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Currently Unstable: Daily Ups and Downs in E-I Balance

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Balance between excitation and inhibition (E-I balance) in neural circuits is believed to be tightly regulated. To the contrary, in this issue of *Neuron*, Bridi et al. (2020) reveal that inverse oscillations of synaptic inhibition and excitation lead to peaks and valleys in E-I balance across the 24 h day.

Stable function in the central nervous system is something of a miracle. Without careful coordination and compensation, Hebbian plasticity and recurrent connectivity can quickly drive circuits toward saturation or silence (Ma et al., 2019). While synaptic and cellular mechanisms constrain neuronal activity on the timescale of hours to days (Ibata et al., 2008; Hengen et al., 2016), it is generally recognized that inhibitory signaling rapidly stabilizes dynamics. In other words, careful calibration of inhibition prevents problems of runaway gain. The strength of synaptic excitation relative to synaptic inhibition, often termed E-I balance, is believed to be tightly regulated about a set point and necessary for normal response properties in individual cells. At the network level, appropriate E-I balance is a key ingredient of a "critical" state that maximizes information processing, dynamic range, and entropy in the neural population (Ma et al., 2019). With the computational consequence of proper E-I balance being clear, it comes as no surprise that alterations in E-I balance are suspect in neurological diseases and disorders, including autism, epilepsy, and Alzheimer disease.

Stable E-I balance implies co-regulation of excitation and inhibition. Alterations in excitatory synaptic strength (for example) might be expected to drive parallel changes in inhibitory synaptic strength. Modeling studies suggest that inhibitory plasticity maintains function following experience-dependent changes in excitatory synaptic strength. *In vivo* and *ex vivo* works demonstrate that inhibitory changes can gate excitatory plasticity (e.g., Kuhlman et al., 2013). Importantly, E-I balance itself is sensitive to experience and development in complex and circuit-specific patterns (Tatti et al., 2017).

An emerging body of work underscores systematic fluctuations in excitatory synaptic transmission across the 24 h day (e.g., Vyazovskiy et al., 2008). If E-I balance is indeed regulated around a set point, decreased excitatory tone across the light cycle predicts similarly decreased inhibitory tone (to maintain the ratio). In this issue of *Neuron*, Bridi et al. (2020) directly tested this hypothesis (Figure 1).

The authors first recorded excitatory and inhibitory minis (miniature post-synaptic currents, mEPSCs and mIPSCs) in pyramidal neurons in slices of mouse visual cortex. Slices were harvested at either zeitgeber time 0 (ZT0; lights on) or ZT12 (lights off). While mini amplitudes, a measure of synaptic strength, were equivalent at both time points, mEPSC frequency was higher at ZT0 than ZT12. Surprisingly, changes in mIPSC frequency were inverted, resulting in different E-I ratios at the light-to-dark and dark-to-light transitions. The authors noted that pyramidal cell excitability was stable, indicating that their findings were not accompanied by shifts in intrinsic excitability. To address regional specificity, Bridi et al. (2020) repeated their experiments in prefrontal cortex and hippocampus and noted similar effects.

Next, the authors recorded in the absence of synaptic blockers to facilitate circuit activity in their slices, quantifying inhibitory strength as the total charge in 1 s. In a dedicated series of recordings, the authors collected slices every 4 h across the day. This allowed them to dissect whether synaptic inhibition changed gradually across the circadian cycle or stepwise at light-dark transitions. While inhibitory charge nearly doubled across the light cycle, the data suggested a rapid increase over the first 4 h of light.

Bridi et al. (2020) then prevented animals from sleeping during the first 4 h of light and recorded significantly lower inhibitory charge in slices from sleep-deprived animals than controls (*ad libitum* sleep). However, the question of disentangling sleep-dependent effects from circadian effects in rodent models is extremely difficult, in part due to ethological constraints: rodent sleep is fragmented and generally observable in each hour of the day. Apropos this point, brief sleep deprivation produces equally robust increases in sleep drive across the light-dark cycle (Brüning et al., 2019). Unequivocally separating effects of sleep, time of day, and light and dark is a core challenge for the field interested in understanding how these variables influence neural plasticity. Bridi et al. (2020) provide rigorous evidence of powerful changes in inhibitory synaptic transmission across these cycles, and their data raise the possibility that sleep is a mediator of these changes.

