

NEUROSCIENCE



Neurons



Anxiety

The adult brain generates neurons every day. These cells help us to distinguish one memory from another—a finding that could lead to novel treatments for anxiety disorders

By Mazen A. Kheirbek and René Hen

IN BRIEF

To keep memories from becoming jumbled, the brain must encode the distinct features of events and situations in a way that allows them to be distinguished from one another—a process called pattern separation. **Pattern separation** enables us to distinguish danger-

ous situations from similar ones that pose no risk. People with defects in this ability may be prone to anxiety disorders.

The process occurs in one of the two regions of the brain that generate neurons throughout life. These

fledgling cells seem to be critical to pattern separation. **Interventions** that specifically boost the ranks of rookie neurons could provide new ways to regulate mood and possibly treat conditions such as post-traumatic stress disorder.



Mazen A. Kheirbek is assistant professor of clinical neurobiology in psychiatry at Columbia University and a research scientist at the New York State Psychiatric Institute.



René Hen is professor of psychiatry, neuroscience and pharmacology at Columbia and director of the division of integrative neuroscience in the department of psychiatry at the New York State Psychiatric Institute. The authors have worked together to explore the role that the dentate gyrus—and its newborn neurons—plays in memory and mood since Kheirbek joined Hen's laboratory as a postdoctoral student in 2009.



FOR CENTURIES THE NOTION THAT THE ADULT BRAIN COULD NOT MAKE NEW NEURONS stood as a central tenet of neurobiology. Even Santiago Ramón y Cajal—the Barcelona-based histologist who essentially invented modern neuroscience at the end of the 19th century—declared such neural renewal impossible. After decades of careful observation and painstaking illustration of the microscopic architecture of nerve cells and their connections, Ramón y Cajal concluded that in the adult brain, “the nerve paths are something fixed, ended, and immutable; everything may die, nothing may be regenerated.”

So, when Joseph Altman, then at the Massachusetts Institute of Technology, published a series of papers in the 1960s showing that new neurons cropped up in the brains of adult guinea pigs, he was largely ignored. This disregard was perhaps not surprising, because from a logical standpoint, adding new neurons into a fully developed brain would be a recipe for disaster. After all, if the brain stores information in specific webs of neural connections, it would seem that randomly inserting inexperienced cells into these preexisting networks could cripple our ability to properly encode and recover information and thus garble our memories.

But logic is no match for experimental results, and in the 1990s the data began to roll in. Looking carefully at the brains of adult rodents, monkeys and even humans, investigators turned up evidence that new neurons continue to appear throughout life in two brain regions—one involved in smell and the other, the hippocampus, involved in learning, memory and emotion.

Since then, researchers have wondered what, exactly, these newborn neurons do. Although the role that neophyte neurons play in the olfactory system is still somewhat obscure, the hippocampus has begun to serve up its secrets. Work by our research group and others suggests that fledgling cells may be involved in helping to record memories in a way that distinguishes them as unique, preventing them from blurring, one into the next. This realization could lead to novel approaches to treating a variety of anxiety disorders, including post-traumatic stress disorder (PTSD), because people who suffer from such conditions have trouble telling the difference between situations that merit fear and those that are innocuous.

TRICKS OF MEMORY

AT ITS HEART, memory involves recalling as well as recording. Most often it is the former process—by which a vivid, detailed

memory can be summoned by a single sight, smell or taste—that inspires wonder. The flavor of a cake dunked in a cup of tea instantly transported the narrator of Marcel Proust's *Remembrance of Things Past (À la recherche du temps perdu)* back to the Sunday mornings of his childhood:

Once I had recognized the taste of the crumb of madeleine soaked in the decoction of lime-blossom which my aunt used to give me ... immediately the old gray house upon the street, where her room was, rose up like a stage set to attach itself to the little pavilion opening on to the garden...; in that moment ... the whole of Combray and of its surroundings ... sprang into being, town and gardens alike, all from my cup of tea.

