Chronicle / *Lab Book*

Random behavior often helps animals escape predators.

Decisions, Decisions

A stress hormone can flip the switch between strategic and random behavior in rats.

WE'RE HARDWIRED TO learn from our mistakes. But sometimes the brain abandons lessons learned and acts less strategically. In the wild, for example, animals often move unpredictably to escape predators. How the brain switches between paying attention to past experiences and proceeding randomly is clearer now, thanks to new findings from Janelia Group Leader Alla Karpova.

"Normally, we determine the best course of action strategically, by making a model of the world based on all the information available to us—in other words, our experience," explains Karpova. "But sometimes it can be better not to use all—or, for that matter, any—of that information."

Karpova and postdoc Gowan Tervo created a game in which rats were presented with two holes, only one of which contained food. The food's location was determined by one of three increasingly tough computer programs that learned from the rat and attempted to thwart its strategy. The first was predictable and fairly easy to beat, the second a bit trickier, and the third essentially unbeatable because the program used a very sophisticated prediction algorithm that was hard for the animal to counter-model.

The animals used a strategic approach when playing the easy opponent. But when they went up against the sophisticated competitor, they stopped basing choices on previous experience, and their decisions became haphazard. In fact, many of the rats got stuck in random mode for hundreds of trials, even when the food became easy to find—an effect resembling a psychological condition called learned helplessness.

As Karpova and Tervo reported September 25, 2014, in *Cell*, they discovered that altering levels of a stress hormone called norepinephrine in an area of the brain called the anterior cingulate cortex changed the rats' operating mode. Increasing norepinephrine suppressed the rats' strategic mode and caused them to behave randomly; inhibiting release of the hormone had the opposite effect.

"Just by manipulating a single neuromodulatory input into one brain area, you can dramatically enhance the strategic mode," says Karpova. "The effect is strong enough to rescue animals out of the random mode and successfully transform them into strategic decision makers."

Karpova suspects that the same neural mechanisms govern the way humans act, which could lead to a future therapy for learned helplessness. *–Nicole Kresge*

IN BRIEF

A GROWING FAMILY TREE

About 8,000 years ago, a band of brown-eyed, pale-skinned farmers made their way to Europe from the Near East. They mingled with the indigenous blue-eyed, swarthy hunter-gatherers, and their offspring eventually became modern Europeans. But that might not be the entire story. HHMI Investigator David Reich of Harvard Medical School has found evidence that modern Europeans have a third branch in their family tree.

Reich and a team of more than 100 collaborators worldwide compared the DNA of 2,345 modern Europeans to DNA in the remains of a 7,000-year-old farmer from Germany and eight 8,000-year-old hunter-gatherers from Luxembourg and Sweden. "What we find is unambiguous evidence that people in Europe today have all three of these ancestries: early Near Eastern farmers who brought agriculture to Europe, the indigenous hunter-gatherers who were in Europe prior to 8,000 years ago, and ancient north Eurasians," says Reich.

Although nearly all modern Europeans tested showed evidence of DNA from the third group—north Eurasians—the team did not find that same DNA in ancient hunter-gatherers or ancient farmers. This means the north Eurasians came to Europe after agriculture had been established—a scenario most archaeologists had believed unlikely. The scientists' data, published September 18, 2014, in *Nature*, also reveals that the first Near Eastern farmers to reach Europe had ancestors from a previously unidentified lineage, which Reich's group named the Basal Eurasians.

CRUNCHING BIG DATA

Nowadays, microscopes capture images of the brain in unprecedented detail. But with that detail come mountains of complex data that can slow even the fastest computer to a crawl. On a single machine, "you can load the data, start it running, and then come back the next day," explains Janelia Group Leader Jeremy Freeman. "But if you need to tweak the analysis and run it again, then you have to wait another night." For larger data sets, the lag time might be weeks or months.

Freeman joined with Janelia Group Leader Misha Ahrens to find another way. The scientists realized that a new distributed computing platform called Spark, which divvies up tasks across a cluster of computers, was particularly well suited to the challenges of neural data. Building on the technology, Freeman and Ahrens developed an open-source library, dubbed "Thunder," for analyzing large-scale neuroscience data. With their library, tasks that before would take days can be completed in hours or minutes—ideal for supporting high-throughput, exploratory analysis of large data sets.

