during spatial trajectories fired in a time-compressed sequence that occurred over one theta cycle (~120 ms). These theta sequences, which occurred both in the maze and on the wheel, were abolished by medial septal inactivation. What is the relationship between these theta sequences and episodic or spatial trajectories? To examine this question, the authors implemented a computational model of hippocampus with asymmetric excitatory connections and short-term synaptic plasticity. This network architecture results in oscillatory excitatory drive from the medial septum forming a bump of activity that sweeps forward through the network, serving as the mechanism for the emergence of temporal sequences. In parallel, sensory inputs bind spatial sequences to the cues in the environment. Thus, their model presents a circuit architecture that predicts that a loss of medial septal theta will abolish temporal and episodic trajectories, but allow spatial trajectories to remain intact. The model also predicts, however, that if sensory cues are substantially dampened, spatial trajectories will also be abolished. Consistent with this hypothesis, the authors demonstrated that medial septal inactivation disrupts spatial representations in large novel environments, where the distance between environmental boundaries is very large and the salience of a major sensory cue is therefore reduced. This computational framework also predicts a differential importance of internal versus external input on the formation and maintenance of spatial trajectories as the animal explores and learns about the sensory cues in a given environment.

One internal source of spatial information might come from upstream medial entorhinal cortex grid cells, which could provide a neural metric for distance traveled by firing in multiple, regularly spaced locations[9,10]. Previous work has demonstrated that the inactivation of medial septum abolishes grid cell firing patterns, but does not affect place cells, raising the possibility that place cells might be primarily driven by sensory inputs[11,12]. Wang *et al.*[3] take this idea further and dissociate the effect of internal and external cues from experience on place cell firing. Place cells were recorded in either novel or familiar open arenas of different sizes, as well as the linear track. The authors found that place cells remain impervious to medial septal inactivation only when boundary cues in the environment are proximal, such as in the linear track or a small open arena. In large novel environments, where the boundary distance increases, stable place cells were not observed during medial septal inactivation. This result has two key implications. First, experience may shift the balance between internal and external drive on spatial representations in the hippocampus. Second, boundaries in the environment may act as sensory stimuli capable of supporting stable place cell representations in the absence of medial septal–driven activity.

Several interesting questions are also raised by the findings of Wang *et al.*[3]. Does the loss of theta sequences depend exclusively on the loss of medial septal–driven theta? As mentioned above, medial septal inactivation is also known to perturb upstream medial entorhinal grid responses[12]. It will be of interest to determine whether the medial-septal effects on sequences reflect changes in neuromodulatory, rhythmic or entorhinal drive. In addition, what kinds of sensory cues can determine the formation of place fields in the absence of septal inputs? The authors discuss the importance of boundaries in the environment, but future work should examine how capable diverse sensory inputs, such as odor, can inform spatial representations. And finally, does the inactivation of medial septum affect other types of temporally compressed sequences that are thought to be involved in memory, such as hippocampal replay events[13–15]? Wang *et al.*[3] point the way for future research by identifying the medial septum as a key component in generating at least one of the proposed neural correlates of episodic memory.

Episodic memory is an intrinsic and mysterious process, but, in many ways, it defines the core of our cognitive experience. The findings of Wang *et al.*[3] offer a tantalizing glimpse into the mechanisms and circuits that build the cell assemblies crucial to the formation of episodic memories.

COMPETING FINANCIAL INTERESTS
The author declares no competing financial interests.