The ability of sensory cues to invoke the recollection of a previous experience—a process called pattern completion—is one of the most important functions of the brain's hippocampus. Yet before a memory can be retrieved, it must be laid down properly. Recording the details of an event in a way that allows us to distinguish one from another—pattern separation—is the other basic job of the hippocampus. Thanks to this ability, which appears to be linked to the production of new neurons, we can (in most cases) remember where we parked the car this morning, as opposed to where we left it yesterday or last week.

Such discrimination is essential not only for keeping memories organized but also for guiding our behavior—for example, allowing us to head toward where we last remember seeing the car. Unlike pattern completion, which seems to occur primarily in a region of the hippocampus called CA3, pattern separation takes place in a wedge of cells called the dentate gyrus.

The two of us decided to explore the role that new neurons play in distinguishing memories in part because these rookie

cells are known to arise in that exact wedge. Inside this part of the hippocampus, neural stem cells—the parental cells that churn out new neurons—are packed into a thin layer of cells called the subgranular zone. Newborn cells then migrate out of this neural nursery into the rest of the dentate gyrus, where they become integrated into existing neural circuits. In mice, newborn cells can account for up to 10 percent of the neurons in the dentate gyrus. And a recent study using a form of carbon dating to estimate cells’ “birth dates” showed that humans continue to produce fresh neurons in the hippocampus at a steady rate well into old age, adding about 1,400 every day.

SEPARATION ANXIETY

TO TEST WHETHER NEW NEURONS participate in pattern separation, in 2009 we began to study the question in mice. First, we either eliminated young, immature neurons by shutting neurogenesis down or boosted their numbers by promoting the cells’ survival. Then we asked whether these manipulations affected the ability of the test animals to differentiate among similar situations.

Like many behavioral investigators, we made use of a type of conditioning developed by Russian physiologist Ivan Pavlov in the early 1900s. Pavlov found that if he rang a bell as he fed his dogs, the animals would come to associate the sound with the food—and begin to salivate on hearing the ding. Over the past 100 years this simple form of learning has been widely exploited to test the neural basis of memory.

In our experiments, instead of ringing a dinner bell to herald the appearance of food, we trained mice to anticipate receiving a mild foot shock when they were removed from their home cage and placed in an unfamiliar box. After a few exposures, an animal learns to associate that new environment with the shock, so that each time it is placed in this enclosure, it will freeze in fear.

Next, to test the ability of the mice to engage in pattern separation, we placed them in a box that was very similar to the first one—but not exactly the same. If the “shock box” were square with silver walls, blue lighting and a distinct smell of anise, the lookalike box might be the same shape and color but carry a scent of banana or lemon. At first the animals are afraid. Yet when no shock is forthcoming, they soon learn to tell the two situations apart—standing immobile in the shock box but relaxing when they visit the version that is a little different.

If the production of new neurons were critical to pattern separation, we reasoned, eliminating neurogenesis in an animal’s dentate gyrus would make it difficult to distinguish the two situations. And that is what we saw. Animals lacking new neurons remain overly skittish, reacting with alarm in both environments, even after repeated trips to the harmless box proceed without incident. Without the ability to perform pattern

separation, the animals generalize their fear of the original location—allowing their anxiety to spread to any place that resembles the site of the unpleasant experience.

Conversely, we can experimentally boost the number of new neurons in the mouse dentate gyrus by eliminating a gene that would otherwise encourage any unneeded young cells to die. The resulting mice, which have a beefier dentate gyrus, are better able to distinguish between the shock box and its lookalike, becoming comfortable more quickly in the enclosure that has proved safe. These observations confirm that newborn neurons play a part in encoding and distinguishing among memories that are related but distinct.

Other laboratories have obtained similar results. Investigators led by Fred H. Gage of the Salk Institute for Biological Studies, whose work helped ignite the explosion of research on neurogenesis in the 1990s, and by Timothy Bussey of the University of Cambridge have shown that eliminating new neurons in the brains of adult mice impairs their ability to discriminate among closely spaced objects—as assessed by their ability to choose the

correct arm in a maze or to touch the correct image with their nose on a computerized screen. Bussey’s lab has further demonstrated that enhancing neurogenesis improves animals’ performance in the touch-screen test. Also, using a conditioning protocol similar to the one we have employed, M.I.T.’s Susumu Tonegawa and his colleagues have confirmed that mice lacking new neurons demonstrate an inability to discriminate between safety and danger.

