

CLINICAL IMPLICATIONS OF BASIC RESEARCH

Elizabeth G. Phimister, Ph.D., *Editor***On Complement, Memory, and Microglia**

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We all forget things, and sometimes it would be better if we did not. Normal forgetting occurs more frequently as we age, but more serious, progressive, retrograde forgetting, which can have severe, life-threatening consequences, occurs in patients with Alzheimer's disease or other dementias. Most of us assume that memory formation is an active process and that forgetting reflects a combination of defects in initial memory coding, localization of memories, or memory retrieval. Memory loss is believed to be pathologic, as in diseases of dementia. However, studies in healthy animals indicate that the formation and loss of memories are both active processes that involve the strengthening and weakening of synapses through actions directed by the neurons themselves. Activity-dependent strengthening of synapses is known to underlie memory formation. A recent study by Wang et al.¹ showed that active depletion of weak synapses, resulting in memory loss, takes place in the adult mouse brain.

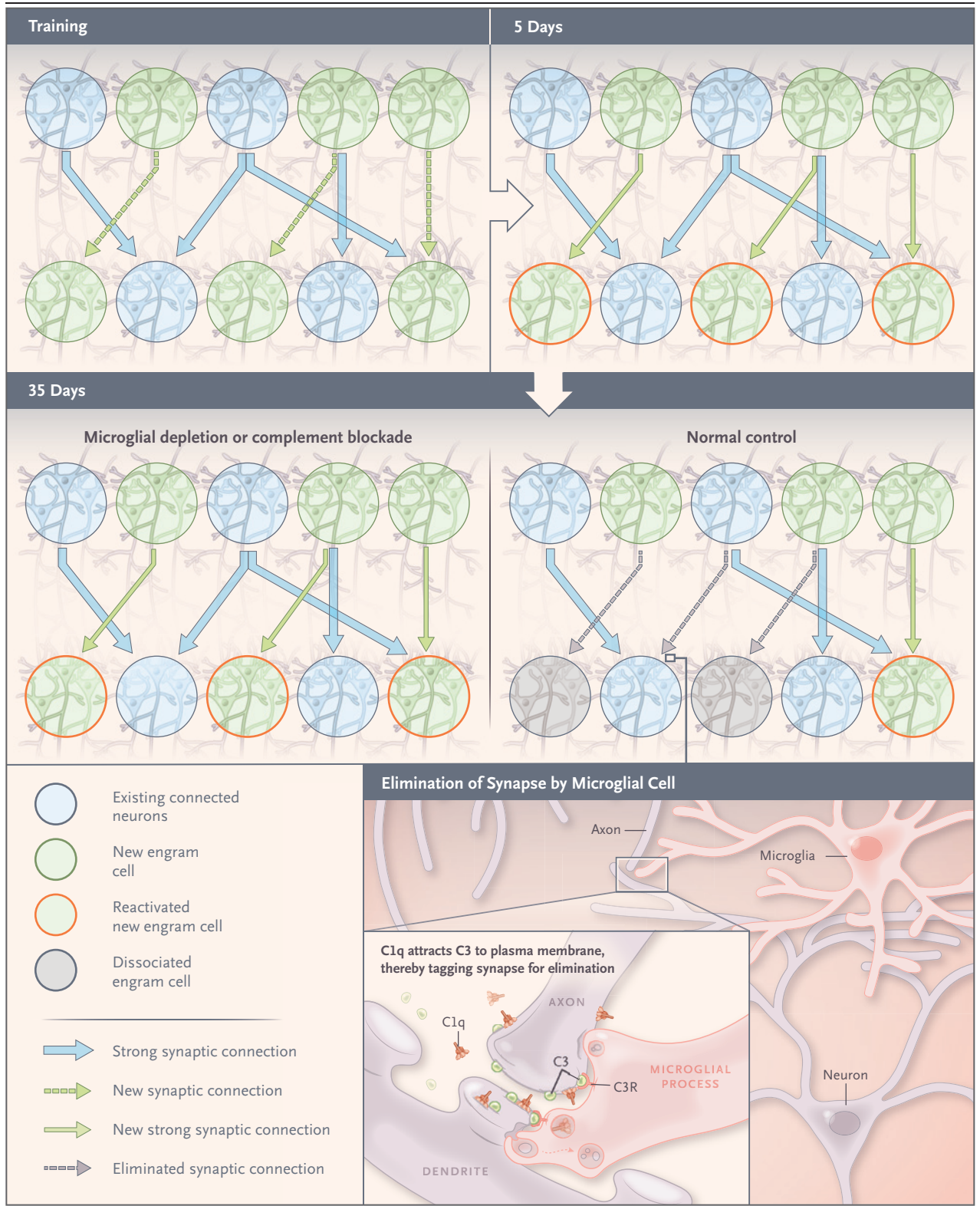
In the developing brain, weak synapses are pruned away by microglia (resident phagocytic myeloid cells) through a process involving the neuronal expression of classic complement proteins such as C1q and C3, which tag weak synapses for elimination by complement receptor-

expressing microglia.² Synaptic pruning refines neuronal networks such that the signal-to-noise ratio is enhanced. In the hippocampus, where memory traces are formed, the same process of synapse elimination has been linked to diseases of memory in adults,^{3,4} which suggests that the aberrant activation of this developmental tool in adults may be pathologic. The study by Wang et al. suggests that the exact same process also mediates normal forgetting in adult animals.¹

