Synapses Let Loose for a Change: Inhibitory Synapse Pruning throughout Experience-Dependent Cortical Plasticity

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In this issue of *Neuron*, Chen et al. (2012) and van Versendaal et al. (2012) used fluorescently tagged gephyrin to track inhibitory synapses in the mouse visual cortex in vivo. Their studies show that visual experience-dependent plasticity is associated with clustered and location-specific pruning of inhibitory synapses.

Studies of cortical plasticity have classically focused on glutamatergic, excitatory synaptic changes. A large fraction of the excitatory synapses in the neocortex are impinging on dendritic spines. This allows researchers to monitor the formation and elimination of excitatory synapses by watching the appearance and disappearance of fluorescently labeled dendritic spines in live neurons. Similarly, large glutamatergic axonal varicosities are often used as anatomical surrogates for vesicular presynaptic boutons. The turnover of these structures occurs throughout life even in virtually naive animals, and newly added synapses stably integrate into cortical circuits upon changes in experience or learning (Fu et al., 2012; Hofer et al., 2009; Holtmaat and Svoboda, 2009).

Similar to their excitatory counterparts, inhibitory synapses are thought to display continuous structural changes. Synaptic inhibition in the neocortex is governed by a diverse group of interneurons that transmit GABA or glycine in spatially and temporally discrete manners (Markram et al., 2004). Inhibitory inputs can modulate excitatory neuronal membrane potentials, enforce spike timing, and effectively restrain the summation of postsynaptic excitatory potentials (Isaacson and Scanziani, 2011). Therefore, regulated inhibition through the formation and elimination of synapses could efficiently leverage excitatory activity and hence cortical network processing or plasticity.

Studies of inhibitory synapse dynamics on excitatory cells have been complicated due to the lack of postsynaptic anatomical proxies that can be resolved by light microscopy. Recent time-lapse imaging studies in vivo have described experience-dependent and structural remodeling of GABAergic interneuron axonal boutons, suggesting that some excitatory cells are subject to changes in inhibitory synaptic input (Chen et al., 2011; Keck et al., 2011). However, from these studies it is difficult to deduce the identity let alone the dendritic compartments of the postsynaptic cells that may be affected. In this issue of *Neuron*, Chen et al. (2012) and van Versendaal et al. (2012) present an elegant method for studying inhibitory synapse dynamics in excitatory cells in vivo based on fluorescently tagged gephyrin. This synaptic scaffolding protein is highly enriched in GABAergic and glycinergic postsynaptic compartments, and when expressed in neurons, fluorescent puncta can be observed, which are likely to represent inhibitory synapses (Moss and Smart, 2001). Tagged gephyrin DNA constructs were electroporated into cortical layer (L) 2/3 pyramidal cell progenitors in mouse embryos in utero, and fluorescent puncta were imaged in adults using two-photon laser scanning microscopy. A cytosolic fluorescent protein of a different color was coexpressed to visualize dendritic morphology. The auxiliary expression of a synaptic protein implicates two potential risks. An excess of protein could disturb a neuron's physiology and integration in the network, or result in ectopic accumulations that are not associated with synapses. Both studies controlled for such artifacts. The density of puncta fell in the range of previously reported inhibitory synapse densities, and miniature inhibitory postsynaptic responses were unaffected. Both studies also verified the result by using immunoelectron microscopy (EM), and confirmed that fluorescently tagged gephyrin localizes at presumptive inhibitory synapses. Chen et al. (2012) even went to the extent of reconstructing an in vivo imaged dendrite in 3D using serial section EM. A perfect match was found between the location of the imaged puncta and the ultrastructural markers for inhibitory synapses. All in all, the studies found no obvious signs of disturbed neuronal function and provide a strong case for the use of fluorescently tagged gephyrin as a tracking reagent of inhibitory synapses in vivo.

Inhibitory Synapses Are Differentially Distributed along the Dendrite

Consistent with previous reports, both studies show that approximately 30%–40% of the gephyrin-associated synapses are localized on dendritic spines (Figure 1). Chen et al. (2012) found this density to be almost twice as high along distal apical dendrites as compared to proximal locations. This stands in contrast to the uniform distribution of dendritic spines and shaft inhibitory synapses. Since almost all spines receive excitatory inputs, this means that those bearing gephyrin puncta were almost certainly

