Neighborly synapses help each other out

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Cortical circuits are shaped by sensory experience. These changes have now been visualized with single-synapse resolution *in vivo*, revealing clustered potentiation along stretches of dendrite.

Rodents rely on their whiskers to move around in complex and poorly lit environments. The whisker system is also a favorite with neuroscientists; its distinctive properties make it an attractive model for the study of cortical plasticity. The anatomical arrangement of whisker input is preserved along thalamic relay stations so that a single area of neocortex, called a barrel, receives information from a single whisker. This alleviates the 'needle in a haystack' problem of systems neuroscience: experience changes the brain, but where exactly should we look for these changes? Using the whisker-barrel projection, it had been shown that neurons in the stimulated barrel change their response properties during sensory deprivation[1]. It is generally assumed that altered synaptic transmission is behind such changes in cellular responsiveness, but locating the modified synapses in a living animal has appeared to be an intractable problem.

In this issue of *Nature Neuroscience*, Zhang *et al.*[2] visualized activity-induced changes in exquisite detail. They used two-photon microscopy in anesthetized mice to monitor synapses in barrel cortex before and after wiggling the connected whisker for 1 h. To monitor changes in AMPA-type glutamate receptor density at spines and dendrites of cortical layer 2/3 pyramidal neurons, they used a pH-sensitive tag that reveals receptors on the surface of neurons[3]. After the intense whisker stimulation, the fluorescence signal from tagged AMPA receptors increased. Given that AMPA receptor insertion has been firmly established as the primary mechanism for long-term potentiation (LTP) in brain slice experiments[4], we can assume that the synapses that showed increased fluorescence after stimulation underwent LTP. In accordance with *ex vivo* studies[5], potentiation occurred in clusters at a large fraction of synapses and, surprisingly, even dendritic shafts showed an increased density of receptors (**Fig. 1a**). What could lead to AMPA receptor insertion in entire dendritic segments?

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A recent study investigated the mechanisms underlying whisker stimulation–evoked LTP in barrel cortex[6]. Frequent deflections of the principal whisker at frequencies that mice use to sample objects evoked local, dendritic NMDA receptor–mediated plateau potentials without triggering somatic spikes. These local dendritic events caused by the activity of thalamocortical projections apparently provide an associative signal for direct sensory synaptic inputs. Zhang *et al.*[2], using similar stimulation patterns, found that potentiation occurred at many spines along entire dendritic segments. When Zhang *et al.*[2] blocked NMDA receptors, stimulation-induced AMPA receptor insertion was prevented. This suggests that local NMDA receptor–mediated plateau potentials, although not shown in the current study, may have triggered the AMPA receptor insertion.

Zhang *et al.*[2] report that neighboring spines often changed their AMPA receptor density in unison and rarely in opposite directions. One possible interpretation is that an ample supply of synaptic building blocks, such as AMPA receptors, needs to be available locally for successful LTP. This is supported by the observation that elevated surface expression on the dendritic shaft had returned to basal levels after 2 d, whereas AMPA receptors remained enriched in the spines (**Fig. 1b**). The exact location of AMPA receptor exocytosis is not entirely clear. Do fresh receptors get exocytosed at the dendritic shaft and then drift into spines by lateral diffusion, or do receptors get inserted at a few potentiated spines and then spill out of them? In the first scenario, elevated AMPA receptor surface expression would initially not be synapse specific. To establish synapse specificity, additional mechanisms, such as actin polymerization and insertion of PSD proteins, would be required to capture and stabilize AMPA receptors in potentiated spines. In the latter scenario, AMPARs would specifically be delivered to synapses undergoing LTP and dendritic receptor density would be locally elevated as a secondary effect. In either case, the transiently elevated pool of AMPA receptors might constitute some form of local plasticity tag, making it easier to potentiate synapses on AMPA-rich dendritic segments within a certain time window.