In a report published July 27, 2014, in *Nature Methods*, the Janelia team illustrated Thunder's capabilities by using it to rapidly identify patterns of biological interest in high-resolution images of the brains of mice and zebrafish.

Thunder is designed to run on a private computer cluster or on Amazon's cloud computing services. It is totally open source; information and tutorials can be found via the GitHub project page at freeman-lab.github.io/thunder.

HIPPOCAMPAL STORAGE SPACE

The neurons in the brain's hippocampus store recent information about people, places, and events. But how does the hippocampus avoid running out of storage space

Photo: Stephen Dalton/Science Source; Illustration: Leif Parsons

Cancer's Niche Problem

In some bone marrow cancers, a stem cell's surroundings play a surprising role.

UNTIL NOW, SCIENTISTS believed that cancer was driven by changes inside cells: genes become mutated, cells grow unchecked, and a tumor forms. But there may be more to the story. New research from HHMI International Early Career Scientist Simón Méndez-Ferrer indicates that, in some cases, what goes on just outside a cell also plays a role in cancer formation.

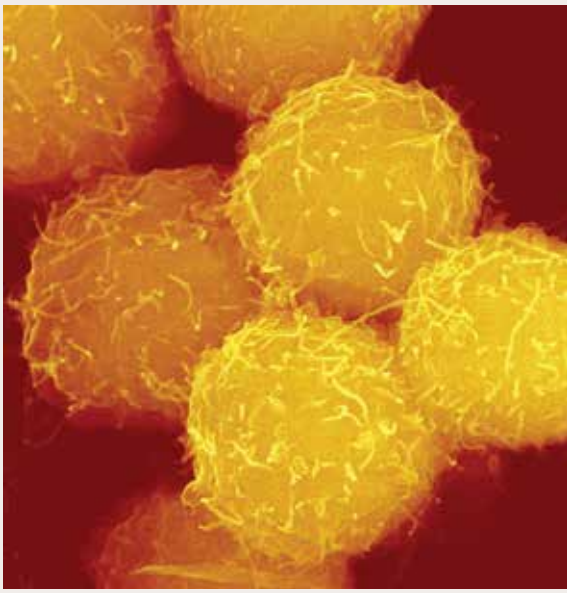
In a group of bone marrow disorders known as myeloproliferative neoplasms, a defective gene causes hematopoietic stem cells (HSCs) to make too many blood cells. As the blood cells build up, the disease worsens, sometimes evolving into cancer. The only real cure is a bone marrow transplant. However, some transplant patients develop a new bone marrow cancer originating from the donor transplant cells, hinting that something other than the patient's mutant HSCs are to blame.

Studying the process in mice, Méndez-Ferrer's team discovered that the mutant HSCs produced an abundance of small inflammatory proteins called cytokines that were damaging nearby neurons. This damage, in turn, prevented the nerves from activating other cells that help regulate HSCs. The end result: unchecked growth of HSCs and the potential of myeloproliferative neoplasms.

"Even though the disease is initiated by a mutated blood stem cell, this stem cell cannot drive the disease until it destroys particular elements of the microenvironment," explains Méndez-Ferrer at the Spanish National Center for Cardiovascular Research. "First, it has to damage the nerve cells in the marrow."

When the researchers added an analog of the neurotransmitter adrenaline to compensate for the damaged neurons' inability to fire, the mouse cells were once again able to control HSC proliferation. The team reported its findings August 7, 2014, in *Nature*.

Most efforts at treating myeloproliferative neoplasms are focused on blocking the mutant HSCs, according to Méndez-Ferrer. "We showed that an alternative approach might be to protect the niche—the environment—and control the expansion of the mutated cell," he says. With that in mind, Méndez-Ferrer, Radek Skoda, and other colleagues will soon start a clinical trial to see if adrenaline analogues can be used to effectively treat patients with myeloproliferative disease. *–Nicole Kresge*



The environment around these hematopoetic stem cells plays a role in cancer formation.

if, say, an environment is larger than predicted or an experience goes on longer than expected? According to Janelia Group Leader Albert Lee, it's all about division of labor.