To determine whether microglia are required for normal forgetting, Wang et al. evaluated fear-induced freezing behavior in contextual fear-conditioned mice that had undergone ablation of microglia with the use of genetic or pharmacologic approaches. Contextual fear conditioning is a standard method used in the evaluation of short-term and long-term memory in animals; in the study by Wang et al., the evaluation involved measuring the extent of freezing behavior at various time points after the animals received mild foot shocks in a training apparatus. The investigators found that normal mice reintroduced into the apparatus after a comparatively long interval (35 days) after training froze for a shorter duration than did mice that were reintroduced into the apparatus after 5 days. This difference reflects a normal amount of forgetting

Figure 1 (facing page). Microglial, Complement, and Cell Activation in Memory Formation, Recall, and Loss.

Microglial depletion or inhibition of complement activation affects synaptic connectivity and engram-cell reactivation during memory formation and forgetting in the hippocampus. Existing connected neurons (blue circles) are present in consolidated networks (blue arrows). During contextual fear conditioning, small numbers of new engram cells (green circles) are recruited into new networks (dashed green arrows), which become consolidated (solid green arrows). Reactivation of these new engram cells (green circles with orange borders) occurs when animals reencounter cues that triggered fear. Forgetting involves the disruption of these new synaptic connections (dashed gray arrows) with disconnection of their engram cells (grey circles), a process that can be reversed with microglial depletion or complement inhibition. C3R denotes C3 receptor.



during the longer interval. The microglia-depleted mice, however, had durations of freezing at 35 days that were similar to those in mice without microglia depletion tested at 5 days after training, a finding that shows that forgetting was prevented in the microglia-depleted mice. These data indicate that microglia are critical for the process of forgetting after contextual fear conditioning. However, given that neuronal activation underlies learning and forgetting, it was important in this field of research to determine whether microglia-mediated forgetting depends on neuronal activity.

Engram cells — initially controversial as an entity and as being important for the establishment of memory — are a population of neurons that become activated during learning and require reactivation for memory recall. In fact, it is the synaptic connections between engram cells, and not the cells themselves, that are the physical sites where memories are stored.⁵ Genetic markers of neuronal activity, including *c-fos*, are powerful tools for labeling engram cells on the basis of their history of neuronal activity. Using transgenic mice in which reactivated engram neurons are permanently labeled by induction of *c-fos* expression, Wang et al. showed that the rate of neuronal activity within the hippocampus correlated positively with freezing behavior and increased with microglial depletion. The levels of synaptic proteins within microglia were also observed to be higher at 35 days than at 5 days after contextual fear conditioning in the normal mice, which suggests that synapse elimination underlies normal forgetting. Administration of minocycline, an antibiotic agent that dampens microglial activation, reduced microglial engulfment of synapses and ameliorated forgetting. Together, the findings from these experiments support that microglial-mediated synapse elimination normally underlies the act of forgetting, at least for certain noxious stimuli.

Does complement mediate this process? It would appear to be so. Wang et al. showed that the complement protein C1q colocalized with synaptic proteins within microglia during nor-

mal forgetting. Then they injected an engineered virus that expresses CD55, an inhibitor of complement activation, into the brains of mice. These mice showed increased freezing behaviors 35 days after contextual fear conditioning and had higher levels of activation of engram cells and decreased levels of synaptic proteins within microglia. Further experimentation showed that suppressing the activity of engram cells was associated with increased forgetting, which could be abolished by microglial ablation or CD55 expression (Fig. 1).

These experiments indicate that the act of forgetting is exactly that — an active process in which weaker synapses are tagged for removal; a process that occurs throughout life to strengthen networks and parsimoniously retain memories. The finding that complement and microglia contribute to normal forgetting suggests that pathologic forgetting, as occurs in patients with dementias, or pathologic remembering, as may occur in patients with post-traumatic stress disorder, may be due to defects in the maintenance of appropriate networks. New treatments that can regulate microglial- and complement-mediated synapse elimination might someday prevent or promote memory loss, depending on the clinical need.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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1. Wang C, Yue H, Hu Z, et al. Microglia mediate forgetting via complement-dependent synaptic elimination. *Science* 2020;367:688-94.
2. Schafer DP, Lehrman EK, Kautzman AG, et al. Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. *Neuron* 2012;74:691-705.
3. Vasek MJ, Garber C, Dorsey D, et al. A complement-microglial axis drives synapse loss during virus-induced memory impairment. *Nature* 2016;534:538-43.
4. Hong S, Beja-Glasser VF, Nfonoyim BM, et al. Complement and microglia mediate early synapse loss in Alzheimer mouse models. *Science* 2016;352:712-6.
5. Yang G, Pan F, Gan WB. Stably maintained dendritic spines are associated with lifelong memories. *Nature* 2009;462:920-4.

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