sleep. One possibility is that random modifications of RA burst patterns over periods of sleep induce an error in morning singing that is subsequently adjusted during the day. The daytime adjustment brings the network into a slightly more stable state that then constrains the range of random modifications that are induced by the following period of sleep. From this perspective, the magnitude of the error is the critical feature of the sleep-induced change. Alternatively, the structure of the sleep-induced changes, *i.e.*, which bursts are changed and how they are changed, carries information. In that case, there is information in the state space explored by the song system in bursting activity during sleep.

In humans, dreams with vivid imagery are associated with rapid eye movement (REM) sleep. Neural plasticity during REM sleep is thought to help consolidate memory. There are direct data to support the REM plasticity hypothesis[9], but the larger body of evidence tends to be correlative, and the hypothesis remains controversial[10]. In any case, the different roles of sleep stages in consolidation or other aspects of procedural learning are not well established.

REM sleep has been described in birds but is reported as occurring in extremely short intervals (circa 4 s) that are relatively rare. Although numerous species have been examined, virtually none of them were 'true' (oscine) songbirds, but when oscine birds were examined, a pattern with more REM was observed[11]. Recent work in zebra finches confirms and extends this work. In preliminary results, sleep staging in zebra finches was reported to include slow wave sleep (SWS), intermediate stages and far more and longer periods of REM than observed in non-passerine birds (P.S. Low, S.S. Shank & D.M., *Soc. Neurosci. Abstr.* 769.5, 2003). There are systematic changes in the relative frequency of non-REM and REM sleep throughout the night, and there is structure to the patterns of transitions between stages. This opens the possibility for examining the roles of sleep stages in song learning. The nuclear pattern of anatomical organization of the song system also should facilitate the design of sleep-stage perturbation experiments that spare whole-animal sleep. This can address issues of stress and other non-specific effects of sleep deprivation.

The results of Derégnaucourt *et al.*[2] focus on sensorimotor learning but do not exclude the possible roles of sleep, presumably in the form of memory consolidation, on sensory learning. Auditory activity in the song system is highly state dependent[12]. A recent playback experiment with juvenile zebra finches reported that auditory responses were stronger for the tutor song during the day and were stronger for the developing bird's variable 'plastic' song at night[13]. This could be part of the mechanism that assesses error on the basis of auditory feedback during the day and modifies the network on the basis of song-like activity at night.

It is uncommon, particularly from a neuro–ethological standpoint, for physiological observations to suggest behavioral phenomena that have yet to be observed. Derégnaucourt and colleagues[2] bring a welcome behavioral focus to the analysis of how sleep affects the developmental learning of bird song. Coupled with the strong physiological insight that the field enjoys, it seems likely that a mechanistic explanation for the actions of sleep on several forms of learning is likely to emerge.

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Trafficking in emotions

Dan Ehninger, Anna Matynia & Alcino J Silva

Postsynaptic receptor trafficking is associated with long-term synaptic plasticity, but whether this mechanism actually mediates learning is unclear. A new study shows that fear learning drives AMPA receptors into synapses in the lateral amygdala.

Changes in synaptic strength are critical for learning and memory, but much remains to be understood about the molecular and cellular events that accompany learning and are required for memory. In a recent paper, Rumpel *et al.*[1] used powerful molecular tools to demonstrate that fear conditioning in rats triggers the insertion of AMPA receptors into synapses in the lateral amygdala, causing increases in synaptic strength required for emotional memory. These findings have far-reaching implications for the role of the amygdala in fear learning and, more generally, for the role of postsynaptic receptor trafficking in memory.

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The ability to remember pleasant and aversive events (emotional memory) is critical for survival, and, not surprisingly, many of the underlying mechanisms seem to be evolutionarily conserved. One of the most studied forms of emotional memory, auditory fear conditioning, depends on a subject's ability to associate a tone with an aversive stimulus, such as an electric foot shock (**Fig. 1a**). When re-exposed to the tone, trained subjects show fear responses, including 'freezing', the cessation of all but respiratory movements. Freezing is a reliable measure of fear conditioning, and the amygdala is important in this type of memory.

