dures (see the figure). Given its advantages over conventional survey methodologies, eDNA is likely to quickly become the method of choice for detecting rare and elusive marine species and determining their distributions and ranges.

Estimation of the relative and absolute abundance of mobile aquatic organisms is an ongoing challenge for eDNA and conventional study methods alike. Because of their long history of use, conventional techniques currently have clear advantages over eDNA for assessing abundance. However, given its rapidly increasing use and the expected concomitant advances in knowledge, the utility of eDNA may catch up quickly and may ultimately overtake that of conventional techniques because of its greater sensitivity and nondestructive character. In the interim, finding ways to incorporate eDNA information into existing comprehensive analysis frameworks is likely to improve abundance estimates.

The greatest limitation of eDNA methodologies is that they can only inform us about attributes of the living world that are discoverable from genetic material. Many species, community, and ecosystem traits and interactions are not currently discernible from analysis of eDNA, including life stage, size, age, growth rates, physiological processes, behaviors, trophic dynamics, and the relationships between organisms and their environment. Understanding these complex aspects of our living world is vital, and eDNA can thus only complement, rather than replace, other research approaches. ■

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SUPPLEMENTARY MATERIALS

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NEUROSCIENCE

Crystallizing a memory

Researchers identify and modulate synaptic correlates of a memory engram

By **Steve Ramirez**

What is the physical basis of memory? What does it take to retrieve a memory in the brain? What would it take to activate or erase memories? In the early 20th century, the German zoologist Richard Semon coined the term "engram" to denote the physical manifestation of a memory in the brain (*1*). Two decades later, Canadian psychologist Donald Hebb posited a physiological correlate for learning and recollection: The process of learning strengthens the connections, or synapses, between neurons, which leads to the development of brain-wide cell assemblies that undergo changes in their structural and functional connectivity (*2*). The coordinated activity of these assemblies—called ensembles, traces, or engrams—that occurs during learning (memory formation) is thought to be reengaged during recall and thereby forms a stable neuronal correlate of memory (*2*). As subsequent memories are formed, the dynamics of these assemblies evolve and provide preexisting scaffolds to influence how the brain processes the variety of memories an organism forms. Studies by Abdou *et al.* (*3*) on page 1227 of this issue and by Choi *et al.* (*4*) develop new technologies to visualize discrete engrams in the brain and modulate them in a synapse-specific manner to understand memory strength and memory restoration from an amnestic state. This improved understanding could eventually be translated to modulate memories to alleviate maladaptive memory states.

Hebb's conceptualization of memory in the brain became an oft-quoted creed in brain science: Neurons that fire together wire together. In the spirit of Semon, cells that are active during learning, that undergo enduring learning-induced changes, and that facilitate recollection are referred to as engram cells. A physical manifestation of Hebb's principle, of engram cells communicating and linking with one another during learning, has been recently demonstrated in mice (*2*): A discrete ensemble of hippocampal cells that were simultaneously active during learning preferentially strengthened their structural and functional connectivity relative to quiescent cells. It was a remarkable demonstration of hippocampal engram cells firing together at the time of learning and physically interlinking together to facilitate memory retrieval.

Choi *et al.* developed an activity-dependent strategy to tag and visualize not just active engram cells but also active synapses between engram cells and non-engram cells. They used a clever trick: They engineered a system in which presynaptic and postsynaptic membranes of a neuron had complementary green fluorescent protein (GFP) fragments that reconstituted a functional GFP on synapse formation. Excitingly, by using fluorescent proteins of different colors, the researchers were able to visualize two different presynaptic neurons that projected to a single postsynaptic cell. This allowed the authors to measure how learning modulates connectivity between engram cells, engram to non-engram cells, between non-engram cells, and non-engram to engram cells. Their results are striking: Learning induced preferential increases in synaptic connectivity specifically between engram cells and not between non-engram cells. They also found that, although weak and strong fear memories activated a similar proportion of cells in the hippocampus, a stronger fear memory elicited stronger connectivity (that is, a higher density of synapses and potentiation) specifically between engram cells.

> ***"...modulating memories... reveal both how memory naturally works and, when artificially controlled, how memory can work."***

A cell ensemble can process multiple memories, but how the same population of cells can encode separate memories has remained unclear. Abdou *et al.* combined cutting-edge techniques to visualize and directly modulate discrete memories in a synapse-specific manner. They used an

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auditory fear conditioning task in which a tone is paired with a foot shock such that mice subsequently display fear responses to hearing the tone without foot shock. Of the myriad of neural circuits involved in a memory, the auditory cortex (AC), the medial geniculate nucleus (MGN), and the lateral amygdala (LA) are key nodes involved in processing an auditory fear memory.

