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The Downs and Ups of Sensory Deprivation: Evidence for Firing Rate Homeostasis In Vivo

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Homeostatic adjustment of neuronal firing rates is considered a vital mechanism to keep neurons operating in their optimal range despite dynamically changing input. Two studies in this issue of *Neuron*, Hengen et al. (2013) and Keck et al. (2013), provide evidence for firing rate homeostasis in the neocortex of freely behaving rodents.

The nervous system has the remarkable ability to undergo adaptive changes in response to sensory experience during development and learning. Experience-dependent circuit refinements have been studied extensively in cortex and are thought to rely heavily on synapse-specific, associative "Hebbian" plasticity mechanisms such as synaptic strengthening through long-term potentiation (LTP) and synaptic weakening through long-term depression (LTD). It has long been recognized that these Hebbian plasticity mechanisms, when left unchecked, could lead to saturation of synaptic strengths and thus threaten the stability of neural networks. To solve this problem, non-Hebbian, "homeostatic" forms of plasticity have been proposed to act in concert with Hebbian mechanisms, globally regulating neuronal activity levels toward an optimal set point and thus providing stability despite ongoing fluctuations in synaptic strength. In this issue of *Neuron*, Hengen et al. (2013) and Keck et al. (2013) provide the first glimpses that homeostatic mechanisms act to regulate firing rates within neocortical circuits in vivo.

Research over the past few decades has solidly established that cortical neurons possess mechanisms that maintain firing around a homeostatic stable point in vitro (Turrigiano, 2011). One classic example of homeostatic regulation demonstrated that cultured neocortical neurons exposed to pharmacological activity blockade for prolonged periods exhibit increased spontaneous firing rates when network activity is resumed. Reciprocally, neurons compensate after network activity is elevated for many hours, restoring firing rates to baseline. Notably, these activity manipulations induced bidirectional compensatory changes in the unit strength of synaptic inputs, globally increasing or decreasing the strength of all synapses in a multiplicative manner referred to as "synaptic scaling," thus allowing the preservation of information stored in the distribution of synaptic weights (Turrigiano et al., 1998).

More recently, focus has turned to whether and how homeostatic plasticity operates in intact neocortex in vivo. Experiments to address these questions have monitored activity changes in response to sensory manipulations, using ex vivo electrophysiological recordings in acute slices or in vivo calcium or intrinsic signal imaging in anesthetized animals. One classic model of experience-dependent cortical plasticity has been the postnatal development of visual cortex (Levelt and Hübener, 2012). Original studies, primarily in cats, showed that depriving one eye of visual input (monocular deprivation [MD]) during a critical period of development produces a loss in visual cortical responsiveness to inputs through the deprived eye, followed by a temporally delayed increase in responsiveness to inputs through the nondeprived eye. While the initial component of these shifts in ocular dominance have been shown to rely on LTD of excitatory synapses (Smith et al., 2009), several studies support that the second phase of the cortical response, namely the increase in responsiveness to the nondeprived eye, could be regulated by homeostatic forms of plasticity. Indeed, it has been shown that visual deprivation leads to global multiplicative scaling of miniature excitatory postsynaptic current (mEPSC) amplitudes in L2/3 and L4 in visual cortical slices ex vivo (Desai et al., 2002; Goel and Lee, 2007). In addition, two-photon calcium imaging of visually evoked responses in visual cortex of anesthetized animals showed a delayed, presumably homeostatic, response potentiation after MD (Mrsic-Flogel et al., 2007). Furthermore, the increase of responsiveness after MD is dependent on TNFα, a molecule shown to be necessary for synaptic scaling in vitro (Kaneko et al., 2008). Yet the central hypothesis that homeostatic mechanisms act in the neocortex in vivo to regulate firing rates around a critical set point had never been tested. In this issue of *Neuron*, Hengen et al. (2013) and Keck et al. (2013) describe these long-awaited experiments, and in doing so provide several new insights into how cortical activity levels are regulated in freely behaving mice in response to sensory deprivation.

