Journal Club

Editor's Note: These short, critical reviews of recent papers in the *Journal*, written exclusively by graduate students or postdoctoral fellows, are intended to summarize the important findings of the paper and provide additional insight and commentary. For more information on the format and purpose of the Journal Club, please see http://www.jneurosci.org/misc/ifa_features.shtml.

A Role for Short-Lived Synapses in Adult Cortex?

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Review of Cane et al.

Longitudinal imaging studies of individual neurons *in vivo* have greatly enhanced our understanding of the rewiring that the adult brain is capable of, both during normal experience and in response to altered sensory input or learning. Most of these studies have focused on dendritic spines, tiny femtoliter-sized protrusions from the dendritic shaft that receive the majority of excitatory synapses onto pyramidal neurons. Electron microscopy (EM) studies have established a strong positive correlation between spine and postsynaptic density (PSD) size. PSD size, which is activity-dependent and varies between synapses, in turn provides a good measure of synaptic strength. Hence, dendritic spines have become a well established and frequently used proxy for the number and strength of cortical excitatory synapses.

In vivo two-photon imaging studies have shown that while most dendritic spines persist for weeks or months, ongoing formation and elimination of spines occurs even in the adult cortex (Trachtenberg et al., 2002). This spine turnover is thought to underpin experience-dependent reorganization of cortical circuitry as well as some forms of learning and memory

(Holtmaat et al., 2006; Keck et al., 2008; Xu et al., 2009; Yang et al., 2009). However, fundamental questions remain: How frequently do new spines form synapses? What is the temporal relationship between spine and synapse formation, and how does the presence of a PSD affect spine survival? Finally, and most tantalizing: what is the function of transient spines that live for only hours or days?

In a recent study published in *The Journal of Neuroscience*, Cane et al. (2014) set out to address many of these questions. They used *in utero* electroporation to label both PSDs and neuronal morphology, including dendritic spines of mouse layer 2/3 cortical pyramidal neurons, by coexpressing PSD-95-eGFP and DSRRedExpress. They then implanted cranial windows over somatosensory cortex and used chronic *in vivo* two-photon microscopy to repeatedly image the same dendritic structures in layer 1 over hours and days. This enabled a systematic comparison of the temporal dynamics of spines and PSDs. PSDs were detected using a method based on localized accumulation of PSD-95-eGFP fluorescence. By reconstructing a stretch of dendrite imaged *in vivo* using Focused Ion Beam Scanning EM, the authors provided compelling evidence that they could detect *in vivo* the smallest PSDs discernable with EM.

Determining the relationship between spine and synapse formation is an important task that is not without technical difficulties. Previous correlative EM studies have established that not all spines have a

PSD, at least not all of the time: whereas spines ≥ 4 -d-old always possessed PSDs, younger spines frequently did not (Knott et al., 2006). However, EM by necessity provides a “snapshot” assessment of PSD presence. By incorporating an *in vivo* PSD marker and using short (6–24 h) intervals between imaging sessions, Cane et al. (2014) were able to gain considerable new insight into this relationship. They confirmed that both spines and PSDs appear and disappear over time, even without overt manipulation of sensory experience. Most new spines lacked a PSD when first observed. However, the proportion of new spines carrying a PSD increased with spine lifetime, from 19% at ≤ 6 h old to 30% at ≤ 24 h old and 42% at ≤ 96 h old. Therefore, while PSDs can form within 6 h, delayed or more gradual PSD acquisition is also possible.

One intriguing point not discussed by the authors is that late PSD acquisition was rarely seen. Indeed, 32% of spines lacking a PSD at < 6 h old acquired one over the ensuing 18 h, whereas none of the spines lacking a PSD at 24 h old acquired one over the subsequent 24 h. Therefore, PSD acquisition may be predominantly confined to the first 24 h after spine formation, matching the time course for PSD insertion at new spines in hippocampal slice cultures (De Roo et al., 2008). How the temporal window for PSD formation is regulated is unknown, although it appears that the pattern of cortical activity is the key determinant of spine stability *in vivo* (Wyatt et al., 2012).

Received March 19, 2014; revised April 9, 2014; accepted April 9, 2014.

C.E.J.C. is funded by a Long-Term Fellowship from the Human Frontier Science Program.

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DOI:10.1523/JNEUROSCI.1108-14.2014

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It has been widely thought that PSD formation is required for spine survival, such that persistent spines always contain a PSD. As described above, Cane et al. (2014) show that the proportion of spines carrying a PSD increases with spine age, indicating that the presence of a PSD does indeed promote spine stability. However, PSD formation was not sufficient to render a spine persistent: 25% of spines present at only a single time point had a PSD, while 70% of new spines that bore a PSD at least once still disappeared within 4 d. This suggests that there is not a simple binary relationship between PSD formation and spine survival. In further support of this idea, only 83% of new spines that survived for ≥ 4 d displayed a PSD once or more. Although nonsynaptic spines have been described previously, they have been widely thought of as “intermediates,” i.e., new spines yet to form a synapse or disappearing spines that have lost their synapses (Arellano et al., 2007). Data in Cane et al. (2014) suggest that some PSD-95-lacking spines are in fact persistent, although what role they play remains to be determined.