1. Buzsáki, G. & Moser, E.I. *Nat. Neurosci.* **16**, 130–138 (2013).
2. Pastalkova, E., Itskov, V., Amarasingham, A. & Buzsaki, G. *Science* **321**, 1322–1327 (2008).
3. Wang, Y., Romani, S., Lustig, B., Leonardo, A. & Pastalkova, E. *Nat. Neurosci.* **18**, 282–288 (2015).
4. Stewart, M. & Fox, S.E. *Trends Neurosci.* **13**, 163–168 (1990).
5. Petsche, H., Stumpf, C. & Gogolak, G. *Electroencephalogr. Clin. Neurophysiol.* **14**, 202–211 (1962).
6. Green, J.D. & Arduini, A.A. *J. Neurophysiol.* **17**, 533–557 (1954).
7. MacDonald, C.J., Lepage, K.Q., Eden, U.T. & Eichenbaum, H. *Neuron* **71**, 737–749 (2011).
8. Hasselmo, M.E. *Hippocampus* **15**, 936–949 (2005).
9. Fyhn, M., Molden, S., Witter, M.P., Moser, E.I. & Moser, M.B. *Science* **305**, 1258–1264 (2004).
10. Hafting, T., Fyhn, M., Molden, S., Moser, M.B. & Moser, E.I. *Nature* **436**, 801–806 (2005).
11. Brandon, M.P., Koenig, J., Leutgeb, J.K. & Leutgeb, S. *Neuron* **82**, 789–796 (2014).
12. Koenig, J., Linder, A.N., Leutgeb, J.K. & Leutgeb, S. *Science* **332**, 592–595 (2011).
13. Wilson, M.A. & McNaughton, B.L. *Science* **265**, 676–679 (1994).
14. Pavlides, C. & Winson, J. *J. Neurosci.* **9**, 2907–2918 (1989).
15. Kudrimoti, H.S., Barnes, C.A. & McNaughton, B.L. *J. Neurosci.* **19**, 4090–4101 (1999).

Neddylation is needed for synapse maturation

Amy K Fu & Nancy Y Ip

A study reports for the first time on the importance of post-translational modification by neddylation in postnatal brain development. In particular, it is critical to synapse maturation and stability, and thus to cognition.

Precise control of synapse maturation is critical for establishing connections between neurons and proper brain functioning. Although the molecular mechanisms underlying synaptic maturation and elimination remain largely unknown, proper brain development requires precise regulation of different protein modifications. In this issue of *Nature Neuroscience*, Vogl *et al.*[1] elegantly demonstrate that neddylation, a post-translational protein modification, is critical for controlling dendritic spine maturation and synapse maintenance. The authors found that neddylation deficiency in mouse brains led to the elimination of excitatory synapses in adult hippocampal circuits and contributed to impairments in learning and memory. This study reveals that, unlike other post-translational modifications, neddylation in the brain is specifically confined to synapse maturation and maintenance. Furthermore, the authors provide mechanistic insight into the regulation of synapse maintenance via

Amy K. Fu and Nancy Y. Ip are in the Division of Life Science, Molecular Neuroscience Center, and State Key Laboratory of Molecular Neuroscience, The Hong Kong University of Science and Technology, Clear Water Bay, Hong Kong, China.
e-mail: boip@ust.hk

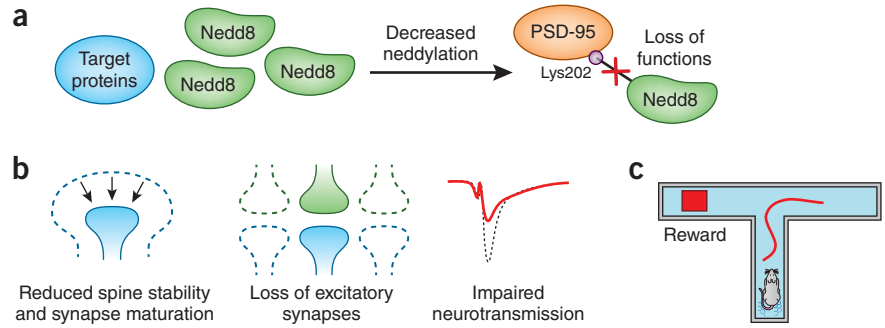

Figure 1 Inhibition of neddylation compromises dendritic spine stability, synapse maturation and cognitive function. (**a**) Neddylation results in the conjugation of Nedd8 to its target proteins. Blocking the neddylation of target protein, such as PSD-95, perturbs its functions. (**b**) Blocking neddylation notably reduces structural stability of dendritic spines, which inhibits development of excitatory synapses and leads to impaired transmission. (**c**) Neddylation deficiency causes memory impairment in mouse models.

neddylation by identifying the postsynaptic protein PSD-95 as a neddylation target (**Fig. 1**).