When it comes to potentiation, it now seems clear that neighboring synapses are not completely independent. Collaborative interactions between neighboring synapses have been demonstrated during spontaneous activity[7] and in response to single-spine stimulation in slice culture[8]. The current study suggests that clustered plasticity is strongly driven by sensory inputs in intact animals, although we do not know the degree of input clustering that was already present before the period of experimental whisker stimulation. If cortical synapses cannot be changed independently, this reduces the number of patterns that could be stored and retrieved from a synaptic matrix. However, it puts great importance on the location of a synapse on the synaptic shaft: in a poorly synchronized neighborhood, it is hard to get by and even harder to get ahead. As the dendritic neighborhood gentrifies, contacts made by neurons with unrelated activity patterns will be pushed out.

Interestingly, only a relatively small fraction of dendritic segments (21%) in the stimulated barrel displayed elevated surface AMPA receptor

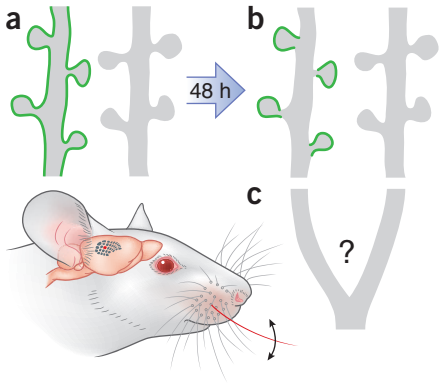

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Figure 1 Repetitive stimulation of a single whisker triggered enhanced surface expression of AMPA receptors in somatosensory cortex. (**a**) 21% of dendritic segments showed elevated AMPA receptor densities on their surfaces. Illustration shows single-whisker stimulation model and the corresponding barrel (red) in somatosensory cortex. (**b**) 48 h after stimulation, dendritic spines still maintained high AMPA receptor densities, whereas dendritic surface expression returned to control levels. (**c**) Whether potentiated and non-potentiated spine clusters coexist on individual neurons is not yet known.

levels after whisker stimulation. This suggests that axons carrying information about the principal whisker were indeed wired specifically to those dendritic segments. Such clustering of coactive inputs would be the expected outcome of clustered plasticity acting over long periods of time[7,9]. As Zhang *et al.*[2] did not reconstruct entire neurons, we cannot be sure about the connectivity of the imaged segments. If non-potentiated and potentiated dendrites indeed coexist at individual neurons (**Fig. 1c**), this would strongly support the hypothesis that dendritic branches serve as independent information storage units[10]. Experiments to directly test this hypothesis are now within reach.

Direct observation of AMPA receptor insertion allowed Zhang *et al.*[2] to assess synaptic potentiation independently of spine volume changes. The volume of the spines carrying the most strongly potentiated synapses did not in fact increase particularly. AMPA receptor enrichment in spines was stable during the 48 h following stimulation, but this functional strengthening did not affect spine morphology in an obvious way. Whether this sensory-evoked plasticity also increased the long-term stability of synapses remains to be seen. More dramatic manipulations, such as whisker trimming[11] or monocular deprivation[12], have been shown to affect spine turnover. However, the time lag between plasticity induction and spine loss can be considerable[13], suggesting that potential effects on synaptic stability might not manifest in the 2-d observation window of the current study[2].

Although direct imaging of AMPA receptor insertion *in vivo* is an attractive strategy for measuring the spatiotemporal dynamics of plasticity events at the level of single synapses, Zhang *et al.*'s results[2] must be taken with a grain of salt. All of the observations were made by overexpressing the two AMPA receptor subunits GluA1 and GluA2, of which GluA1 contained the fluorescent label. The authors thoroughly characterized expression levels of those subunits and found no overexpression artifacts. Still, we cannot know for sure whether all labeled GluA1 molecules at the surface were indeed assembled into functional AMPA receptors, or whether increased fluorescence merely indicated elevated rates of local exocytosis. Fluorescent labeling of endogenous GluA subunits—for example, using gene editing[14]—would reduce the risk of disturbing expression levels and subunit composition.