Hengen et al. (2013) set out to probe firing rate homeostasis in the neocortex using chronic multielectrode recordings in monocular visual cortex (mV1) to record neural activity prior to and after MD induced by lid suture in juvenile rats. Multiunit recordings of cells across all cortical layers in freely behaving animals were separated into putative parvalbumin (PV)-positive, fast-spiking inhibitory neurons (pFS) and regular spiking units

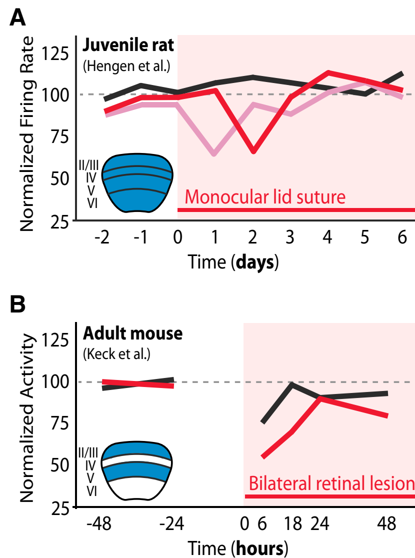

Figure 1. Schematic Representation of Evidence for Firing Rate Homeostasis In Vivo

(A) Hengen et al. (2013) used chronic multielectrode recordings from all layers of the visual cortex of freely behaving juvenile rats to show that firing rates of putative inhibitory neurons (pink line) and putative pyramidal neurons (red line), which decreased after monocular lid suture, returned to baseline 24 hr later despite continued visual deprivation. Controls (black line) were unchanged.

(B) Keck et al. (2013) used calcium imaging of GCaMP signals from neurons in L2/3 and L5 of the visual cortex of awake adult mice to show that overall activity levels, which decreased within 6 hr of bilateral retinal lesions (red line), returned to mock-lesioned control levels (black line) within 24 hr, despite the irreversible loss of visual input.

(RSUs), putative excitatory pyramidal neurons. Hengen et al. (2013) observed an initial decrease in average ensemble firing rate of RSUs after 2 days of MD. Despite ongoing deprivation, firing rates restored to baseline within 24 hr (Figure 1A), supporting homeostatic regulation. Remarkably, this homeostatic regulation of firing rates was observed across sleep and wake behavioral states. Interestingly, inhibitory pFS cells also underwent biphasic modulation after MD, although with a more rapid timescale. After 1 day of deprivation, pFS cells showed a significant drop in firing rate, followed by a rapid return to baseline by day 2 (Figure 1A). Thus, both excitatory and inhibitory neocortical neurons show homeostatic recovery of baseline firing rates after monocular deprivation.

It may seem surprising that Hengen et al. (2013) did not observe a drop in firing rate of putative excitatory neurons until the second day after monocular deprivation. Hengen et al. (2013) suggest that a drop in firing rates might be masked by a release from inhibition due to decreased firing rates of pFS cells 24 hr after MD. Consistent with this hypothesis, Hengen et al. (2013) observed a significant anticorrelation between firing rates of inhibitory and excitatory neurons from the same electrode, suggesting indeed that the inhibitory neurons were suppressing firing of the excitatory neurons. Notably, a recent study reported a drop in visually evoked firing rates of PV neurons in L2/3 in vivo after 1 day of MD, leading to a doubling of visually evoked monocular responses and an overall conservation of firing rate (Kuhlman et al., 2013).

Which cellular mechanisms support the homeostatic recovery of firing rates in these putative pyramidal neurons? Hengen et al. (2013) hypothesized that the recovery of firing rates could involve homeostatic scaling of mEPSC amplitudes. To test this possibility, Hengen et al. (2013) measured mEPSC amplitudes on layer 2/3 pyramidal neurons in acute slices of mV1 after 2, 4, or 6 days of MD. They found that mEPSC amplitudes were depressed after 2 days of MD, rebounded to baseline by day 4, and were elevated above baseline by day 6. These changes matched the time course of RSU response measured across all cortical layers and suggest that synaptic scaling could be one of the mechanisms at play to support firing rate homeostasis in the neocortex in vivo.

Keck et al. (2013) used the latest technological approaches to examine neocortical activity levels in awake, behaving animals in response to sensory deprivation. In these experiments, Keck et al. (2013) probed changes in the activity of neocortical neurons in adult mice after bilateral retinal lesion using two-photon calcium imaging of GCaMP3 or GCaMP5 in L2/3 and L5 cells of mV1. Notably, imaging data were obtained as the animals experienced virtual environments while moving on a spherical treadmill, as recent studies have shown that locomotion affects the gain of cortical responses in primary visual cortex (Niell and Stryker, 2010). Keck et al. (2013) observed that activity of excitatory neurons in mV1 was rapidly decreased by 50%–60% within 6 hr of lesioning. Remarkably, despite the irreversible retinal lesions, neuronal activity levels were restored to baseline within 24 hr postlesion (Figure 1B), supporting homeostatic adjustment of firing rates in the neocortex of adult mice in vivo.