How did the authors first link the neddylation pathway to synapse maturation? Neddylation is the process of conjugating the ubiquitin-like protein Nedd8 to substrate proteins. Like ubiquitination, neddylation has its own activating enzyme (Nedd8-activating enzyme, NAE), conjugating enzyme (Ubc12) and substrate-specific ligase that covalently conjugates Nedd8 to its target substrates[2,3]. Nedd8 (neural precursor cell–expressed, developmentally downregulated protein 8) was originally identified as being highly expressed in the embryonic brain, with its mRNA level reportedly being downregulated during development[4]. However, its function in the nervous system has not been explored. Vogl *et al.*[1] report that the mRNAs encoding Nedd8 and Ubc12 are highly expressed in neurons and are enriched in the CA1 region of the hippocampus. In contrast with previous reports[4], they found that levels of these mRNAs remained relatively constant throughout development, whereas neddylation increased in brain tissues and neurons. These findings suggest a function for neddylation during postnatal development.

To explore the possible functions of neddylation in neurons, the authors used three approaches to inhibit neddylation in primary cultures: expressing short hairpin RNAs against Nedd8 and Ubc12, overexpressing a dominant negative mutant of Ubc12, and treating with a well-characterized NAE inhibitor. Blockade of neddylation by all three approaches inhibited the development of mature dendritic spines, leaving immature spines (filopodia) on the dendrites. These results suggest that inhibition of neddylation abolishes the maturation of dendritic spines.

The authors also asked whether neddylation affects the establishment of synaptic contacts other than by attenuating spine morphogenesis. Although dendritic spines are the sites where excitatory synapses reside, transient synaptic contacts can be formed between filopodia and presynaptic sites[5,6]. The filopodia resulting from neddylation blockade were unable to establish even transient synaptic contacts with presynaptic neurons, and synaptic contacts were formed only on the dendritic shafts of affected cells. Furthermore, the specific reduction in clustering of the excitatory postsynaptic marker PSD-95, accompanied by a reduction in the frequency of miniature excitatory postsynaptic currents (mEPSCs), indicates that neddylation specifically influences the development of excitatory synapses.

Having revealed that neddylation is integral to the regulation of excitatory synapse development *in vitro*, the authors then demonstrated a specific function of neddylation in hippocampal neurons *in vivo*. After expressing a dominant-negative mutant of Ubc12 in hippocampal neural progenitors using *in utero* electroporation, they observed an increase in filopodia in hippocampal pyramidal neurons at postnatal day 5, followed by a reduction in dendritic spines by postnatal day 28. Live imaging analysis revealed that neddylation affected the structural stabilization of spines rather than the dynamics of the filopodia: blocking neddylation with the NAE inhibitor shrunk mature dendritic spines. Interestingly, neddylation is a reversible process, and although ~57% spines recovered to their original size, ~34% did not. This suggests that, although the morphology of most of the dendritic spines is regulated by neddylation, the extent and manner in which the spines are influenced by neddylation varies. The underlying mechanisms responsible for this differential responsiveness of individual spines to neddylation, be it subcellular localization of Nedd8, variations in other critical components of the neddylation pathway or differential sensitivity to the particular stimulus that triggers neddylation, remain to be elucidated.

To further confirm the role of neddylation in maintenance of mature spines, the authors next utilized an elegant conditional mutant approach by using the CaMKII$\alpha^{CreERT2}$ mice, with which they expressed a dominant-negative mutant of Ubc12 specifically in hippocampal neurons in response to treatment with tamoxifen. Tamoxifen treatment blocked Ubc12 function in mature neurons and reduced spine density, further supporting the notion that inhibition of neddylation destabilizes mature spines. To confirm the role of neddylation in spine elimination, the dominant-negative Ubc12 was overexpressed hippocampally in CaMKII$\alpha^{CreERT2}$ mice at postnatal day 35. This reduced spines in CA1 pyramidal neurons and dentate granule neurons, confirming the *in vivo* role of neddylation in spine maintenance.