WHEN LESS IS MORE

STUDIES EXAMINING the effects of interrupting or enhancing neuron generation have not been conducted in human volunteers. But if neurogenesis were important to pattern separation in people, one would expect to find that disruptions

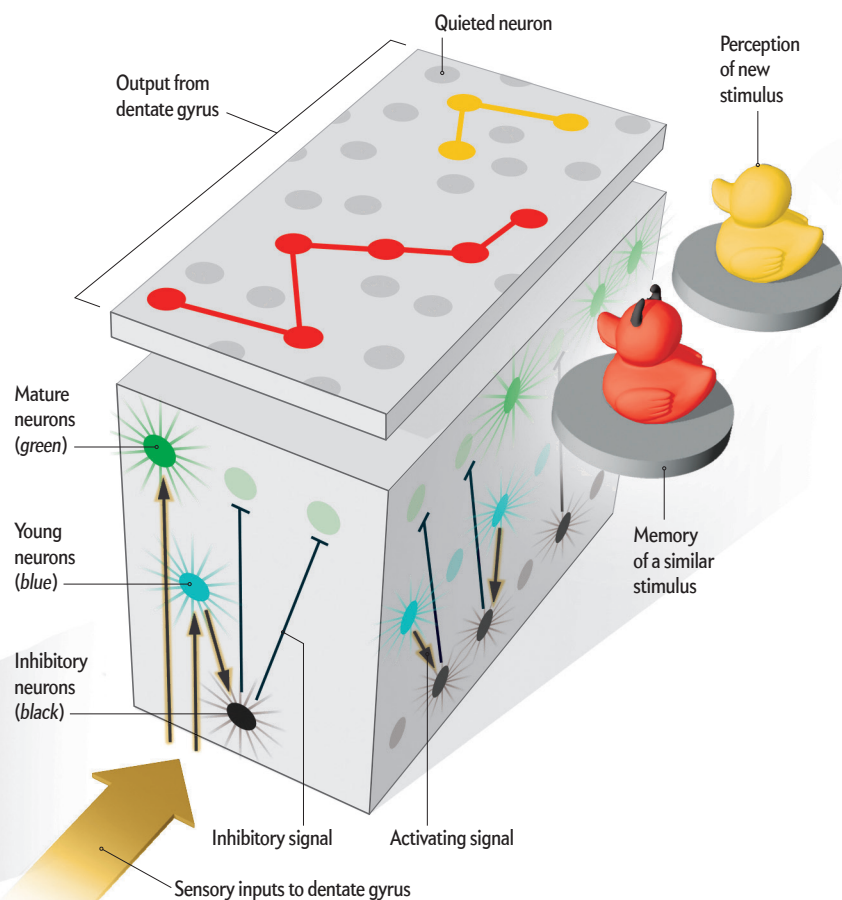
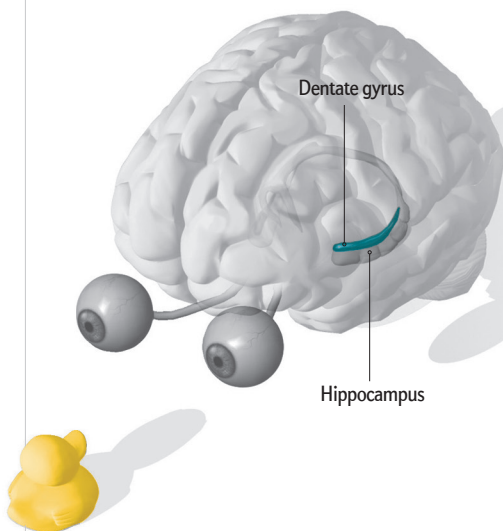
in the process would be tied to some detectable disturbance in the activity of the dentate gyrus, where new neurons are born and reside. Indeed, such a connection has been seen in human subjects. Using functional MRI to track neural activity, Michael Yassa of Johns Hopkins University and Craig Stark of the University of California, Irvine, demonstrated that individuals who show an impaired ability to differentiate among similar items display elevated activity in the dentate gyrus.

