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Neuronal Competition and Selection During Memory Formation

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Competition between neurons is necessary for refining neural circuits during development and may be important for selecting the neurons that participate in encoding memories in the adult brain. To examine neuronal competition during memory formation, we conducted experiments with mice in which we manipulated the function of CREB (adenosine 3',5'-monophosphate response element–binding protein) in subsets of neurons. Changes in CREB function influenced the probability that individual lateral amygdala neurons were recruited into a fear memory trace. Our results suggest a competitive model underlying memory formation, in which eligible neurons are selected to participate in a memory trace as a function of their relative CREB activity at the time of learning.

Competition is a fundamental property of many biological systems and creates selective pressure between individual elements. For example, competition between bilateral monocular neural inputs mediates ocular dominance plasticity (1, 2). The transcription factor CREB (adenosine 3',5'-monophosphate response element–binding protein) has been implicated in the developing brain (3, 4). The finding that only a portion of eligible neurons participate in a given memory (5–8) suggests that competition between neurons may also underlie plasticity in adult brain.

Plasticity within the lateral amygdala (LA) is required for auditory conditioned-fear memories (7, 9–11). Although ~70% of LA neurons receive the necessary sensory input, only one-quarter exhibit auditory fear conditioning–induced plasticity (6, 7). We found that a similar proportion of LA cells show activated CREB (phosphorylation at Ser133) after auditory fear conditioning (Fig. 1A), which suggests a role for CREB in determining which neurons are recruited into the fear memory trace. To examine this result, we manipulated CREB function in a similar portion of LA neurons by microinjecting replication-defective herpes simplex viral vectors expressing endogenous or dominant-negative CREB (CREBWT and CREB133A, respectively) fused with green fluorescent protein (GFP) (12).

To maximize the relative difference in CREB function between neurons, we first increased CREB levels in a subset of LA neurons in mutant mice that have reduced CREB function. Mice lacking the major isoforms of CREB (α and δ; CREB-deficient mice) show deficits in developmental and adult plasticity, including auditory fear memory (13, 14) (Fig. 1B and fig. S1A). We microinjected CREBWT or control vector into the LA of CREB-deficient or wild-type littermate mice before fear conditioning and assessed memory (the percentage of time mice spent freezing during subsequent tone presentation) 24 hours later. Although CREBWT vector

Fig. 1. Auditory fear conditioning activates CREB in ~20% of LA cells in wild-type (WT) mice; increasing CREB function in a similar portion of LA neurons rescues the fear memory deficit in CREB-deficient mice. (A) Percentages of LA cells expressing phosphorylated CREB after fear conditioning (tone + shock, n = 6) was higher than after control conditions [tone alone (n = 4), immediate shock (n = 4), exposure to chamber (n = 4), or homecage control (n = 4)] (Fig. 1A, B, C, and D). Error bars in all figures represent SEM. (B) CREB-deficient (CREB−/−) mice show impaired auditory fear memory [F(1,20) = 24.23, P < 0.05; WT n = 12, CREB-deficient n = 10]. (C) Left: Outline of the LA. Right: Roughly 20% of LA neurons expressed GFP after infusion of CREBWT vector [top, nuclei stained with 4,6-diamidino-2-phenylindole (DAPI); bottom, GFP]. Scale bar, 250 μm. (D) Microinjection of control vector (Cntr; n = 8) or CREBWT vector (n = 9) did not change the high freezing in WT mice, whereas microinjection of CREBWT vector (n = 9), but not control vector (n = 8), into the LA of CREB-deficient mice rescued this memory deficit [Genotype × Vector F(1,30) = 6.64, P < 0.05].
infected only 18.0 ± 3.2% of LA neurons (Fig. 1C), the memory impairment in CREB-deficient mice was completely rescued: CREB-deficient mice infused with CREB<sup>WT</sup> vector froze at levels similar to those of wild-type mice infused with either CREB<sup>WT</sup> or control vector (Fig. 1D). Moreover, microinjecting CREB<sup>WT</sup> vector into the LA of CREB-deficient mice failed to rescue the memory impairment observed in a parallel-context fear-conditioning task (fig. S2) that also critically depends on intact hippocampal function (15). This finding indicates that infusing CREB<sup>WT</sup> vector into the LA does not simply increase freezing.

We next examined whether neurons containing CREB<sup>WT</sup> vector were disproportionately represented in the fear memory trace. To visualize the memory trace, we used the activity-dependent gene Arc (activity-regulated cytoskeleton-associated protein; also termed Arg3.1) (8). Neuronal activity induces a rapid but transient increase in Arc transcription, such that nuclear-localized Arc RNA serves as a molecular signature of a recently (5 to 15 min) active neuron (8). Five minutes after the fear memory test, we removed the brain and used fluorescence in situ hybridization to detect Arc RNA (8). Only neurons active in the preceding minutes (during the memory test) would have Arc RNA localized to the nucleus (“Arc+”).