What are the targets that mediate the effect of neddylation in dendritic spines? The authors tested various synaptic scaffold proteins, which are critical for organizing the postsynaptic density (PSD), as potential substrates for neddylation. PSD-95 was the only protein that could be neddylated *in vitro*, whereas endogenous neddylated PSD-95 was found in the synaptosome. Elevated neddylated PSD-95 resulted from the blockade of deneddylation, suggesting that PSD-95 is endogenously neddylated at synapses. PSD-95 is regulated at the PSD by a variety of post-translational modifications. In particular, ubiquitination of PSD-95 is mediated by the ubiquitin ligase Mdm2 (ref. 7), which can also neddylate its target proteins. Interestingly, expression of a dominant-negative mutant of Mdm2 was able to block the neddylation of PSD-95, suggesting that neddylation of PSD-95 is mediated through Mdm2. Given that Mdm2 mediates both the ubiquitination and neddylation of PSD-95, it would be interesting to explore whether there is crosstalk between the two pathways.

Consistent with its role as a core structural component of PSD, PSD-95 resides in the PSD and is highly stable[8]. It has been shown that ubiquitination of PSD-95 causes its degradation[7]; however, the authors demonstrated that neddylation did not affect the expression level of PSD-95. Instead, neddylation caused the diffusion of the protein away from mature spines, resulting in

the observed reduction in PSD-95 clusters. It will be interesting to explore whether the ubiquitination and neddylation of PSD-95 are coordinately regulated and how such coordination may modulate the activity and functions of PSD-95.

What are the functional consequences of PSD-95 neddylation? PSD-95 is critical to the basal stability, as well as activity-dependent structural plasticity, of the PSD[9,10]. Overexpression of PSD-95 increases the number of dendritic spines and enhances maturation of excitatory synapses[11]. The multiple domains of PSD-95 contribute in various ways to stabilizing the PSD and organizing its components[10]. In particular, PSD-95 contains two N-terminal PSD-95/Disc large (Dlg)/zona occludens-1 (ZO-1) (PDZ) domains, which are critical for the stability and the activity of PSD-95. PSD-95 is neddylated at residue Lys202, which is located at the second PDZ domain of PSD-95, but the functions of this neddylation remain unclear. Like blockade of neddylation, mutation of Lys202 blocked the ability of PSD-95 to induce spine growth following expression in mature neurons. In addition, overexpressing PSD-95 enhanced AMPA receptor–mediated mEPSC frequency, an indicator of an increase in the number of synapses, whereas mutating the Lys202 site reduced mEPSC frequency. Thus, neddylation of PSD-95 at Lys202 is necessary for its functions. To our knowledge, Vogl *et al.*[1] are the first to identify a synaptic protein as a substrate of Nedd8 and to show synaptic functions of neddylation. However, how neddylation of PSD-95 regulates the activity of PSD-95 remains to be explored. Investigation of such a mechanism could focus on three possibilities, as neddylation of proteins can trigger a conformational change, preclude interaction with partner proteins or enhance recruitment of proteins by generating new binding sites[2].

What is the *in vivo* role of neddylation in forebrain excitatory circuits? The authors addressed this question by using the CaMKII$\alpha^{CreERT2}$ mouse line to delete *Nae1* specifically in forebrain regions. Consistent with the notion that neddylation is critical for synapse maturation, the authors observed reduced dendritic spine density and fewer excitatory synapses. The reduced strength of synaptic transmission and the lower activity-induced gene expression in the hippocampus indicate diminished neuronal activity in the neddylation-compromised mouse hippocampus. These results also further confirm the specific functions of neddylation in spine morphology and synapse maintenance. The resulting mice were also impaired in a battery of behavioral tasks that are associated with hippocampus-dependent working memory and memory retrieval. However, neddylation deficiency did not cause defects in emotional or social behavioral performance.