In the future, the elegant approach of following the complement of receptors at individual synapses *in vivo*[2] could be combined with sophisticated learning experiments. Perhaps the rodent barrel cortex will indeed be the first system in which the potentiated synapses that constitute a new memory can be identified. The search for the memory trace, or engram, has come a long way from Lashley's original lesion experiments[15], and the current study marks an important step toward this ultimate goal.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

1. Margolis, D.J. *et al. Nat. Neurosci.* **15**, 1539–1546 (2012).
2. Zhang, Y., Cudmore, R.H., Lin, D.T., Linden, D.J. & Huganir, R.L. *Nat. Neurosci.* **18**, 402–407 (2015).
3. Ashby, M.C. *et al. J. Neurosci.* **24**, 5172–5176 (2004).
4. Shi, S.H. *et al. Science* **284**, 1811–1816 (1999).
5. Makino, H. & Malinow, R. *Neuron* **72**, 1001–1011 (2011).
6. Gambino, F. *et al. Nature* **515**, 116–119 (2014).
7. Takahashi, N. *et al. Science* **335**, 353–356 (2012).
8. Harvey, C.D. & Svoboda, K. *Nature* **450**, 1195–1200 (2007).
9. Iannella, N.L., Launey, T. & Tanaka, S. *Front. Comput. Neurosci.* **4**, 21 (2010).
10. Branco, T. & Häusser, M. *Curr. Opin. Neurobiol.* **20**, 494–502 (2010).
11. Holtmaat, A., Wilbrecht, L., Knott, G.W., Welker, E. & Svoboda, K. *Nature* **441**, 979–983 (2006).
12. Hofer, S.B., Mrsic-Flogel, T.D., Bonhoeffer, T. & Hubener, M. *Nature* **457**, 313–317 (2009).
13. Wiegert, J.S. & Oertner, T.G. *Proc. Natl. Acad. Sci. USA* **110**, E4510–E4519 (2013).
14. Hsu, P.D., Lander, E.S. & Zhang, F. *Cell* **157**, 1262–1278 (2014).
15. Lashley, K.S. *Science* **73**, 245–254 (1931).

Attention: feedback focuses a wandering mind

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Neurofeedback that tracks attentional focus in real time using fMRI and alerts subjects to impending lapses by modulating the difficulty of the task itself has been demonstrated to improve behavioral performance.

Brief lapses of attention while performing daily tasks are ubiquitous. Whether it's adding salt instead of sugar to your coffee or missing a stop sign, these attentional lapses can result in unintended consequences ranging from minor nuisances to outright catastrophes[1]. A challenge for controlling such lapses is that humans often are not very good at immediately noticing when their mind has drifted off from the task at hand[2]. However, deBettencourt *et al.*[3] have now developed an approach that uses fMRI in real time to detect when the subject's brain is no longer in an attentive state and provides them with continuous feedback to get them back on track. This neurofeedback approach yielded reliable increases in behavioral performance relative to a sham feedback condition, demonstrating the value of online feedback for optimizing performance in attention-demanding situations.

The authors required subjects to attend to either the face or scene aspect of a composite stimulus (**Fig. 1**) while tracking the strength of task-relevant information in each subject's brain. The task required them to make a response on 90% of trials, but to withhold that response on the rare trials in which non-target stimuli were presented; this task is well known to tax one's ability to sustain attention over time and to inhibit prepotent responses. As the subjects performed this attentionally demanding task, the authors used the ongoing neural signals from each subject's brain to provide moment-to-moment feedback using a clever and direct method: the weight of each image in the composite stimulus started out equal, but when ongoing neural activity indicated that attention to the relevant stimulus was waning, the percentage of the task-relevant aspect (face or scene) in the composite mixture was reduced. Conversely, when neural activity indicated increasing attentional focus, the relevant face or scene aspect of the physical stimulus was amplified (**Fig. 1**). Thus, the feedback signal that informed subjects of their current attentional state was integrated into the very stimulus subjects were attempting to attend. deBettencourt *et al.*[3] suggest that this feedback scheme served to reward subjects with an easier stimulus display when

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