Learning is associated with changes in synaptic strength required for memory, as many molecular and cellular studies demonstrate[2]. Synapses modify their strength by changing the number of postsynaptic AMPA-type glutamate receptors[3,4]. For example, AMPA receptors with GluR1 subunits are driven into the synapse in response to plasticity-inducing stimuli (**Fig. 1b**), including electrical stimulation that increases synaptic strength in a hippocampal slice preparation[5] and sensory input from whiskers in rodents, which induces plasticity in the developing somatosensory cortex[6]. Before the study by Rumpel *et al.*, however, it was unclear whether AMPA receptor trafficking was associated with learning and memory[1].

The authors applied elegant experimental tools developed in previous studies[5,6] to test

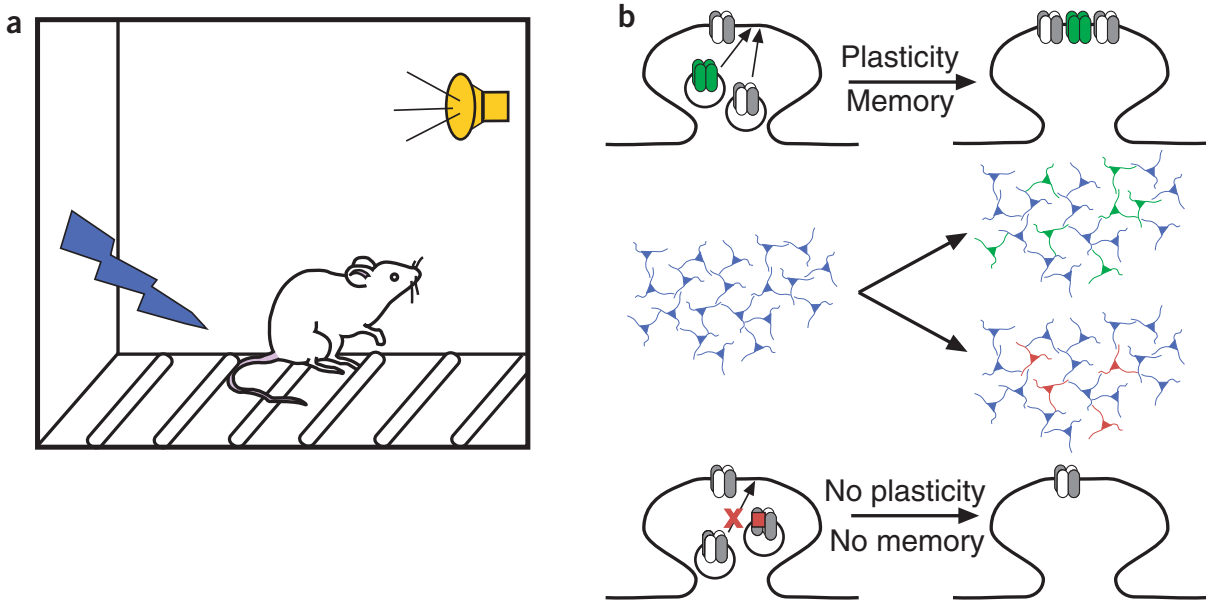

Figure 1 The role of synaptic AMPA receptor insertion in emotional memories. (**a**) Pavlovian fear conditioning. In auditory fear conditioning, a tone (neutral stimulus) is paired with a foot shock (aversive stimulus). The rat learns that the tone predicts the shock and will show appropriate, biologically relevant fear responses when later presented with the tone, including freezing (the cessation of all movement except for respiration). (**b**) Fear memory–related plasticity requires AMPA receptor insertion in a large proportion of the neuronal network. A 'plasticity tag' (green AMPA receptor) was used to show that GluR1-containing AMPA receptors are inserted at thalamo-amygdala synapses after auditory fear conditioning. A 'plasticity block' (red subunit of AMPA receptor) prevents insertion of GluR1-containing AMPA receptors after auditory fear conditioning and consequently prevents learning of the predictive association between the tone and the foot shock. Approximately 36% of infected neurons in the lateral amygdala showed learning-related plasticity, whereas blocking plasticity in only about 20% of neurons disrupted fear learning.