To tease out mechanisms driving their observations, the authors turned to manipulation of endocannabinoids. Endocannabinoids can modulate inhibitory tone, and subsets of endocannabinoid receptors maintain circadian fluctuation. Pharmacological inhibition and stimulation of endocannabinoid receptors in slices at ZT0 and ZT12 revealed suppression of inhibitory tone at ZT0. Further, the authors measured an increase in

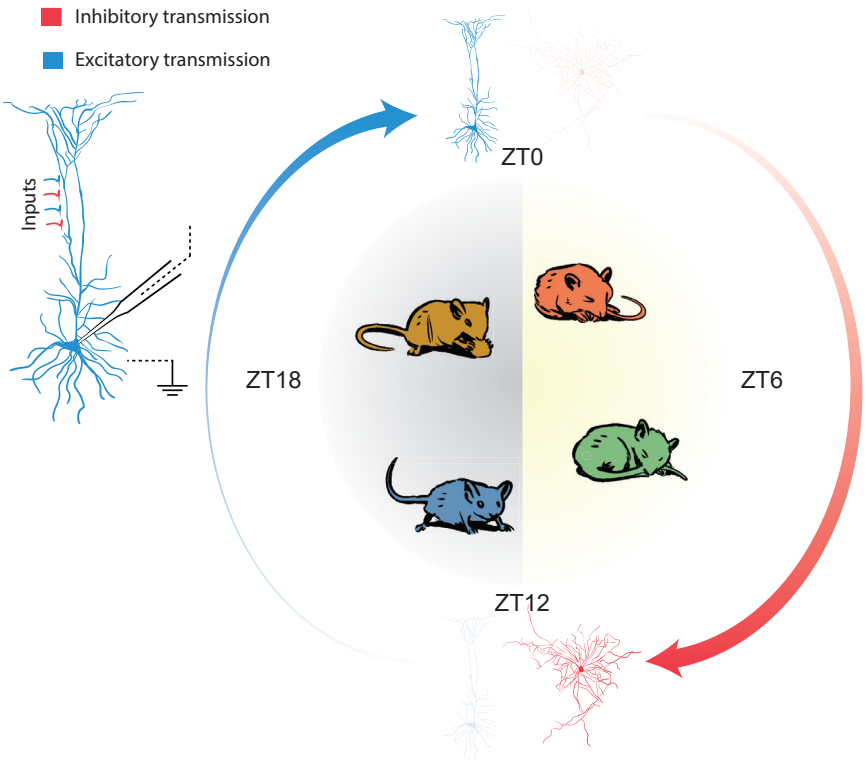

Figure 1. Anticorrelated Changes in Inhibitory and Excitatory Transmission across the 24 h Cycle Result in Systematic Variability in E-I Balance

Left: schematic of recording E-I balance in acute brain slices. The strength and frequency of inhibitory (red) and excitatory (blue) input currents are measured in a post-synaptic pyramidal neuron (also excitatory). The ratio of excitatory relative to inhibitory synaptic transmission is calculated. Right: the E-I balance at zeitgeber time 0 (ZT0, lights on) is typified by stronger excitatory tone. Across the 12 h light cycle, excitatory tone decreases and inhibitory tone increases, resulting in a significantly lower E-I balance at ZT12 (lights off). These effects are disrupted by sleep deprivation and manipulation of endocannabinoids.

ligand at the end of the dark phase. Together, these findings point to endocannabinoid signaling as key in suppressing inhibitory transmission in the dark. These experiments set the stage to dissect the role of endocannabinoids in sleep and circadian neurobiology.

Importantly, cortical inhibition arises from a variety of sources, most notably, feedforward inhibition from input layers (layer IV) and feedback inhibition from intermediate layers (layer 2/3). These motifs have important differences in inhibitory timing relative to excitation and thus have unique computational effects. To ask whether cyclic changes in E-I balance are global or circuit specific, Bridi et al. (2020) stimulated each pathway while recording from pyramidal neurons in layer 2/3. Alternately clamping neurons at the reversal potential for GABA and AMPA receptors, the authors measured evoked excitatory and inhibitory potentials, respectively, and calculated E-I ratio. There was significant cyclic variation in the E-I balance of synaptic potentials arising from the lateral (layer 2/3) pathway; E-I ratio was higher at ZT0 than ZT12. In contrast, the E-I ratio of potentials driven by the feedforward (layer 4) pathway was constant at lights on and lights off. The authors concluded that daily oscillations in E-I balance were largely the result of circuit-specific changes.