Could synaptic scaling also support homeostatic regulation of activity levels in adult neocortex? Earlier studies using acute slices from dark-reared adult mice found that cells of layer 2/3 retain a form of synaptic scaling into adulthood (Goel and Lee, 2007). However, Ranson et al. (2012) showed that open eye response potentiation after MD persists in adult TNFα knockout animals, suggesting that TNFα-mediated synaptic scaling is not required. To examine a role for synaptic scaling, Keck et al. (2013) measured mEPSC amplitudes from L5 pyramidal neurons in acute slices of mV1 from animals with bilateral retinal lesions or mock-lesioned controls. They found that mEPSC amplitudes were unchanged at 6 and 18 hr postlesion but then increased at 24 and 48 hr, closely matching the time course of activity rate homeostasis. Because spine size is correlated with synaptic strength, and changes in a predictable manner when circuits are weakened or strengthened in response to MD in vivo (Hofer et al., 2009), Keck et al. (2013) hypothesized that in vivo scaling of synaptic strengths should have a structural correlate in altered dendritic spine size. Remarkably, they indeed found that spine size on L5 pyramidal neurons increased 24 hr after the retinal lesion and was maintained at 48 hr, thus following the same time course as the changes in mEPSC amplitude and cortical activity in vivo. Altogether, these data and those obtained by Hengen et al. (2013) are consistent with the hypothesis that synaptic scaling could underlie homeostatic adjustments in neocortical firing rates in vivo.

The studies by Hengen et al. (2013) and Keck et al. (2013) provide much anticipated evidence supporting that neuronal activity levels are homeostatically regulated in the neocortex in vivo. While both studies report an initial drop in activity levels in response to sensory deprivation, followed by a subsequent rebound, the time courses of the two observations are dramatically different. Interestingly, the

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rapid sensory deprivation induced drop in overall activity levels observed by Keck et al. (2013) recovered to control levels within 24 hr, which is when Hengen et al. (2013) obtained their first measurements also showing baseline firing rates in excitatory neurons. Discrepancies between the two studies are evident only at 48 hr, when Hengen et al. (2013) see significant depression of firing rates in excitatory neurons, whereas Keck et al. (2013) observe baseline activity levels. Most likely, differences are due to the widely diverse experimental conditions in the two studies—including deprivation protocols (monocular lid suture versus binocular retinal lesion), species (rat versus mouse), and ages (juvenile versus adult; Figure 1). Future experiments utilizing similar paradigms, while independently varying the individual parameters, will shed light on the mechanisms and origins of these differences.

Several testable predictions arise from these studies and lead to exciting new avenues of research. While these studies support that synaptic scaling could be responsible for homeostatic regulation of firing rates in the neocortex, they do not exclude that alternative mechanisms of synaptic plasticity, such as plasticity of intrinsic excitability, anti-Hebbian mechanisms, or Hebbian modifications of excitatory or inhibitory synapses, are also at play. One prediction is that a homeostatic set point should operate bidirectionally; and consequently, enhanced firing rates due to sensory overstimulation should be homeostatically downregulated. Clearly, bilateral retinal lesions cannot be bidirectional; however, lid suture can be reversed, and firing rates immediately after eye reopening are expected to be heightened above normal. Similar approaches utilizing other sensory modalities (auditory, somatosensory) that are potentially more amenable to bidirectional manipulations would provide further support and also establish how generalizable the findings are. The hypothesis that synaptic scaling is responsible for homeostatic regulation of firing rates in vivo leads to the prediction that knockouts that interrupt synaptic scaling in response to monocular deprivation (Kaneko et al., 2008) would also be expected to interrupt firing rate homeostasis in vivo. Ultimately, the utilization of patterned optogenetic stimulation (Wyatt et al., 2012) of identified cells in the LGN or V1 should provide a wealth of information that will help elucidate the activity patterns, combinations of inputs, and plasticity mechanisms leading to firing rate homeostasis in vivo.

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