the hypothesis that AMPA receptor trafficking underlies memory associated with fear conditioning. One of the tools was a recombinant GluR1 subunit fused with a green fluorescent protein (GFP) gene packaged in a herpes simplex virus (HSV) vector. The GFP marker allowed the authors to identify and record from cells that had been infected with their unique construct, and the distinct biophysical properties of homomeric GluR1 AMPA receptors (greater conductance when passing inward current than when passing outward current) provided an ingenious way to determine whether these receptors had been inserted into synapses. By stimulating auditory fibers from the thalamus and recording from labeled (infected) neurons in the lateral amygdala in brain slices, Rumpel *et al.* showed that recombinant GluR1-containing receptors were clearly detectable in thalamo-amygdala synapses after auditory fear conditioning. This result is consistent with the idea that learning is associated with AMPA receptor trafficking that causes changes in synaptic strength required for memory. Importantly, recombinant GluR1 receptors were not detected in synapses of rats that had not been conditioned, confirming the behavioral specificity of the plasticity and justifying their label of the vector as a 'plasticity tag'. Clearly, this is not the last time we will hear about these plasticity tags, as there are many obvious uses for a tool that can mark cells and synapses activated by learning!

The second molecular tool used by Rumpel *et al.* consisted of a C-terminal fragment of GluR1 fused with a GFP gene in an HSV vector. This C-terminal fragment blocks synaptic plasticity by interfering with the synaptic insertion of GluR1 receptors[7]. Importantly, electrophysiological experiments showed that this 'plasticity block vector' did not affect basal AMPA receptor–mediated transmission or disrupt freezing immediately after training. Thus, the plasticity block vector did not affect general amygdala function. Strikingly, the vector did impair memory for the association between tone and shock. Unlike control rats, in which an unrelated protein was cloned in the vector, conditioned rats infected with the plasticity block vector showed a profound deficit in tone conditioning. Together with the plasticity tag results described above, these findings strongly suggest that learning is associated with the insertion of AMPA receptors into activated synapses and that this mechanism is responsible for the synaptic potentiation required for memory.

The plasticity tag experiments provide a rare glimpse into how many neurons might be involved in the formation of emotional memories. Previous methods[8,9] have been used to estimate the fraction of neurons activated or altered by different experiences or stimuli, but the distinct rectification properties of the recombinant GluR1 receptors used by Rumpel *et al.* allowed them to identify neurons in which synapses were specifically modified after fear learning. The authors showed that conditioning triggered the insertion of plasticity tags in a large proportion of lateral amygdala neurons (at least 36% of infected neurons). Because learning is thought to involve both increases and decreases in synaptic strength, and their method could not identify decreases in synaptic AMPA receptors, the number of neurons whose synapses were modified in association with a single conditioning memory may be even larger.

However, when the authors stimulated separate bundles of fibers from the auditory thalamus, they found that for most neurons, only synapses activated by one pathway showed evidence of receptor insertion. This suggested that despite the large fraction of affected neurons, plasticity was still restricted to only a subset of those neurons' synapses, perhaps to those directly involved in encoding the learned association. Furthermore, in

the plasticity block experiments, by comparing the fraction of infected neurons with conditioned fear responses, the authors estimated that blocking AMPA receptor insertion in about 20% of neurons was sufficient to prevent fear learning. Thus, synaptic modifications were widely distributed but with apparently limited redundancy.

Previous studies had already provided hints that fear conditioning led to the potentiation of a large number of synapses. Conditioning causes a large potentiation of AMPA-mediated synaptic transmission, which could have been observed only if a considerable percentage of synapses were involved[10,11]. This unexpectedly large commitment of amygdala memory space to single emotional memories may have significant implications for the understanding of emotional memory disorders such as post-traumatic stress disorder. Widely distributed representations of traumatic events may function as 'memory attractors' that inappropriately connect neutral stimuli with past traumatic experiences, potentially reinforcing those representations and the trauma associated with them.