The circuit specificity described here is similar to observations that visual deprivation modulates lateral, but not vertical, inputs (Petrus et al., 2015). To investigate the role of visual experience, Bridi et al. (2020) repeated the feedforward/feedback circuit experiments following 24 h of darkness. Even under these conditions, E-I balance was higher at ZT0 than ZT12 but (as previously) only in the lateral pathway. These data suggest that plastic changes observed here are not the result of externally driven, experience-dependent mechanisms. To fully understand the role of visual experience, extended dark adaptation might be required since, once adapted to a light cycle, animals maintain their circadian cycle in constant darkness for days.

The core finding by Bridi et al. (2020)—that the E-I balance set point changes predictably across the day—has significant implications for our understanding of robust neural function. Ultimately, the authors revealed a novel plasticity in the interplay between excitatory and inhibitory neurotransmission, a finding with remarkable computational repercussions. It is increasingly clear that time of day, arousal state, and environmental variables collectively bear influence on the computational regime of cortical circuits. This work firmly topples any notion that E-I balance is as simple as homeostatic tethering to a singular set point. In combination with evidence that cortical computational regimes are stable across the 24 h day (Ma et al., 2019), the data presented by Bridi et al. (2020) are consistent with the idea that E-I balance is a powerful lever of negative feedback. Through this lens, plasticity of E-I balance may directly allow neurons to maintain activity across a range of inputs (e.g., Antoine et al., 2019). If true, *in vivo* manipulations of circuit firing rates would be expected to reveal bidirectional compensatory effects in E-I balance. Arguably, those circuits most prone to experience-dependent modification should exhibit the most labile E-I balances. Further dissection of why these changes are observed in some circuits and not others will provide meaningful insight into mechanisms underlying the variability of circuit modifiability and, potentially, selective vulnerability in pathology.

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Not Fade Away: Mechanisms of Neuronal ATP Homeostasis

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In this issue of *Neuron*, Ashrafi et al. (2020) identify a feedforward signaling mechanism that couples neuronal activity to the homeostatic maintenance of axonal and synaptic ATP production. This mechanism is achieved via changes in cytoplasmic calcium and activation of brain-specific, mitochondrial MICU3.

The generation of a biochemical map detailing the metabolic pathways responsible for the homeostatic maintenance of cellular ATP represents one of the great scientific achievements of the 20th century. The "metabolic map," which appears far more complex than a diagram of the New York City subway system, details the cellular uptake, conversion, storage, breakdown, and use of carbon sources that provide the raw material for ATP production, the evolutionarily conserved power source for all of cell biology. These discoveries touch upon nearly every aspect of modern medicine, including aging, cancer, cardiac disease, epilepsy, and the myriad effects of diet on human health and wellbeing.

The brain is a primary beneficiary of the complexities of metabolic homeostasis. It is well established that the brain consumes a disproportionate amount of ATP compared to other organs, estimated to be as much as 20% of total ATP consumption (Yellen, 2018). This makes sense. The electrochemical reactions that drive neuronal signaling are energetically expensive. Neurons maintain a hyperpolarized resting membrane potential, ship proteins throughout an expansive cellular architecture, maintain an enormous membrane surface area (plasma membrane and smooth endoplasmic reticulum [ER]), and continually release and recycle synaptic vesicles at each of the trillions of synapses throughout the brain. Recently, there has been an attempt to pinpoint the subcellular processes within a neuron that are the primary sink for ATP, with evidence focusing on synaptic vesicle recycling (Rangaraju et al., 2014; Pathak et al., 2015). Regardless, with more than 80 billion neurons and trillions of synapses, the human brain is an energy hog.

Despite the importance of metabolic signaling for the maintenance of normal brain function, our understanding of how the biochemical metabolic map is instantiated within individual neurons and coupled to their changing energetic demands remains poorly understood. For example, we appreciate that supplying the brain with fuel is a dilemma. It appears that the brain does not rely upon β-oxidation, eliminating the breakdown of fatty acids as a major fuel source (Schönfeld and Reiser, 2017). Furthermore, unlike muscle, the brain does not maintain appreciable stores of fuel in the form of glycogen. Although astrocytes maintain a glycogen reserve, neurons appear to rely primarily on fuel delivered in the form of blood glucose (Yellen, 2018). Indeed, this is the source of the signal used in functional magnetic resonance imaging (blood-oxygen-level-dependent [BOLD] signals) (Yellen, 2018). An increase in neuronal activity is associated with increased blood flow, providing both oxygen and glucose and eliminating CO_2, the byproduct of oxidative phosphorylation.