Together, these findings identify new roles for neddylation at excitatory synapses and in synaptic maintenance. By exploring potential roles for neddylation at the synapse, Vogl *et al.*[1] have serendipitously opened the door for future investigations of a process that has only now been found to be crucial for synapse maturation and elimination in the brain. Their studies thoroughly and convincingly demonstrate how this modification can affect the intensely scrutinized postsynaptic scaffolding protein PSD-95 and lay the groundwork for many more studies to come. Several important questions remain. What other targets may be neddylated at synapses? What signals trigger neddylation of substrate proteins at synapses during synaptic maturation? How does neddylation of PSD-95 regulate its functions at the PSD? In addition, neddylation dysfunction has been implicated in Alzheimer's disease[12], a disease associated with synaptic failure and memory impairment[13]. Thus, whether deregulation of neddylated substrates occurs at excitatory synapses and whether neddylation-dependent excitatory synaptic dysfunctions are involved in the pathophysiology of the disease also remain to be uncovered.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

1. Vogl, A.M. *et al. Nat. Neurosci.* **18**, 239–251 (2015).
2. Rabut, G. & Peter, M. *EMBO Rep.* **9**, 969–976 (2008).
3. Herrmann, J., Lerman, L.O. & Lerman, A. *Circ. Res.* **100**, 1276–1291 (2007).
4. Kumar, S., Tomooka, Y. & Noda, M. *Biochem. Biophys. Res. Commun.* **185**, 1155–1161 (1992).
5. Yuste, R. & Bonhoeffer, T. *Nat. Rev. Neurosci.* **5**, 24–34 (2004).
6. Alvarez, V.A. & Sabatini, B.L. *Annu. Rev. Neurosci.* **30**, 79–97 (2007).
7. Colledge, M. *et al. Neuron* **40**, 595–607 (2003).
8. Han, K. & Kim, E. *Prog. Neurobiol.* **84**, 263–283 (2008).
9. Chen, X. *et al. J. Neurosci.* **31**, 6329–6338 (2011).
10. Sturgill, J.F., Steiner, P., Czervionke, B.L. & Sabatini, B.L. *J. Neurosci.* **29**, 12845–12854 (2009).
11. El-Husseini, A.E., Schnell, E., Chetkovich, D.M., Nicoll, R.A. & Bredt, D.S. *Science* **290**, 1364–1368 (2000).
12. Chen, Y., Neve, R.L. & Liu, H. *J. Cell. Mol. Med.* **16**, 2583–2591 (2012).
13. Mucke, L. & Selkoe, D.J. *Cold Spring Harb. Perspect. Med.* **2**, a006338 (2012).

Mobile binding sites regulate glutamate clearance

Robert H Edwards

Glutamate transporters influence the kinetics of synaptic transmission by acutely buffering synaptically released glutamate. In addition to high synaptic density of EAAT2, the transporter's high mobility contributes to function.

Localization regulates the function of many proteins, particularly membrane proteins. In the nervous system, generation of the action potential depends on appropriately localized ion channels. Neurotransmitter release involves tight physical coupling between voltage-gated calcium channels and synaptic vesicles. As a result, the translocation of proteins from one site to another can have dramatic physiological effects. Indeed, glutamate receptor movement into the synapse underlies long-term potentiation (LTP). However, there are few examples where mobility alone, independent of location, is crucial. In this issue of *Nature Neuroscience*, Murphy-Royal *et al.*[1] provide compelling evidence that the time course of excitatory neurotransmission is regulated specifically by the mobility of the principal glial glutamate transporter.

The clearance of classical neurotransmitters generally involves removal by transport. In the case of monoamines, transporters have a profound effect on the activation of receptors since the transmitter is often released from a distant site. In contrast, GABA and glutamate mediate neurotransmission at specific, closely apposed synaptic sites, suggesting that transporters for these transmitters might have a different function. Work over the last two decades has identified and characterized the plasma membrane transporters for

Robert H. Edwards is in the Departments of Physiology and Neurology, University of California, San Francisco, San Francisco, California, USA.
e-mail: robert.edwards@ucsf.edu