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Memory, Synapse Stability, and β-Adducin

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In this issue of *Neuron*, two studies by Pielage et al. and Bednarek and Caroni suggest that the cytoskeleton regulator β-Adducin provides an activity-dependent switch controlling synapse disassembly and assembly at the *Drosophila* neuromuscular junction (NMJ) and the mouse hippocampus. In mice, the β-Adducin switch is required for the improvement of learning and memory induced by enriched environments.

Selective formation of neuronal circuits is central to normal brain function. During development, most neuronal circuits initially develop an excess of synaptic connections that are then refined by activity-dependent rearrangements, including the elimination of unwarranted synapses. In the adult brain, ongoing structural plasticity is thought to underlie aspects of long-term memory formation, adjustment of functional circuits to novel experience, and recovery from brain injuries and disease. In comparison to plastic changes altering the strength of a synapse, structural plasticity provides a greater variability of synaptic connections and thus a large number of potential new circuits that may substantially increase memory storage capacity (Holtmaat and Svoboda, 2009).

Exposure to an enriched environment (EE), where animals experience ample sensory, motor, and social stimuli, significantly improves learning and memory. Even more remarkably, EE can overcome learning deficits in genetically challenged mice and enhances recovery after lesions in adult animals. Increased neurogenesis and chromatin remodeling may at least in part underlie the beneficial effects of EE on memory (Baroncelli et al., 2010; Deng et al., 2010; Fischer et al., 2007; Nithianantharajah and Hannan, 2006).

Under standard housing conditions (that is, in mice housed with same-sex littermates in standard laboratory cages), bouton densities and presumably synapse densities remain stable even though a subpopulation of boutons disappear and reappear (Holtmaat and Svoboda, 2009). The size of this unstable population varies from neuron to neuron. For example, boutons on thalamocortical axons in mouse somatosensory cortex are remarkably stable, with a large fraction persisting for 9 months or more. En passant boutons on intracortical layer 2/3 and layer 5 pyramidal cell axons exhibit a monthly turnover of 20% while small terminal boutons from layer 6 pyramidal cells exhibit a 50% turnover (De Paola et al., 2006). Since EE reversibly increases the density of excitatory synapses and causes circuit alterations that are reminiscent of enhanced structural plasticity in juveniles, an increase in the population of unstable synapses could contribute to the memory improvements induced by EE. However, whether this is the case and how the balance between stable and unstable synapses is controlled on the molecular level remains poorly understood.

Regulation of Adducins provides a switch between dynamically growing actin filaments and the stable spectrin cytoskeleton. Adducins bind (cap) the fast-growing barbed ends of actin filaments and link them to the spectrin cytoskeleton. The actin-binding activity has been mapped to the MARCKS-related domain at the C terminus of Adducins and can be controlled by PKC, PKA, and calcium-calmodulin binding (Baines, 2010). Adducins are highly expressed in the vertebrate nervous system and found at growth cones, axon terminals, and dendritic spines. Knockout of mouse β-Adducin impairs the long-term maintenance of LTP and hippocampal learning (Porro et al., 2010; Rabenstein et al., 2005). Accordingly, regulation of Adducin's actin-binding activity could reversibly switch synapses between a stable and unstable state.

Hts/Adducin Controls Synapse Formation and Elimination at Fly NMJs

The *Drosophila* genome encodes only a single adducin gene (termed hu-li tai shao, hts), which expresses a single MARCKS domain-containing isoform in the larval brain (Hts-M). Examining the glutamatergic NMJ of *Drosophila*, Pielage et al. (2011) (this issue of *Neuron*) found that pre- but not postsynaptic loss of Hts/Adducin destabilizes synapses and increases the rate at which synapses and synaptic boutons are turned over, but also promotes synaptic growth.

Synapse elimination was initiated by a loss of dense core projections (T bars in flies) at active zones (AZs) that was followed by elimination of the presynaptic bouton. Postsynaptic structures including glutamate receptor clusters were retained. Notably, the MARCKS domain was at least in part critical for the synapse stabilizing function of Hts/Adducin, implicating a role for its actin capping activity.

Hts/Adducin, α/β-spectrin, and presynaptic ankyrin2L mutant axon terminals all share an increased rate of synapse elimination. However, Hts/Adducin mutants also showed a striking increase in synaptic growth. Loss of presynaptic Hts/Adducin increased the number of synaptic boutons of large-caliber type Ib axons and triggered an abundant growth of actin-rich, small-caliber protrusions that retained synaptic proteins and likely contained functional synapses.

