all four mouse models of autism (Figure 1A, top).

One expected consequence of such an elevated E-I ratio would be stronger synaptic output, which Antoine et al. (2019) reasoned might account for the hyperexcitability others have observed in autism spectrum disorder (ASD) models. To test this hypothesis, they recorded spontaneous activity of L2/3 neurons but found no increase in firing rates compared to wild-type controls. They then recorded from L2/3 neurons in response to L4 stimulation, and strikingly, L4-evoked postsynaptic potentials (PSPs) and spiking in L2/3 neurons were also normal (Figure 1A, bottom). Thus, the increased E-I ratio did not lead to excessive spiking of cortical neurons. Antoine et al. (2019) also confirmed that the E-I imbalance was also not reflected in the passive neuronal properties at rest, which were largely normal across the mutant mice.

Next, to dig deeper into this puzzling finding, they used their quantitative experimental data to perform computer stimulations and examine the relationship between excitatory (G_{ex}) and inhibitory (G_{in}) conductances and net synaptic depolarization (Figure 1B). Just as with the

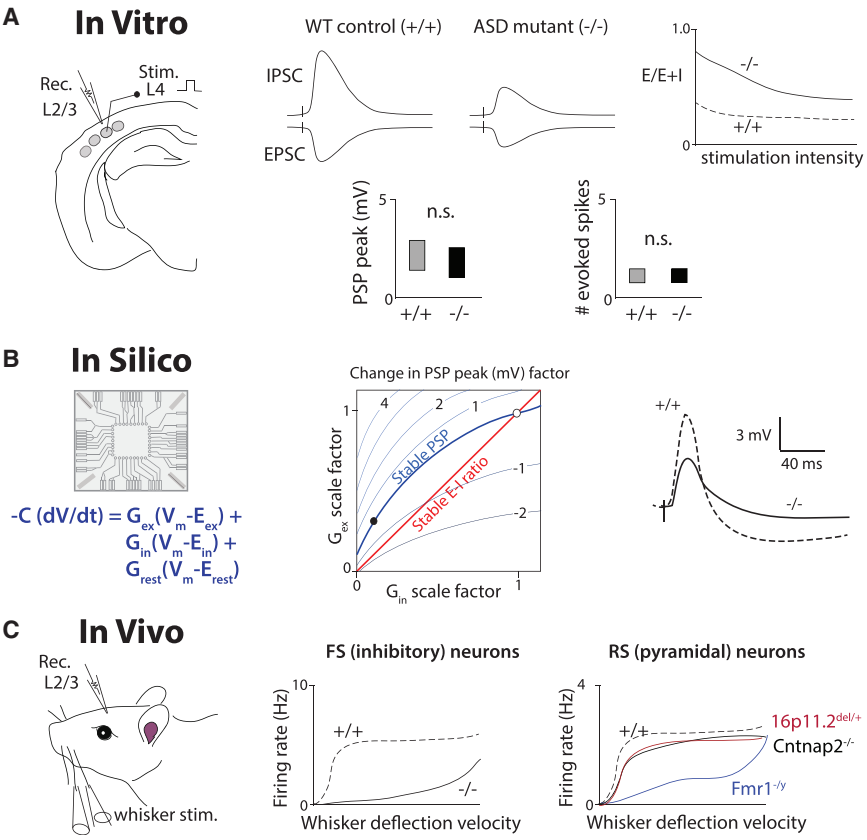

Figure 1. Main Findings from the Antoine et al. Study

(A) Different genetic mouse models of autism all show an elevated E-I ratio but normal L4-evoked postsynaptic potentials (PSPs) and spiking in L2/3 neurons in acute brain slices.

(B) Results from their computational model showing how PSP amplitude is affected by changes in excitatory (G_{ex}) and inhibitory (G_{in}) conductances.

(C) *In vivo* recordings in mutant ASD mice showed that, despite a reduction in whisker-evoked firing rates of fast-spiking (FS) units in L2/3, spontaneous and whisker-evoked firing of regular spiking (RS) units was not elevated.

experimental findings, the modeling data showed that an equal decrease in G_{ex} and G_{in} diminishes the PSP amplitude, while an additional decrease in G_{in} could rescue the decreased PSP. By plugging in the values for G_{ex} and G_{in} that Antoine et al. (2019) had measured *in vitro*, the model could make predictions about the overall PSP, and no change in PSP peak values between normal and mutant mice was found, indicating that the reductions in G_{ex} and G_{in} seen in autism mutants essentially counterbalance each other in order to maintain a stable synaptic depolarization and keep neuronal spiking constant.