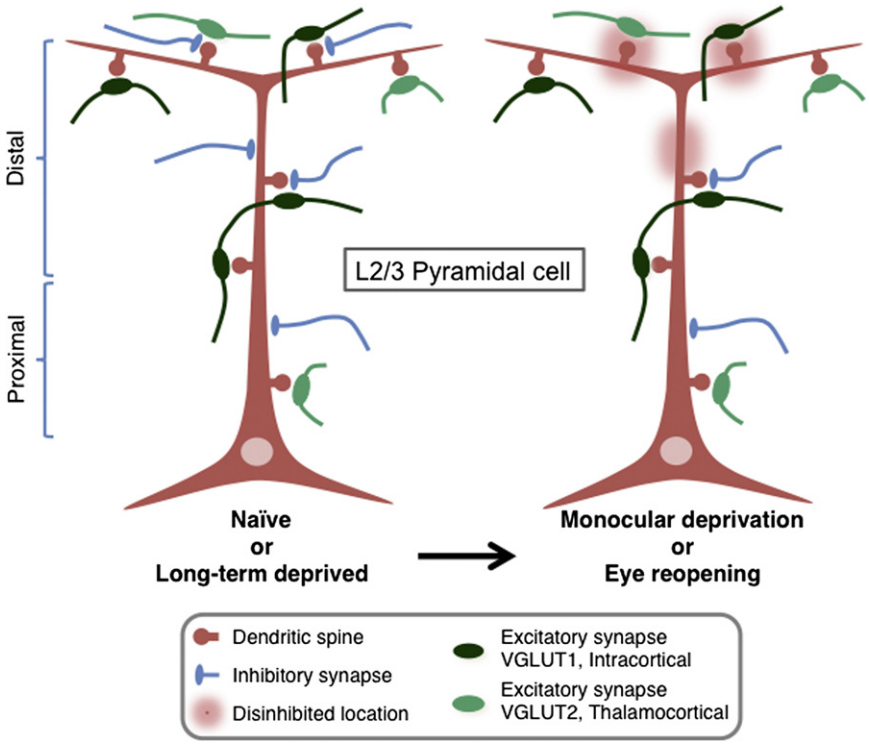

Figure 1. Experience-Dependent Plasticity in Visual Cortex L2/3 Cells Is Associated with Inhibitory Synapse Pruning, Mainly on Distal Dendritic Spines

coinnervated by an excitatory synapse. The finding that such a high fraction of spines on distal dendrites is doubly innervated prompts the question whether inhibitory spine synapses have a specific function in modulating dendritic activity. While proximal inhibitory synapses are thought to be efficient attenuators of more distal excitatory inputs or even Ca^{2+} spikes and back propagating action potentials, the function of distal inhibitory spine synapse may be restricted. An inhibitory synapse on a spine could cause a large increase in chloride conductance that is confined to the spine head, shunting its neighboring excitatory input (Koch, 1999). However, in contrast to the relatively broad temporal window during which inhibitory shaft synapses can shunt more distal excitatory conductances (in the millisecond range), shunting inhibition on spines is thought to operate only over sub millisecond time frames (Koch, 1999). Therefore, both inputs would have to arrive almost instantaneously. Alternatively, an inhibitory spine synapse could directly affect its neighboring excitatory input by hyperpolarizing the spine's membrane, thereby increasing the Mg^{2+} block on NMDA receptors. This effect may be partially mediated by postsynaptic GABA-B receptors, but it is not known whether these receptors cluster together with gephyrin-GABA-A/glycine receptor complexes.

The high density of inhibitory spine synapses on distal dendrites may be a reflection of them being associated with particular afferents that preferentially project to this region. To substantiate this idea, both papers refer to a study by Kubota et al. (2007) describing that a large proportion of cortical doubly innervated spines receive their excitatory input from vesicular glutamate transport (VGLUT) type 2 positive presynaptic partners. In contrast to VGLUT1, which is predominantly located in presynaptic boutons of intracortical axons, VGLUT2 is typically found in thalamocortical projections. van Versendaal et al. (2012) estimated that ~50% of the doubly innervated spines are juxtaposed to VGLUT2-expressing excitatory inputs. Both studies speculate that part of the inhibitory synapse population may therefore serve to specifically gate thalamocortical excitatory inputs (Figure 1). Analogous to the somatosensory system and the cat or monkey visual system, the thalamocortical axons that putatively connect to the most distal parts of pyramidal cell apical dendrites (in cortical layer 1) may have a modulatory function, whereas those that project to cortical layer 4 and lower parts of L2/3 may be drivers of specific activity. If such a divergence in thalamocortical function and projection territory holds to be true for the mouse visual system it would make the densely packed inhibitory spine synapses on the distal dendrites the most likely candidates to gate modulatory sensory information.

