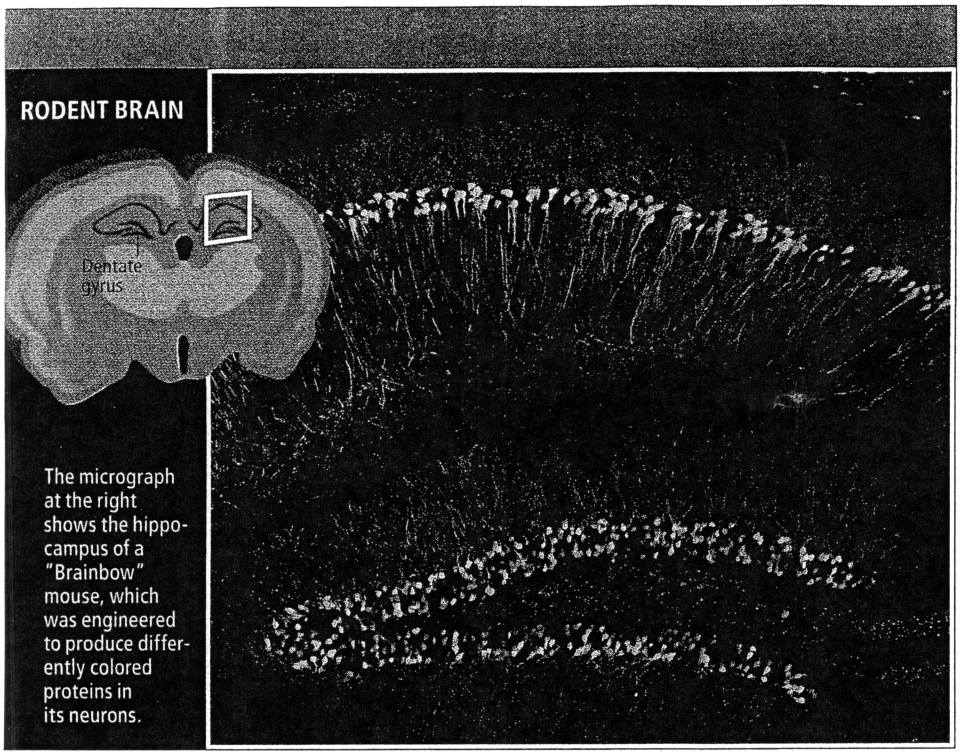


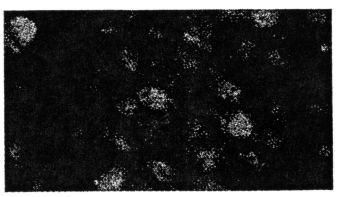
The micrograph at the right shows the hippocampus of a "Brainbow" mouse, which was engineered to produce differently colored proteins in its neurons.

and neurogenesis, all the animals were injected with BrdU at the start of the experiments. One week later half the rats were recruited into the eyeblink training program; the others lounged in their home cages. After four or five days of training, we found that the rats that had learned to time their blink properly retained more BrdU-labeled neurons in the hippocampus than did the animals that had simply remained in their cages. We concluded that learning this task rescued cells that would otherwise have died. In the animals that received no training, very few of the newborn cells that had been labeled with BrdU at the start of the experiment could be seen at the end. And the better the animal learned, the more new neurons it retained. The same thing happens in animals that have learned to navigate a maze.

When we first started doing the eyeblink studies in the late 1990s, we examined the effects of training in animals that had learned well: in other words, rats that learned to blink within, say, 50 milliseconds of the eyelid stimulation—and did so in more than 60 percent of the trials. More recently, we asked whether animals that failed to learn—or that learned poorly—also retained new neurons after training. They did not. In studies published in 2007, rats that went through some 800 trials but never learned to anticipate the eyelid stimulation had just as few new neurons as the animals that never left their cages.

VIEWING NEW NEURONS

The chemical BrdU marks cells that are born after an animal has been exposed to the substance. The image below highlights one newborn cell—the BrdU shows up as red, and the green peeking through identifies the cell as a neuron. Mature neurons surround the new one.



We also conducted eyeblink experiments in which we limited the animals' opportunity to learn. This time we gave rats only one day—200 trials—to get it right. In this situation, some animals learned to anticipate the stimulus, and others did not. Again, the rats that learned retained more of the new neurons than the rats that did not, even though all went through the same training. These data imply that it is the process of learning—and not simply the exercise of training or exposure to a different cage or a different routine—that rescues new neurons from death.

No Pain, No Gain

Although learning must occur if newborn hippocampal neurons are to survive, not all types of learning work. For example, training an animal to swim over to a platform that is visible in a pool of water does not enhance cell survival. Nor does training an animal to recognize that two stimuli, such as a tone and an eyeblink stimulus, occur almost simultaneously.

The reason these tasks fail to rescue new cells from death, we surmise, is that they do not require much thought. Swimming to a visible platform is something rats do readily. After all, they do not want to drown. And if eyelid stimulation overlaps in time with a tone, the animals do not need to form a memory trace of an event that happened in the past—the sound of the tone—to help them predict when the eyeblink stimulus will occur. They simply respond when they hear the sound.

We think that the tasks that rescue the most new neurons are the ones that are hardest to learn, requiring the most mental effort to master. To test this hypothesis, we took a task that is a bit of a no-brainer and made it a little more challenging. We started with the easy eyeblink task, in which the tone precedes but still overlaps in time with the eyelid stimulation. Learning that connection, as indicated above, does not typically rescue new neurons. Then we made this task more challenging by greatly extending the duration of the tone so that now the stimulus arrived toward the end of a very long sound.