Antoine et al. (2019) then turned to *in vivo* recordings to confirm their *in vitro* and *in silico* predictions. They used laminar polytrodes to record single units in L4 and L2/3 from urethane-anesthetized Fmr1$^{-/y}$, 16p11.2$^{del/+}$ and Cntnap2$^{-/-}$ mice (Figure 1C). Consistent with the lower inhibition they observed in slices from Fmr1$^{-/y}$ mice, Antoine et al. (2019) found a significant reduction in the firing rate of fast-spiking (FS; presumed inhibitory) neurons in Fmr1$^{-/y}$ mice *in vivo* but not in the other two mutant mouse models. However, all three autism mutants showed a robust and significant reduction in whisker-evoked firing rates of FS units in L2/3, which suggests that feedforward inhibition is reduced in these mutants. Surprisingly, however, both spontaneous and whisker-evoked firing of regular spiking (RS; presumed pyramidal, excitatory) units was normal (not elevated) in the three mutants, which is also in agreement with their acute slice results. In the case of Fmr1$^{-/y}$ mice, whisker-evoked spiking of L2/3 RS units was actually significantly reduced, both in juvenile animals (postnatal day 20, roughly the age of the slice experiments) and in 2-month-old adults. Even in L4 neurons, both spontaneous and whisker-evoked spiking were normal in the mouse mutants despite a clear increase in E-I ratio. This is presumably due to the nonlinear effects of the relationship between G_{ex} and G_{in} on overall PSP amplitude.

Because inhibition is critical for efficient sensory coding, by reducing noise and sharply tuning pyramidal cell responses, reduced firing of FS interneurons in the mouse models of autism would be expected to bring about a broadening of receptive fields and whisker maps in S1. Indeed, other studies have demonstrated abnormally large whisker maps in the somatosensory cortex (Arnett et al., 2014; He et al., 2018; Juczewski et al., 2016). Consistent with those studies and with the notion of aberrant sensory coding in ASDs, Antoine et al. (2019) found fewer columnar whisker-tuned L2/3 RS units in Fmr1$^{-/y}$ mice compared to controls, as well as a reduction in signal and noise correlations between pairs of simultaneously recorded RS units. However, these deficits were only observed in Fmr1$^{-/y}$ mice and sensory tuning in the other two mutants was normal. Finally, in order to rule out the possibility that anesthesia might influence their results, Antoine et al. (2019) also recorded RS unit responses to passive whisker stimulation in awake Fmr1$^{-/y}$ mice. Again, they found no change in spontaneous spiking of RS units of Fmr1$^{-/y}$ mice, and whisker-evoked firing was reduced (rather than enhanced).

As with any ambitious study of this scope, not all the results in Antoine et al. (2019) fit perfectly well together across experiments; such is science. For example, they find that the fraction of whisker-responsive units and sensory tuning were normal when they recorded in awake fragile X mice, whereas under anesthesia the fraction of responsive units was reduced, and tuning was blurred. But all caveats aside, the Antoine et al. (2019) study is impressive in several ways. For starters, it is the first to systematically survey E-I balance in four distinct models of autism and then record spiking *in vivo* from three of the models. The

experiments that Antoine et al. (2019) performed are elegant, and the presentation of their results is crisp. The data they provide will be a treasure trove for computational neuroscientists, who have been long awaiting these data to plug into their models. In fact, one can only hope that more theorists will be attracted to the field of autism thanks to this paper. There is a dire need for additional modeling studies to make predictions about the usefulness of thinking about autism in terms of E-I balance (O'Donnell et al., 2017). Additionally, the observation that an increase in E-I ratio does not simply mean that neurons will fire more spikes (at least in these four genetic mouse models of autism) is provocative. Those who used to think one could easily explain seizures in individuals with autism simply on the basis of hyperexcitability resulting from elevated E-I ratio will have to reconsider their rationale.

In the discussion, Antoine et al. (2019) provide a bold interpretation of their puzzling results and some thought-provoking ideas for the field to consider. They argue that, because the total number of spikes is ultimately unchanged in the autism models and because the changes in excitatory and inhibitory drive appear to be offsetting each other in very specific ways (e.g., both excitation and inhibition are diminished), it follows that some of the change in E-I balance is likely compensatory in order to homeostatically restore normal synaptic drive in autism. For instance, if a specific mutation results in diminished inhibition, the circuit might maintain cortical firing by lowering the level of excitation accordingly. Why the E-I balance is not fully restored is not clear; perhaps there is a limit to what the network can do or perhaps it finds an optimal compromise that limits potential side effects of full compensation of E-I ratio. A related and perhaps troubling issue is whether it might be futile to correct E-I imbalance in autism (think chemogenetics, pharmacology, brain stimulation, etc.) because it would either lead to another compensation or perhaps might worsen some neuropsychiatric symptoms or trigger new ones.