Although the finding of hyperactivity, rather than reduced function, sounds counterintuitive, it may actually make sense. If every situation evoked widespread stimulation of neurons in the dentate gyrus—activating, say, 95 neurons in a population of 100—the associated memories would blur together, and none would be distinct. Instead the dentate gyrus accentuates the differences between one event and the next by selectively activating discrete, nonoverlapping subsets of neurons. So today’s parking space sparks activity in, say, five neurons out of 100 in

Studies of the antidepressant Prozac lend support to the notion that a deficit in new neuron production can fuel anxiety disorders.

What New Neurons Do

Freshly minted neurons in the brain's dentate gyrus (*below*) participate in "pattern separation," the ability to distinguish between similar experiences. The authors have proposed a hypothesis to explain how new neurons contribute to pattern separation (*right*) and why a lack of them could cause someone to confuse a nonthreatening situation with a scary one from the past (*far right*)—as occurs in post-traumatic stress disorder.



How New Neurons Highlight Differences in Experiences

New neurons might support pattern separation by encoding novel information better than older cells do. But the authors favor a different view: after input from the outside world activates both young and mature brain cells, the young cells induce inhibitory neurons to quell much of the dentate gyrus's activity (*dimmed shading*). This effect throws into sharp relief the distinctive details of both a new experience (*yellow*) and a recollection of a similar experience (*red*) that might be more sinister.

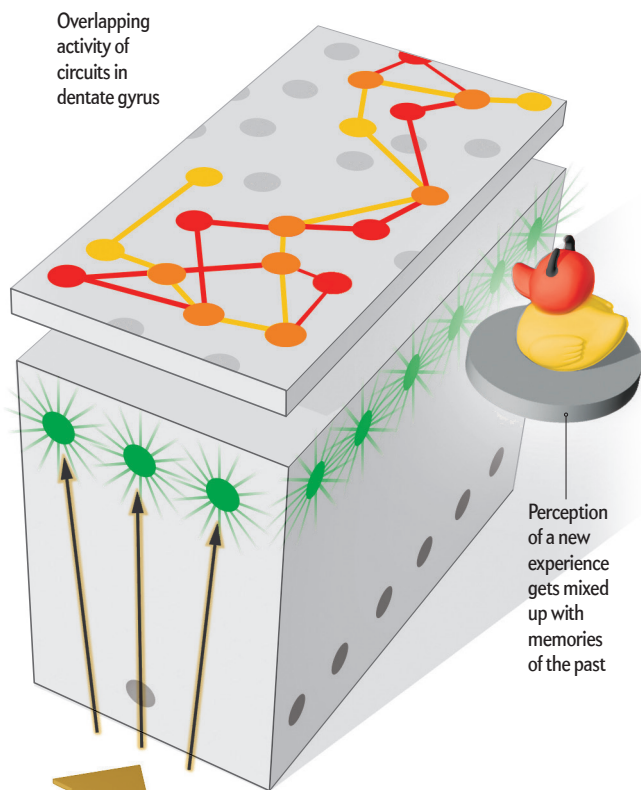
the dentate gyrus, whereas yesterday's parking location fired up a different set of five.

We have begun to speculate that new neurons may promote pattern separation by reining in the overall activity of the dentate gyrus. As newborn cells mature, they appear to interact preferentially with inhibitory neurons. When these inhibitory cells are excited, they dampen the activity of other neurons in the dentate gyrus. This connection between newborn neurons and suppression of the dentate gyrus is borne out in studies of mice in which neurogenesis has been eliminated. These mice, which lack newborn neurons, show elevated spontaneous activity in the dentate gyrus, suggesting that new neurons bear responsibility for keeping the overall neural activity in check.