The paper by Rumpel *et al.*[1] is representative of a growing trend in which molecular tools are used to address key questions that transcend traditional divisions in neuroscience. Indeed, a new field has emerged around studies such as this one that bridge molecular and cellular explanations of cognitive function (see http://www.molcellcog.org[2]). These studies are not only providing detailed information about molecular and cellular events involved in key aspects of cognitive function (such as insertion of AMPA receptors and potentiation of synapses as a result of learning), but they are also offering unique perspectives on important problems in systems and behavioral neuroscience. In this respect, the study by Rumpel *et al.* goes a long way toward resolving the hotly debated issue of whether the amygdala is a memory modulator, or whether this structure actually stores emotional information. These results do not refute the well-demonstrated role of the amygdala in memory modulation, nor do they eliminate the possibility that important facets of emotional experiences are stored in other sites, such as the neocortex. However, they demonstrate that synaptic changes in the lateral amygdala are triggered by learning and are required for emotional memory.

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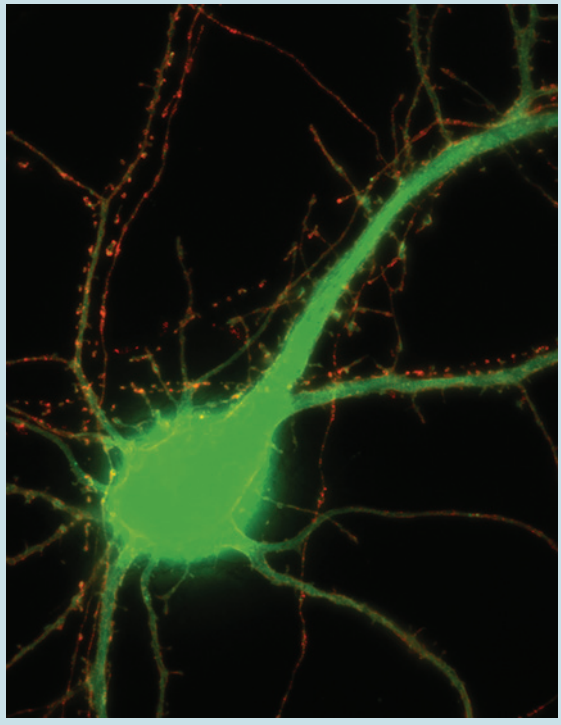
SK channels: a new twist to synaptic plasticity

The cellular mechanisms of long-term potentiation (LTP) have been widely studied because of the attractive idea that LTP may underlie learning and memory. In many parts of the brain, LTP depends on the NMDA receptor class of ionotropic glutamate receptors. Two independent groups show in this issue that NMDA receptors are negatively regulated by small-conductance, calcium-activated potassium channels (SK channels), and that this modulation can affect LTP.

NMDA receptors are normally subject to a voltage-dependent magnesium block, and so make a limited contribution to basal synaptic transmission. However, when the cell is depolarized, these receptors become active, allowing calcium to enter. Activation of NMDA receptors—and the resulting calcium influx—are required for LTP in many brain areas, including the hippocampus and lateral amygdala. The new papers show, however, that this calcium influx activates a negative feedback loop through SK channels that depresses the synaptic potential and turns off the NMDA-receptor response.



John Adelman and colleagues report on page 640 that in hippocampal pyramidal neurons (labeled with green fluorescent protein in the photo), NMDA receptors are colocalized with SK channels (labeled in red) at spines. Using two-photon laser scanning microscopy and two-photon uncaging of glutamate, the authors show that within individual spines, SK channels act to reduce the magnitude of a calcium transient evoked by NMDA receptor activation. During an excitatory postsynaptic potential (EPSP), calcium opens SK channels, which then provide a local shunting current to reduce the EPSP and promote a magnesium-dependent block of NMDA receptors. Blocking SK channels (with apamin, a component of bee venom) enhances NMDA receptor–dependent calcium signals and facilitates induction of long-term potentiation.

This intricate relationship between SK channels and NMDA receptors is not restricted to hippocampal neurons. In a related article, on page 633, Pankaj Sah and colleagues show that a similar mechanism operates in synapses in the lateral amygdala. In pyramidal neurons of the lateral amygdala, Sah and colleagues find that calcium influx via activated NMDA receptors also activates postsynaptic SK channels, and that activation of these SK channels depresses the synaptic potential. They also demonstrate that blockade of SK channels increases LTP of cortical inputs to lateral amygdala pyramidal neurons.

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