Since the newly formed protrusions at Hts/Adducin mutant NMJs are free of microtubules but rich in actin and because Hts/Adducin exhibits actin-capping activity, it is likely that it prevents

the growth of actin filaments to stabilize axon terminals. Consistently, Hts/Adducin overexpression in motor neurons prevents the arborization and growth of small-caliber motor axons (type II–III), which are considered highly plastic and can be strongly altered by neuronal activity.

Consistent with the notion that dephosphorylated Adducin caps actin and is complexed with spectrin, levels of phosphorylated Hts/Adducin are high in actin-rich, small-caliber axon terminals and low in the high-caliber ones. Surprisingly, expression of mutations that disrupt or mimic Ser703 phosphorylation in the MARCKS domain rescues Hts/Adducin loss-of-function defects to a similar degree, even though the synaptic localization of the mutant proteins are different; levels for both mutant proteins are similar in the nerve but the phosphomimicking version is much more abundant at axon terminals than the nonphosphorylated or normal version. This suggests that S703 phosphorylation mainly controls Hts/Adducin levels in axon terminals, which can strongly influence synapse stability (Figure 1A).

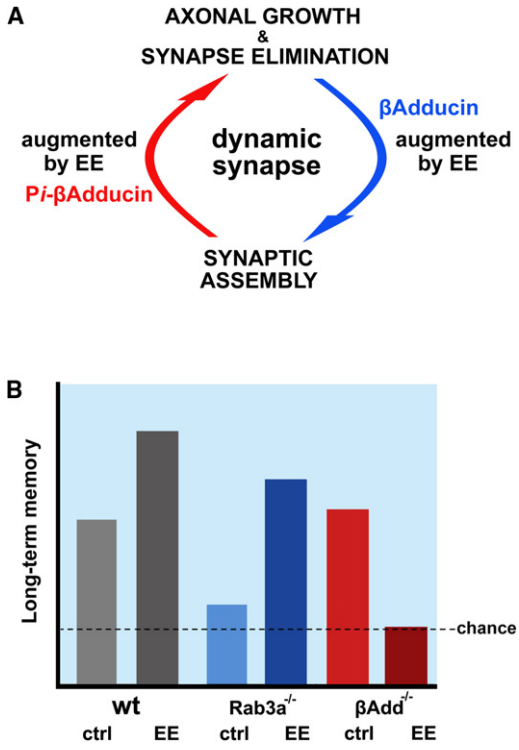

Figure 1. β-Adducin's Role for Learning-Related Synaptic Plasticity

(A) Different phosphorylation states of β-Adducin determine the stability of synapses. EE accelerates the destabilization of synapses and their de novo assembly. Loss of the β-Adducin link between spectrin and actin filaments promotes actin dynamics and protrusive growth.

(B) Learning gains upon environmental enrichment turn into losses in the absence of β-Adducin.

β-Adducin's Synapse Stabilizing Role Is Required for Memory under Environmental Enrichment Conditions

Bednarek and Caroni (2011) (this issue of *Neuron*) examined large mossy fiber terminals (LMTs) in the stratum lucidum of hippocampal CA3 and dendritic spines in the stratum radiatum of CA1. To determine whether EE alters synapse stability, they unilaterally applied the protein synthesis inhibitor anisomycin to the somata of mossy fibers in the dentate gyrus and monitored AZ densities with the AZ marker Bassoon. In mice housed under standard conditions, anisomycin application caused a transient decline of AZ densities after 12 hr that peaked after 24 hr and was fully recovered after 48 hr. Mice kept in EE for 2 weeks showed a similar AZ density before anisomycin application but exhibited an immediate decline in AZ densities after anisomycin application and a much accelerated recovery within 24 hr. Mice kept in EE for 4 weeks showed an even stronger effect as their AZ density was increased almost 2-fold compared to control. Anisomycin application caused an immediate decline in AZ densities to levels similar to control and 2 week EE mice and an accelerated full recovery within 24 hr. EE also increased the structural complexity of LMTs.

Does the accelerated AZ turnover after anisomycin treatment underlie the enhanced plasticity produced by EE? To answer this, Bednarek and Caroni (2011) examined synapse turnover in β-Adducin knockout mice, which exhibit a significantly increased rate of synapse and spine turnover at LMTs but in overall normal AZ densities. Anisomycin application to *β-Adducin*$^{-/-}$ mice raised under standard conditions showed an immediate and accelerated loss of synapses and a slow reassembly of AZs. Pharmacological inhibition of PKC prevented the otherwise observed accelerated reduction of AZ densities and even enhanced AZ reassembly in EE control mice but had no effect in *β-Adducin*$^{-/-}$ mice. Notably, *β-Adducin*$^{-/-}$ mice kept in EE showed a dramatic delay in reassembling synapses. Hence, phosphorylation of β-Adducin is critical for synapse disassembly, and nonphosphorylated β-Adducin is critical for the assembly of labile synapses (Figure 1A). Notably, EE still increased the complexity of spines in the absence of β-Adducin, even though synapse assembly was compromised at those spines.