An outstanding question from the current studies is which types of inhibitory interneurons provide the presynaptic input to the various gephyrin-marked inhibitory synapses? Parvalbumin expressing fast-spiking neurons and in particular the basket cell subpopulation could target the proximal synapses that are electrotonically close to the soma. Theses synapses are thought to provide thalamocorical driven feedforward inhibition and thereby shape the timing and dynamic range of cortical activity (Markram et al., 2004). Somatostatin-expressing Martinotti interneurons often project to upper layers in the cortex and mediate cross-columnar inhibition. They could be a source for the distal, and often inhibitory spine synapses. Ionotropic serotonin-receptor 3A-expressing cells, the third main subpopulation of inhibitory interneurons, are enriched in the upper cortical layers and may also provide distal dendritic inhibition. Future studies based on optophysiology or correlative light and electron microscopy may be able to identify the exact nature and composition of the presynaptic inhibitory inputs to spines and various parts of L2/3 cell dendrites.

Experience-Dependent Dynamics of Inhibitory Synapses

Both studies observed that inhibitory synapses were highly dynamic. Inhibitory shaft as well as spine synapses were added and eliminated at rates comparable to the turnover of spines and inhibitory boutons (Chen et al., 2011; Holtmaat and Svoboda, 2009; Keck et al., 2011). Interestingly, the turnover of

inhibitory spine synapses occurred on otherwise stable spines. This is different from the dynamics of excitatory synapses, which are thought to go hand-in-hand with the physical removal or addition of spines (Holtmaat and Svoboda, 2009). It raises the possibility that the turnover of inhibitory synapses is regulated by excitatory activity. On the other hand, a study by Knott et al. (2002) has suggested that the addition of GABAergic synapses onto spines stabilizes them. This implies that inhibitory spine synapse turnover may affect excitatory spine synapse lifetimes.

Similar to previous studies (Chen et al., 2011; Keck et al., 2011), Chen et al. and van Versendaal et al. investigated whether inhibitory synapse dynamics increase throughout cortical plasticity. They turned to a popular model for cortical plasticity, referred to as the ocular dominance shift that occurs in response to monocular deprivation. In the mouse binocular region, i.e., the part of the visual cortex that receives input from both eyes, the closure of the contralateral eye causes a rapid increase in the sensitivity towards the open ipsilateral eye. Although the potential for this plasticity decreases after the critical period, map shifts can still be induced in adults albeit with longer delay times as compared to young mice. Not surprisingly the structural rearrangements that are generally observed in the excitatory synaptic pathway during the critical period become less obvious in adulthood. Some structural synaptic remodeling remains present. For example, monocular deprivation has been found to cause rapid and long lasting additions of dendritic spines on L5 but not L2/3 cells (Hofer et al., 2009). The current studies build on this by speculating that in the adult other mechanisms may join in to govern plasticity of L2/3 cells, and they envision a role for inhibitory synapses. Indeed, they found that a short period of monocular deprivation (1–4 days) caused the pruning of a significant complement of the inhibitory synapses, mainly on dendritic spines (Figure 1). This is the first live observation of the physical removal of inhibitory synapses on cortical pyramidal cell dendrites in response to changes in sensory input. The massive removal of inhibitory synapses suggests that these cells are disinhibited as part of the plasticity response. However, the studies did not assess if the pruning of inhibitory synapses on one part of the dendrite was compensated by the growth or strengthening of inhibition on other parts. Optophysiological or whole-cell recordings will be needed to assess the levels of disinhibition in more detail.

The pruning of inhibitory synapses could constitute a homeostatic response of the pyramidal cells to compensate for the loss in excitation that is likely to happen immediately after monocular deprivation. This is in line with the study by Knott et al. (2002) describing the opposite effect. Here, increased sensory input caused the addition of inhibitory synapses in layer 4 of the barrel cortex, which was interpreted as a compensatory mechanism to excessive excitation. Inhibitory synapse pruning may also be intrinsic to the interneurons and constitute a response to a reduction in excitatory synapses onto themselves (Chen et al., 2011; Keck et al., 2011). Nonetheless, the reduction in inhibition may depolarize the membrane potential and facilitate sensory-evoked spiking (Isaacson and Scanziani, 2011). This may open the gate for excitatory synaptic plasticity, for example by changing the window for spike timing dependent plasticity or other LTP and LTD like processes (Sjöström et al., 2008), which in turn could further sculpt the ocular dominance shift.

van Versendaal et al. (2012) found that reopening of the eye caused another wave of predominantly inhibitory spine synapse loss (Figure 1). This was surprising since eye reopening rebalances the excitatory inputs from both eyes and was therefore expected to restore inhibitory synapse numbers. The authors measured visually evoked intrinsic optical signals in the binocular visual cortex. They found, perhaps not to their surprise, that reopening of the deprived eye reinstated the ocular dominance of the contralateral eye through an increase of the signal evoked by the reopened eye rather than a decrease of the response to the previously undeprived eye. Therefore, the authors interpret the wave of inhibitory synapse loss as a generalized reactive response that increases cortical excitation. Future studies may be able to test if sensory deprivation or recovery of the ipsi versus the contralateral eye causes inhibitory synapse loss on a differential population of spines. Should this be true, it would argue for inhibitory synapse pruning to gate eye-specific excitatory pathways. If, on the other hand, both manipulations induce pruning of the same pool of synapses it would make a case for plasticity to be initiated by an unspecific and rather homeostatic disinhibitory response.