If neurogenesis is, in fact, involved in pattern separation in humans, the finding could offer insights into the cause of anxiety disorders such as PTSD. Psychologists have long suspected that

an overgeneralization of memory contributes to anxiety disorders, which are marked by an exaggerated, sometimes crippling fear response, even when the environment holds no immediate threat. Such inappropriate generalization could be the result of a diminished ability to distinguish between a past trauma and an innocuous situation that shares some similarity with the traumatic event—for example, a picnic that is interrupted by an unexpected loud noise. Individuals with a normal capacity for pattern separation might flinch at the sudden boom but quickly realize that the park is not a war zone and continue with their lunch. A veteran with an impaired ability to carry out pattern separation, on the other hand, may be unable to separate the sound of a car backfiring from the memory of the battlefield—a mistake that could precipitate a full-blown panic attack.

Experiments have lent support to the proposed connection between impaired pattern separation and anxiety disorders in



Overlapping activity of circuits in dentate gyrus

Perception of a new experience gets mixed up with memories of the past

Without New Neurons, Confusion Reigns

In the authors' hypothesis, the absence of new neurons eliminates the cells' inhibitory effects in the dentate gyrus. Thus, more cells fire in response to new inputs and to the memories they evoke. As a result, the neural representations of the events may overlap excessively, thus causing the perception of the two events to merge inappropriately.

humans. Shmuel Lissek of the University of Minnesota and his colleagues have shown, for instance, that people afflicted by panic disorders have a tendency to become startled when viewing an object similar to one that has been associated with a mild shock to the wrist.

Studies of the antidepressant Prozac offer further support for the notion that a deficit in new neuron production can fuel anxiety disorders. Prozac relieves anxiety in both animals and people. Mice treated with the drug are much less nervous and more adventurous when placed in a novel environment, and this drug-induced boost in boldness, we find, is totally dependent on new neurons. Treatments that staunch the birth of new neurons abolish Prozac's antianxiety effects—work we published in *Science* in 2003.

Since then, one of us (Hen) has shown that neurogenesis is also required for Prozac to relieve depressive behaviors in adult

macaques—a study that was performed in collaboration with his colleagues at Columbia University. We are also beginning to explore the role of new neurons directly in people. By examining brains that were donated postmortem, we have so far determined that treatment with antidepressants increases the number of neural stem cells—those that produce new neurons—in the dentate gyrus of patients who have major depressive disorder. Whether neurogenesis is necessary for these drugs to effectively treat depression and anxiety in people remains to be seen.

EASING THE PAIN

GIVEN THE GROWING APPRECIATION of the role that the dentate gyrus—and its newborn neurons—plays in pattern separation and potentially in the ability of antidepressants to quell anxiety, we suspect that many people who grapple with depression, PTSD and the cognitive decline that comes with aging could benefit from interventions aimed at boosting neurogenesis in the adult brain. One method that has already proved to encourage neurogenesis in adult animals is exercise. In fact, Gage's discovery that access to a running wheel boosted the numbers of neurons in the adult mouse brain is what rekindled interest in neurogenesis in the late 1990s. Physical activity and antidepressants such as Prozac, however, probably also influence behavior and neural activity in ways unrelated to their effects on neurogenesis—for example, promoting strengthened and more numerous neuronal interconnections.

A more targeted approach to enhancing the production of new neurons might help to specifically reverse the deficits in pattern separation that we think precipitates panic in some cases of PTSD or other anxiety disorders. A recent screen for chemicals capable of boosting neurogenesis in the dentate gyrus of adult mice turned up a promising candidate, called P7C3, which promotes the survival of newborn neurons. Coupled with our own studies showing a reduction of anxiety in mice when we inhibited the death of new neurons, such work makes us hopeful that advances in pharmacological approaches to encourage neurogenesis could help those suffering from anxiety.

Although Ramón y Cajal never imagined that the adult brain could generate new neurons, he could envision the therapeutic potential of such neuronal rejuvenation. As he noted in his 1914 book *Degeneration and Regeneration of the Nervous System*, "It is for science of the future to change, if possible, this harsh decree." ■

MORE TO EXPLORE

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