For animals housed under standard conditions, lack of β-Adducin had no effect on learning (contextual fear conditioning and novel object recognition). However, under EE conditions lack of β-Adducin abolished the beneficial effects on learning induced by EE and reduced it to levels below standard conditions (Figure 1B). This phenotypic effect was mimicked by the pharmacological application of a PKC inhibitor. Since EE improved learning in Rab3A knockout mice, the failure of EE in *β-Adducin*$^{-/-}$ mice was not just due to an impaired LTP. Lack of β-Adducin did not interfere with the EE-induced increase in neurogenesis and short-term memory. Taken together, the study by Bednarek and Caroni (2011) suggests that β-Adducin is critical for long-term memory under EE but not standard conditions and that both synapse elimination and assembly are central to the EE-induced improvement of long-term learning.

Together, the featured studies identified a critical activity-dependent switch that underlies synapse stability and memory and likely provides a promising avenue to further dissect the powerful influence of sensory experience on learning and memory.

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Olfactory Bulb: Odor Signals Put into Context

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In this issue, Doucette and colleagues demonstrate that information related to whether an odor is currently linked to reward can be observed uniquely in population activity in the olfactory bulb, changing our understanding both of what is coded by the first olfactory relay in the CNS and of how this coding is instantiated.

The lights drop, the baton rises, and the concert begins with one lone note from the altos. The note itself is lovely and well sung, but the audience waits, unsure of what to think…until the tenors join in, and in the cooperation of the two notes everything changes and a mood is struck. A sad mood if the chord is minor, a happy mood if the chord is major. The emotional information delivered by the music, information that lies at the core of the composition's purpose, is hidden until at least two voices are heard together.

It has long been suspected that aspects of neural population coding work similarly, with information revealed in the cooperation of neurons that cannot be observed in single-neuron activity. Certainly, a host of studies have reported that the *amount* of information (roughly speaking, different magnitudes of spiking activity associated with distinct stimuli or behaviors) available in sets of synchronous spikes or in specific between-neuron patterns of spikes or spike rates often exceeds that found in the spiking patterns of each neuron considered separately (e. g., Womelsdorf and Fries, 2006; Jones et al., 2007). In this issue of *Neuron*, Doucette and colleagues (2011) demonstrate a phenomenon that is more striking and exciting: as awake mice learn that one of two proffered odors predicts the presence of reward at a lick spout, the number of synchronous spikes (SS) fired by pairs of olfactory bulbar (OB) neurons comes to reflect whether the odor is associated with reward; SS dips below spontaneous activity for unrewarded odors and hops above spontaneous for rewarded odors. This dissociation is unavailable in the firing rates of the individual OB neurons in the same trials.

The beauty of this work lies in the two basic ways in which it challenges dogma. First, the results represent unusually powerful evidence for population temporal coding. Information here is uniquely available in pairs of neurons which, while typically located in the same region of the bulb, may be separated by multiple glomeruli (the functional processing units of OB spatial coding, see e.g., Wang et al., 1998). This is an easily understood and implemented population temporal code, the decoding of which simply requires downstream coincidence detectors, connected to decision-making networks, that take input from both members of the neuron pair. Such coincidence-detecting neurons would by their very nature be preferentially sensitive and responsive to the incoming reward-related spikes.

Second, these responses reflect not odor identity per se, but rather learned reward relationships. Thus, these are important, novel data added to a growing corpus suggesting that "sensory" coding is as much about the stimulus in context as what the stimulus physically is (Kay and Laurent, 1999; Haddad et al., 2010). The fact that the authors are recording from putative OB mitral cells, the direct recipients of olfactory information from receptor neurons in the nose, serves to drive home the point that the dividing line between sensation and perception may be found outside the brain. That is, while receptor neurons may respond to purely physical aspects of sensory stimuli, even the earliest stages of neural processing intrinsically pertain to what that stimulus means to the organism under current contingencies. Clearly, neural responses to a stimulus do not need to undergo extensive hierarchical processing to reach a point at which their relationship to reward can be identified.

Note, however, that the expression of this code by OB neuron pairs does not mean that OB works alone in figuring out learned reward relationships. The authors demonstrate that adrenergic feedback to the bulb may somehow control the tendency of these neuron pairs to fire synchronously, suggesting the exciting possibility that an odor might be