Clustering of Dynamic Events

The clustering of synaptic modifications may be an important feature of experience-dependent plasticity (Makino and Malinow, 2011), and relevant for motor learning (Fu et al., 2012). Fu et al. (2012) found that repeated motor learning induces the formation of clustered L5 apical spines, which presumably synapse with axons that belong to the same neuronal circuit. Chen et al. (2012) found the dynamics of inhibitory synapses also to be clustered with dynamic dendritic spines. This suggests that the removal of inhibitory synapses after monocular deprivation is orchestrated by a local interplay between excitation and inhibition. It will be interesting to further dissect the temporal aspects of these interactive dynamics. Do inhibitory synapse dynamics precede those of the excitatory ones or vice versa? It is tempting to speculate that the removal of an inhibitory shaft synapse allows a subsequent local increase in excitatory activity to induce the addition of a nearby spine.

In summary, the studies by Chen et al. (2012) and van Versendaal et al. (2012) convincingly show that inhibitory synapses in the adult brain display profound structural dynamics of their own. By means of the tracking of individual postsynaptic inhibitory synaptic scaffolds in vivo they were able to reveal that L2/3 cell ocular dominance plasticity may be initiated by the pruning of predominantly inhibitory spine synapses on apical dendrites. This pruning occurs close to dynamic spines and may regulate plasticity of circuits that preferentially impinge on distal dendrites. These studies firmly establish that inhibitory structural remodeling has its share in visual cortex plasticity and provide a framework for future endeavors to unravel its mechanisms.

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A Taste of What to Expect: Top-Down Modulation of Neural Coding in Rodent Gustatory Cortex

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A central aspect of sensory perception is the anticipation of forthcoming stimuli, allowing for a faster and more accurate assessment of the surrounding environment. A new study by Samuelsen et al. (2012) in this issue of *Neuron* highlights the neural mechanisms underlying the expectation of an imminent taste.

In the 1998 film *The Truman Show*, a group of television producers labors with Herculean passion to manufacture an artificial but believable world for an insurance salesman, Truman Burbank (played by actor Jim Carrey), who unwittingly stars in his own reality show. As each new day dawns, or is meant to dawn, in the town of Seahaven, the order is shouted within the TV control room to "cue the sun!" The well-timed appearance of a heavenly orb—perhaps the most reliable and dependable sensory cue known to roosters and humans alike—signals morning and launches Truman out of bed.

Hollywood actors notwithstanding, human and nonhuman animals of all sorts readily utilize sensory cues to predict events and guide behavior. External cues, typically arriving in visual, olfactory, auditory, or verbal format, may announce a general state-based change in behavior or in the environmental milieu, for example, the sound of a dinner bell signaling that food is imminent. Alternatively, external cues may forecast more specific information about the identity of an upcoming event, enhancing sensory discrimination, response speed, and perceptually based decisions. The roasted smell of coffee in the morning sets up an expectation of coffee flavor that is met upon sipping from your breakfast mug. Not infrequently, an external cue can be uninformative or misinformative, or absent altogether. Having learned to predict the presence of something that is actually not there has adverse behavioral consequences, reducing discrimination and response speed, and creating cognitive dissonance. Finding that the same coffee smell leads not to coffee but, unexpectedly, to black tea (sipping from the wrong mug, for example) may result in breakfast dismay.

The majority of neuroscientific research on sensory expectation, awareness, and prediction has focused on the visual system (Gilbert and Sigman, 2007; Kouider and Dehaene, 2007; Summerfield and Egner, 2009), whereas comparable studies of the chemical senses—smell and taste—are, well, to be unexpected. In this issue of *Neuron*, Samuelsen et al. (2012) systematically explore how prestimulus cues can modulate network properties of the rodent gustatory system to shape sensory responsiveness at the perceptual level. By bringing together electrophysiological recordings in awake behaving rats, an elegant psychophysical paradigm, and pharmacological inactivation techniques, these investigators were able to show that cue-triggered expectation modulates activity in gustatory cortex (GC) in an amygdala-dependent manner, with consequent enhancement of taste coding.