Lee and his team created a 48-meter-long maze and recorded which neurons in the hippocampus fired when a rat explored a small area of the maze for the first time. Then the researchers incrementally increased the portion of the maze the animals could access and monitored how the brain added the new information to its spatial map.

As they reported August 15, 2014, in *Science*, the team discovered that each neuron contributes to the map at its own rate. As the rat explored the maze, some cells fired early and often and associated themselves with the new space immediately, while others held back. These slower cells rarely contributed unless the space expanded beyond the original size captured by the early-firing neurons.

"Instead of the hippocampus having to adjust in time as the animal notices that the maze gets larger, it anticipates all different sizes of mazes from the beginning," Lee says. "It does this by dividing up its population of neurons so that certain cells are ready to represent smaller mazes, others are ready to represent medium-size mazes, and still others, large mazes." Lee believes that similar mechanisms are at work when the human brain records a new experience.

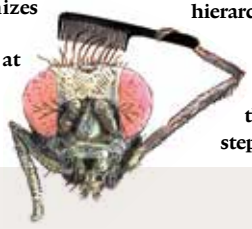
HABITUAL GROOMING

Fruit flies are very fastidious when it comes to cleanliness. A dust-covered fly may spend upwards of 20 minutes removing grit and grime in a habitual sequence that starts at its eyes and ends at its thorax. Studying this structured grooming has helped Janelia Group Leader Julie Simpson explain how the brain organizes sequential behavior.

"You can't do everything at once," says Simpson. "How do you manage competing demands on your brain and limbs so that you execute things in order of importance?" As she reveals in the August 19, 2014, issue of *eLife*, it's all about hierarchy.

Simpson, postdoctoral researcher Andrew Seeds, and their colleagues devised a way to trigger particular grooming tasks by turning on different sets of neurons. They discovered that dust-covered flies always started their grooming program at the beginning—cleaning their eyes—but weren't able to progress beyond the point that the scientists switched on. For example, when the researchers activated neurons for abdominal cleaning, the fly would clean its head and then its abdomen but then would continue cleaning its abdomen, ignoring the dust on the rest of its body.

"It progresses through the hierarchy to that particular point, and then it gets stuck," Simpson explains. This means that earlier grooming steps have the ability to halt those that happen later. These findings may help explain other behaviors that involve sequences of tasks, like nest building in birds.



SENSITIVE SEQUENCING

Techniques known as Sanger sequencing and whole exome sequencing are great tools for identifying defective genes that are present in all of an individual's cells. But they aren't as reliable when mutations occur in just a few cells. Although not as common, these "mosaic" mutations are increasingly recognized as a cause of disease, especially in the brain. "If a mutation is in only 5 or 10 percent of the cells, then that's going to be in a very small fraction of the data, which will be hard to separate from the noise," explains HHMI Investigator Christopher Walsh of Boston Children's Hospital.

To overcome that challenge, Walsh and his colleagues developed a new strategy that flushes out these hard-to-find mutations. The team focused on a set of genes associated with brain

Photo: SPL/Science Source; Illustration: Leif Parsons

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Jamming Jumping Genes

Cells have a rapidly evolving arsenal to counter wayward DNA.

FOR MILLIONS OF YEARS, bits of DNA called retrotransposons have been duplicating and inserting themselves randomly throughout the genomes of different organisms, including humans. Sometimes the jumping genes alight in places that help species evolve new traits. Other times they do nothing. But all too often they touch down in the middle of a gene, altering the way it's regulated or knocking it out of commission. Fortunately, as HHMI Investigator David Haussler recently showed, cells have evolved a way to keep these wayward sequences in check.

"Over generations, our genome becomes bloated with copies of retrotransposons," explains Haussler. "They do damage by disrupting normal genetic mechanisms. Something has to be the security patrol to try and shut these things down."

In humans, one form of security patrol comes from more than 400 rapidly evolving genes that produce watchdog proteins called KRAB zinc-fingers. These watchdogs scan the genome, clamp onto retrotransposons, and then call on other proteins to silence them.