The E-I ratio often refers to the relative contributions of excitatory and inhibitory synaptic inputs corresponding to resting-state spontaneous activity (as might be seen during quiet wakefulness) or to a response evoked by sensory stimulation. It is important to consider, however, that significant plasticity of cortical E-I balance allows for the dynamic online equilibrium between excitatory and inhibitory inputs within different temporal windows (Froemke, 2015). Thus, pyramidal neuron firing rates *in vivo* may be quite different when the animal is attending to a particular behavioral task compared to when the animal is in quiet wakefulness. In the case of the task-engaged animal, one has to take into account not just instantaneous depolarizations triggered by a single stimulus (i.e., stimulation of L4 in slices or single deflections of whiskers *in vivo*), but also changes over much longer timescales. In other words, it is possible that pyramidal spiking might be different in ASD mice when they are performing a behavioral task or simply exploring their environment. In those settings, the effects of brainstem neuromodulators on neuronal spiking can be considerable. For example, firing rates of pyramidal neurons in visual cortex of normal wild-type mice are significantly higher when mice are attending to visual stimuli. This depolarization of pyramidal neurons is driven by cholinergic inputs through a disinhibitory circuit in visual cortex (Fu et al., 2014). Thus, it is conceivable that E-I imbalances might only affect pyramidal cell output in special circumstances, leading to loss of adaptation in response to repetitive tactile stimuli (He et al., 2017) or to broader orientation tuning (Goel et al., 2018), even if at baseline spiking is normal. It would also be interesting to examine whether, developmentally, there is a change in the magnitude of the E-I imbalance, such that at the earliest postnatal ages, one might see a very large imbalance in favor of excitation (due to loss of inhibition) that is perhaps then later followed by a homeostatic decrease in excitation, which achieves near normal firing.

In summary, Antoine et al. (2019) have generated an incredibly useful dataset and have established that changes in E-I ratio in neuropsychiatric disorders do not necessarily translate into changes in neuronal spiking and may instead reflect homeostatic changes to stabilize excitability. While it is challenging for anyone to come up with a single overarching theory of autism based on E-I balance alone, Antoine et al. (2019) have given us a lot to talk about at meetings for years to come.

REFERENCES

Antoine, M.W., Langberg, T., Schnelpel, P., and Feldman, D.E. (2019). Increased excitation-inhibition ratio stabilizes synapse and circuit excitability in four autism mouse models. Neuron *101*, this issue, 648–661.

Arnett, M.T., Herman, D.H., and McGee, A.W. (2014). Deficits in tactile learning in a mouse model of fragile X syndrome. PLoS ONE *9*, e109116.

Contractor, A., Klyachko, V.A., and Portera-Cailliau, C. (2015). Altered neuronal and circuit excitability in fragile X syndrome. Neuron *87*, 699–715.

Froemke, R.C. (2015). Plasticity of cortical excitatory-inhibitory balance. Annu. Rev. Neurosci. *38*, 195–219.

Fu, Y., Tucciarone, J.M., Espinosa, J.S., Sheng, N., Darcy, D.P., Nicoll, R.A., Huang, Z.J., and Stryker, M.P. (2014). A cortical circuit for gain control by behavioral state. Cell *156*, 1139–1152.

Goel, A., Cantu, D.A., Guilfoyle, J., Chaudhari, G.R., Newadkar, A., Todisco, B., de Alba, D., Kourdougli, N., Schmitt, L.M., Pedapati, E., et al. (2018). Impaired perceptual learning in a mouse model of Fragile X syndrome is mediated by parvalbumin neuron dysfunction and is reversible. Nat. Neurosci. *21*, 1404–1411.

He, C.X., Cantu, D.A., Mantri, S.S., Zeiger, W.A., Goel, A., and Portera-Cailliau, C. (2017). Tactile defensiveness and impaired adaptation of neuronal activity in the *Fmr1* knock-out mouse model of autism. J. Neurosci. *37*, 6475–6487.

He, Q., Arroyo, E.D., Smukowski, S.N., Xu, J., Piochon, C., Savas, J.N., Portera-Cailliau, C., and Contractor, A. (2018). Critical period inhibition of NKCC1 rectifies synapse plasticity in the somatosensory cortex and restores adult tactile response maps in fragile X mice. Mol. Psychiatry. Published online April 27, 2018. https://doi.org/10.1038/s41380-018-0048-y.

Juczewski, K., von Richthofen, H., Bagni, C., Celikel, T., Fisone, G., and Krieger, P. (2016). Somatosensory map expansion and altered processing of tactile inputs in a mouse model of fragile X syndrome. Neurobiol. Dis. *96*, 201–215.

O'Donnell, C., Gonçalves, J.T., Portera-Cailliau, C., and Sejnowski, T.J. (2017). Beyond excitation/inhibition imbalance in multidimensional models of neural circuit changes in brain disorders. eLife *6*, 6.