If relative CREB function influences the probability that individual LA neurons are selected to participate in the Arc+ memory trace, then neurons with elevated CREB function (with CREB<sup>WT</sup> vector) should be more likely than neighboring neurons (without CREB<sup>WT</sup> vector) to be Arc+ after the memory test. Indeed, in wild-type mice the probability of detecting Arc+ nuclei was higher by a factor of ∼3 in neurons with CREB<sup>WT</sup> vector (64.1 ± 5.8%) than in their noninfected neighbors (19.5 ± 4.6%). In contrast, neurons containing control vector were no more likely to be Arc+ than their neighbors in either wild-type or CREB-deficient mice (Fig. 2, B and D). The preferential distribution of Arc+ nuclei in neurons with higher CREB function was also observed immediately after training (fig. S3). The bias in Arc+ distribution was even greater in CREB-deficient mice, where the probability of detecting Arc+ nuclei was higher by a factor of ∼10 in neurons with CREB<sup>WT</sup> vector (69.6 ± 2.0%) than in their neighbors (6.9 ± 1.3%) (Fig. 2C).

Because the intense training (0.75-mA shock) used above induced ceiling levels of freezing in wild-type mice, we trained additional groups with a lower-intensity shock (0.4 mA) to examine
the effects of increasing CREB function in wild-type mice. Increasing CREB function enhanced memory (Fig. 3A), consistent with results in flies (16), Aplysia (17), rats (18, 19), and hamsters (20). Furthermore, the probability of detecting Arc+ nuclei was higher by a factor of ~3 in neurons with CREBWT vector (65.8 ± 5.0%) than in neighboring neurons (21.9 ± 4.2%) (Fig. 3A and fig. S3), similar to the distribution of Arc observed in wild-type mice trained with a more intense protocol.

These imaging data could be simply explained if increasing CREB function directly induces Arc transcription. Previous findings do not support this idea (21), likely because the Arc promoter lacks a consensus CRE site (22). Nonetheless, to examine whether neurons with increased CREB function were more likely than their neighbors to be Arc+ independent of fear training, we microinjected CREBWT vector into LA of wild-type mice that were not fear-conditioned. If increasing CREB function is sufficient to induce Arc expression, then neurons with CREBWT vector should be more likely than neighboring neurons to be Arc+. However, the distribution of Arc+ nuclei was similar in neurons with and without CREBWT vector in these home-cage mice (Fig. 3B). Because CREB may not be transcriptionally active under these conditions, we infused a vector encoding a constitutively active form of CREB [CREB134F (23)]. Again, neurons with increased CREB function (with CREB134F vector) were no more likely to be Arc+ than their neighbors (Fig. 3C). Therefore, increasing CREB function in a subset of LA neurons in untrained mice does not affect the distribution of Arc, which highlights the importance of training and learning (fig. S4) in the preferential localization of Arc in neurons with increased CREB function.

Alternatively, neurons with increased CREB function may have a lower threshold for inducing Arc transcription that only becomes apparent in the fear memory test. We therefore microinjected wild-type mice with CREBWT vector 24 hours after training. Mice were tested 4 days after infusion and the distribution of Arc+ was quantified. If the fear memory trace is consolidated in the LA within 24 hours after training (24, 25), a preferential distribution of Arc in neurons with increased CREB function would not be expected. Although Arc+ levels were comparable to those found in previous experiments in which wild-type mice were fear-conditioned (25.4 ± 4.0%), Arc+ neurons were less likely to be Arc+ (14 ± 2.6%) (Fig. 3A and fig. S3), similar to the distribution of Arc observed in wild-type mice trained with a more intense protocol. Increasing CREB function in a subset of LA neurons is recruited to the memory trace. WT mice infused with CREBWT vector (2.7 ± 0.6%) than in neurons without it (33.7 ± 0.9%) (Fig. 3D).

Together, these data provide evidence for neuronal selection during memory formation. The overall size of the Arc+ fear memory trace was both consistent with electrophysiological estimates of the fear memory trace (6, 7) and stable across experiments in fear-conditioned wild-type mice (Fig. 4A). That a constant proportion of LA neurons is recruited to the memory trace, regardless of CREB manipulation, suggests that the rules governing neuronal selection during memory formation are competitive rather than cell-autonomous. If neuronal selection were cell-autonomous, the size of the Arc+ memory trace...
would vary according to CREB manipulation; CREB<sup>WT</sup> vector would induce a larger memory trace, whereas CREB<sup>S133A</sup> vector would induce a smaller one. Therefore, the finding that a fixed portion of winners emerge from a larger pool of eligible neurons suggests a competitive selection process. These studies reveal multiple aspects of this competition by advantaging (CREB<sup>WT</sup>) and disadvantaging (CREB<sup>S133A</sup>) subsets of neurons (Fig. 4B).

The precise mechanism by which CREB confers a competitive advantage to a neuron is unknown. Neurons infected with a vector expressing constitutively active CREB show facilitated long-term potentiation and an increased number of postsynaptically "silent" synapses relative to their noninfected neighbors (26). Silent synapses, containing N-methyl-D-aspartate (NMDA) receptors but not AMPA receptors, are highly plastic and may provide the necessary conditions for participation in new memory traces. Alternatively, CREB could increase neuronal excitability (27) and thus bias these neurons for selection into the memory trace. Nonetheless, our data show that in addition to being necessary for refining neural circuits during development, competition between neurons is fundamental to memory formation in the adult brain.

References and Notes
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Materials and Methods
SOM Text
Figs. S1 to S4
References
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