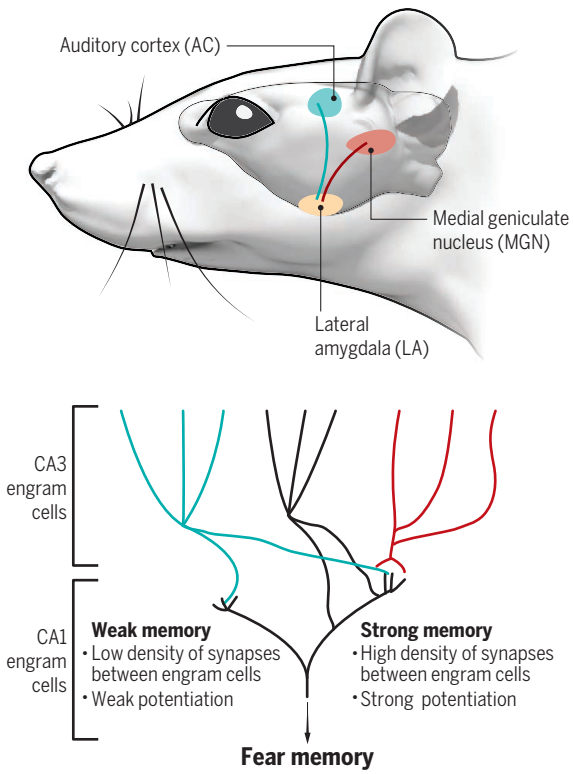
Abdou *et al.* leveraged recent findings supporting the idea that blocking protein synthesis at the time of memory formation induces partial amnesia by impairing memory retrieval (*5*). They tagged engram cells in the AC and MGN and found that stimulating their axonal terminals in the LA still elicited fear responses, even under partial amnestic conditions. In an attempt to induce complete amnesia, the authors both blocked protein synthesis and induced autophagy—a degradation process in which the cytosolic constituents of a cell are recycled. Under these conditions, stimulation of AC or MGN terminals in the LA failed to induce fear. Within these parameters, the memory had been erased—a compelling finding given that, previously (*2*), memories had been fully erased by ablating LA cells rather than by inhibiting cellular processes involved in memory formation.

The authors permanently rescued memories specifically in the partial amnesia cases by repeatedly strengthening, or potentiating, the connections between AC or MGN terminals in the LA. However, a memory could not be recovered after inducing complete amnesia. These fascinating results indicate that memories can be artificially restored, though only under specific cases of partial amnesia when retrieval rather than learning is defective.

In a technical tour de force, Abdou *et al.* then modulated two memories that recruited the same assembly of neurons. They observed that two temporally overlapping fear memories elicited activity in overlapping sets of LA cells, consistent with previous data (*6*). Importantly, they demonstrated that the acquisition of two fear memories engaged two different sets of synapses from the AC and MGN, which interacted with the overlapping neural ensembles in the LA—as such, each memory could be individually, and lastingly, restored or suppressed by modulating the activity levels of each of their respective synapses in the LA.

The microstructure of recollection

The auditory cortex, medial geniculate nucleus, and lateral amygdala are regions in the mouse brain that process auditory fear memory. Strong or weak fear memories depend on the synaptic connectivity between neuron ensembles (engrams) in these regions.



These data provide a tantalizing demonstration of linking and controlling an individual memory amid the ocean of experience that a mouse remembers. They provide a glimpse into the microstructure of recollection. Embedded within and across subregional activity are discrete circuits with unique histories that sculpt the morphological and physiological properties of a neuron and, by extension, of a memory. Engrams are not localized to a single *X-Y-Z* coordinate in the brain, but rather appear to be distributed with key nodes in the brain being necessary, sufficient, or both, to regulate individual components of memory (see the figure). Semon's engram recruits Hebb's assemblies in a brain-wide manner.

Modern neuroscience has isolated cells active during defined periods of learning and recollection to observe their behavior and to test for their necessity and sufficiency for a given behavioral readout of memory. The advances in modulating memories in mice have been extraordinary: Researchers have been able to visualize stable neural correlates of memory (*7*–*9*), to allocate and erase a specific memory (*2*), to reactivate a memory (*10*, *11*), to temporarily inhibit a memory (*12*, *13*), to connect and create artificial memories and spatial maps (*14*, *15*), and to bring back memories once thought to be lost to amnesia (*3*–*5*). They provide conceptual scaffolds for subsequent research aimed at modulating memories to reveal both how memory naturally works and, when artificially controlled, how memory can work. Moreover, researchers can now simultaneously attempt to modulate memories in a therapeutic context to alleviate clusters of symptoms underlying maladaptive states. Although the same techniques cannot yet be used in humans, it is important to translate these concepts from rodent to human memory and back. Once this is achieved, the goals are abundant: for example, to prevent the return of fear memories in posttraumatic stress disorder; to reawaken a memory in Alzheimer's disease; and to manipulate positive memories to boost mood.

The field is burgeoning with questions: In any given circuit, what are the real-time physiological kinetics of engram cells? How do these cells behave as subsequent learning experiences occur? How does the passage of time influence the physical properties of engram cells? How would mimicking the endogenous, learning-induced firing patterns of the brain affect the behavioral expression of a memory compared to artificial parameters that yield a behavioral response? How do different neural circuits differentially process the mnemonic content associated with an experience? Choi *et al.* and Abdou *et al.* have given us exciting new tools and concepts that, together, bring us closer to unweaving the magnificent neural knot we call memory. ■

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