To see KRAB zinc-fingers in action, Haussler's team at the University of California, Santa Cruz, used mouse cell lines containing a copy of human chromosome 11, which includes hundreds of retrotransposons commonly found in primates like humans, but not present in rodents. Since mice have rodent-specific KRAB zinc-fingers to control their retrotransposons, the rodent cells couldn't stop the rogue human retrotransposons from expressing themselves. That is, until the researchers added two human KRAB zinc-fingers—ZNF91 and ZNF93—that halted the primate-specific retrotransposons in their tracks. The team published its findings September 28, 2014, online in *Nature*.

Haussler explains that mutations in retrotransposons allow them to escape detection by the KRAB zinc-fingers, which in turn drives the evolution of new KRAB zinc-finger genes. "By reconstructing molecular evolutionary histories, we can see that these KRAB zinc-finger genes have been major players in this battle with the retrotransposons and will probably continue to be so," he says.

Primates have evolved specialized weaponry to home in on bits of jumping DNA.

There is a silver lining in this seemingly endless arms race within our own DNA. Once KRAB zinc-fingers are no longer needed to suppress retrotransposons, many of these watchdog proteins adopt new roles as regulatory proteins, controlling the activity zof genes near retrotransposon landing sites.

–Nicole Kresge

IN BRIEF

malformations. They isolated DNA from patients with unexplained brain malformations and then sequenced each of these genes hundreds to thousands of times.

"We said we'd shoot to sequence them a thousand times over," Walsh says, "thinking that if a mutation is only present in 5 percent of the cells, it will be obvious that it's a mutation, because we'll see that mutation 50 times." The team reported August 21, 2014, in the *New England Journal of Medicine* that they had found disease-causing mutations in more than a quarter of the patients in their study, demonstrating the effectiveness of their method and further supporting the idea that mosaic mutations are an important cause of diseases that affect just a part of the body.

APPETITE CONTROL

Sometimes science is serendipitous. Researchers—like HHMI Investigator David Anderson—occasionally find something they weren't looking for. In Anderson's case, the accidental discovery was a few thousand cells that turn appetite on and off.

Harnessing a technique called optogenetics, Anderson and his colleagues at the California Institute of Technology used a beam of light to activate a small group of neurons in a part of the mouse brain called the amygdala. In earlier research, Anderson had pegged these nerve cells as part of the fear response. But, as the team reported September 2014 in *Nature Neuroscience*, rather than eliciting fearful or anxious behavior, the light caused the mice to abruptly stop eating. The scientists were able to rule out the possibility that the animals were too scared to eat, and they also established that the neurons were also activated by bad tastes, nausea, and feelings of satiety.

"We think the neurons make up a central node that integrates the influences of multiple [feeding-inhibitory] signals and relays this information to inhibit other brain centers that normally promote feeding," explains Anderson. Many patients with eating disorders also have emotional disorders like depression and anxiety, which may be explained by the neurons' dual appetite and fear functions. Next, Anderson wants to examine how the nerve cells manage to regulate both feeding and emotional behaviors.

QUALITY PATROL

Like intricate pieces of origami, proteins need to be folded in just the right way. An incorrect bend here or an extra loop there can render a molecule useless—or even toxic—to the body. To dispose of these defective proteins, cells detect misfolded proteins and tag them with a protein called ubiquitin, marking them for degradation.

In the yeast *Saccharomyces cerevisiae*, there are two well-established roving protein complexes that add ubiquitin to proteins in the endoplasmic reticulum (ER). One complex is for proteins whose misfolded domains occur in the cytosol, and one is for proteins with misfolded domains in the lumen or membrane of the endoplasmic reticulum.

International Early Career Scientist Pedro Carvalho recently uncovered a third complex that targets proteins associated with a specialized ER domain in the inner nuclear membrane (INM).

When Carvalho and his team at the Center for Genomic Regulation in Barcelona, Spain, removed the two known protein patrol complexes from yeast, they noticed that some molecules were still being disposed of, suggesting that another scout was at work. As they reported September 18, 2014, in *Science*, this mystery warden turned out to be the Asi complex—a gang of three molecules in the INM that not only gets rid of misfolded proteins, but also removes certain correctly folded proteins that are incorrectly directed to the INM.

Carvalho and his team are now looking for other proteins that bind to this patrol complex.

Primate Illustration: John C.W. Carroll / Gladstone Institutes; Quality Patrol Illustration: Leif Parsons