Learning when to blink in this task is more difficult than in the easy test, because in this case blinking soon after the tone begins, like runners taking off after hearing the starting pistol, is not the correct response. The task is also more difficult than the standard, 500-millisecond trace test because the animal cannot use the

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It appears there is a critical window of time in which learning can save new neurons.

end of the tone as a signal to "get ready." Rather the rat must keep track of exactly when the tone started and estimate when the eyelid stimulation will occur—a real challenge for all animals, including humans. And we found that this challenge rescues as many, and sometimes more, new neurons than does the standard trace conditioning task.

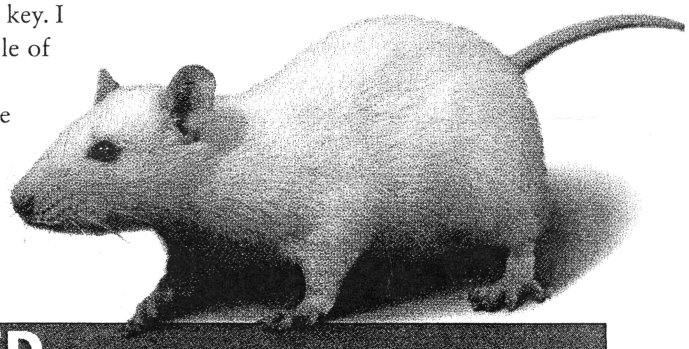
Interestingly enough, among the animals that learned in our conditioning tasks those that were a bit slow—in that they required more trials to learn how to master a task—ended up with more new neurons than animals that learned fast. Thus, it seems that new neurons in the hippocampus respond best to learning that requires a concerted effort.

Timing Counts

Why effortful learning should be critical is not clear. One theory is that tasks requiring more thought—or taking longer periods of training to learn—activate more vigorously the networks of hippocampal nerve cells that include these newborn neurons, and that such activation is key. I tend to favor this hypothesis for a couple of reasons.

First, a number of investigators have demonstrated that tasks involving learning, such as the classical eyeblink conditioning test, generally increase the excitability of neurons in the hippocampus, making them become much more active. Furthermore, this hippocampal hustle and bustle goes hand in hand with learning: the animals that show the most activation are the ones that best learn the task.

Next, it appears there is a critical window of time in which learning can save newborn neurons—in rodents, between about one week and two weeks after the cells arise. One recent study in rats reported, for instance, that learning can rescue cells when the cells are seven to 10 days old. Training that occurs after that time is too late: the neurons are already dying off. And training before that time is too early to help. This learning window corresponds to the period when these newborn cells, which start life unspecialized, begin to differentiate into neurons—sprouting signal-detecting dendrites (which receive impulses from other parts of the brain) and axons (which carry messages to a neighboring region of the hippocampus called CA3). Around this time they also begin to re-

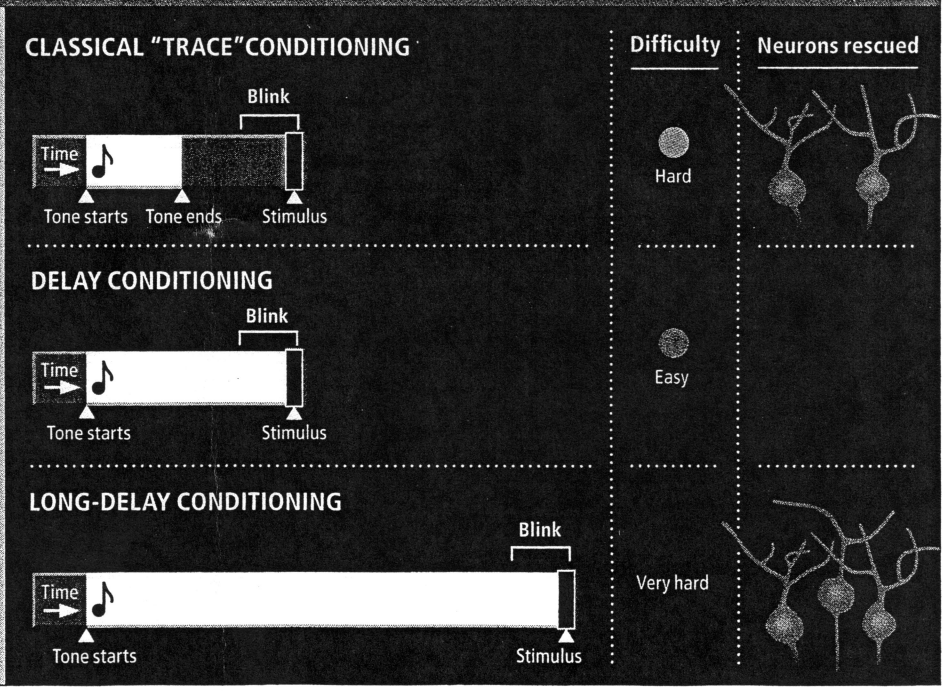


[LEARNING TESTS]

WHAT RAT STUDIES REVEALED

The author and her colleagues relied on "eyeblink conditioning" experiments to discover that working hard to learn something enhances the survival of new neurons. They began with a classical form of the experiment (*top*), in which an animal hears a tone that is followed half a second later by a stimulus that will make it blink. After several hundred trials, most animals learn to blink just before the stimulus arrives. Because the tone and the blink-inducing stimulus are separated in time, figuring out when to blink is difficult; this task rescues a large fraction of newborn neurons.

Rats master readily an easier version of the test—in which the blink stimulus overlaps with the tone (*middle*); this task does not enhance survival of new neurons. Making conditions more challenging—by having the rat wait much longer before the stimulus arrives (*bottom*)—rescues more neurons than even the classical approach does.



NIGEL CATTLIN *Photo Researchers, Inc. (rat)*; JEN CHRISTIANSEN *(illustrations)*