Analysis of spines that were destined to disappear adds further weight to the argument that the presence of a PSD does not always result in a persistent spine. Although PSD loss and spine elimination were temporally matched for the majority of disappearing spines, 20% of disappearing spines survived for ≥ 4 d after PSD loss. In marked contrast, only 5% of new spines that never acquired a PSD lived for ≥ 4 d (Cane et al., 2014). Hence, spines that once had a PSD disappear at a slower rate than those that never contained a PSD. Perhaps some echo or trace of that PSD remains in the spine to promote stability. This could take the form of a weak complement of AMPA or NMDA receptors, meaning that the spine retains some level of neurotransmission. Alternatively, although the authors verified that spines lacking PSD-95 *in vivo* also lacked a detectable PSD by EM, other residual scaffolding or synaptic adhesion proteins may be disassembled more slowly than the PSD itself. Similarly, it is possible that low levels of extrasynaptic receptors could support neurotransmission at new spines before PSD insertion.

Nevertheless, any discussion of the role of new spines is transformed by the finding that some transient spines do acquire PSDs (Cane et al., 2014), and may therefore function as bona fide short-lived synapses. It is possible that new spines, and

Figure 1. Possible roles of transient PSD-carrying spines. **A**, The formation of a transient spine (center) is an entirely postsynaptic process and although it contains a PSD (red) there is no presynaptic partner and thus no functional connectivity. **B**, Transient spines fail to stabilize: after contacting a presynaptic bouton (black), the presynaptic activity pattern is not sufficient to maintain the spine. **C**, Transient spines destabilize existing connections by forming a competing synapse at multisynapse boutons. **D**, Transient spines temporarily increase correlated activity between connected neurons and strengthen preexisting synapses.

even PSDs, are generated by an entirely postsynaptic process that has little consequence for synaptic connectivity (Fig. 1A). However, increased spinogenesis following either motor learning (Xu et al., 2009; Yang et al., 2009) or sensory deprivation (Holtmaat et al., 2006) is testament to the role that new spines likely play in memory acquisition and cortical circuit reorganization. Given the sparse connectivity of excitatory intracortical networks, only a small number of new spines need to be stabilized to dramatically alter network architecture (Chklovskii et al., 2004). In keeping with this idea, new spine stabilization increases after sensory deprivation, but these new persistent spines comprise only 13% of the total spine population (Holtmaat et al., 2006). It is possible that the transient PSD-carrying spines described by Cane et al. (2014) have the potential to stabilize but do not receive appropriate reinforcement in terms of the pattern or frequency of presynaptic stimulation (Fig. 1B). An alternative hypothesis arises from the fact that two-thirds of the boutons contacted by new spines already have a synapse (Knott et al., 2006); hence, new spine addition may result in a competitive interaction that destabilizes the original synapse at these multisynapse boutons. Transient spines may therefore be more than just spines that fail to stabilize, and instead play an active role in cortical reorganization by destabilizing existing networks (Fig. 1C). Finally, transient spines

could temporarily increase correlated activity in connected neurons, promoting strengthening and/or stabilization of existing synapses between those neurons (Fig. 1D). A key next step will be to confirm that transient PSD-carrying spines do form functional synaptic contacts.

Cane et al. (2014) also describe PSDs that were not located in a discernable spine. These represented 20% of imaged structures and although spine synapses that protrude predominantly in the lower resolution axial (*z*) plane may contribute, the authors suggest that these are likely shaft synapses. Approximately 25% of excitatory inputs to cortical pyramidal neurons are in fact formed on dendritic shafts (Knott et al., 2006), yet the structural dynamics and even strength of these shaft synapses are poorly understood. This is in part because they lack a clear structural proxy, rendering longitudinal tracking studies challenging. The strategy used by Cane et al. (2014) provides a solution to this technical problem, and indeed, the authors clearly show that shaft synapses do turn over, and furthermore, that shaft synapse turnover rate is comparable to that of the spine population as a whole. Hence, shaft synapse plasticity is an underappreciated mechanism that could contribute to cortical rewiring.

In conclusion, the data described in Cane et al. (2014) represent an important advance in our understanding of synapse structural dynamics. In particular, the finding that transient spines can carry a PSD hints at a more instructive role for transient spines in cortical reorganization than previously thought. However, interesting questions remain, such as how the temporal progression of synapse formation is affected by sensory deprivation or task-specific repetitive activation. Furthermore, identification and tracking of the presynaptic partners of both new and persistent spines remains an important future goal toward understanding adult plasticity.

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