



Man's best Model

by Michael Mason

It may not seem strange that dogs,
so often anthropomorphized, can enlighten molecular biologists about
behaviors deemed uniquely human.

But apparently, so can other animals:

mice, fish, flies—even snails.

ILLUSTRATION BY JONATHON ROSEN



Emmanuel Mignot is not prepared to let sleeping dogs lie.

In a laboratory hallway at Stanford University, the neurologist and HHMI investigator lays out a small meal for Bear, a fluffy 8-year-old Belgian schipperke. Food is one of Bear's favorite things, along with Mignot himself, and today the combination proves overwhelming. As he tucks into his grub, Bear, the last narcoleptic dog remaining at the university's famous multibreed colony, keels over.

This time the interlude lasts just a moment. After a rousing pat on the head, Bear resumes his meal, apparently none the worse. "They become overstimulated by food, or play, or sex," sighs Mignot. "You can imagine what it was like trying to breed a colony of narcoleptic dogs."

Well, no, actually. Still, while every animal model of human behavior has its own special challenges, they remain essential to investigators. Molecular biologists depend on our evolutionary relatives to illumine the biochemical circuitry that drives us.

"Neurology has been revolutionized by the existence of animal models," says neurobiologist Eric R. Kandel, a Nobel laureate and HHMI investigator at Columbia University. "The brain of a mouse is a prototypic mammalian brain. Evolution has been quite conservative across mammalian species."

An Underused Model

Increasingly, scientists see in animals the underpinnings of behavioral responses once thought to be uniquely human: depression, happiness, even lust. Whether the researchers' experiments lead to new therapies, the very fact that many of these behaviors can be modeled in animals comes as something of a shock.

A depressed mouse? An insomniac fruit fly? C'mon.

Yet these beasts do exist. Scientists have had only to look for them or, more recently, to create them. In the early 1970s, at an American Medical Association conference in San Francisco, sleep researcher William C. Dement of Stanford University School of Medicine described to his audience the bizarre symptoms

of narcolepsy: waves of unstoppable drowsiness, fragmented sleep patterns, episodes of quasi-paralysis at times of strong emotion. An estimated 150,000 people have the disease, he noted, and for them daily life is a perilous obstacle course.

After the talk, a doctor visiting from Canada told Dement that he had seen this syndrome all too recently—in his dog. Far from finding a potential connection laughable, Dement scouted among local breeders for similarly affected animals, ultimately establishing a colony of narcoleptic Doberman pinschers and Labrador retrievers at the university. As it turned out, he could not have picked a better way to study the disease.

Because they are highly inbred and often geographically isolated, dogs harbor more than 300 genetic diseases, researchers now know more than any other species besides humans. At least half of these diseases are believed to be analogous to specific human disorders. Seventeen breeds, including dachshunds and poodles, are known to develop narcolepsy.

"The dog genome is very similar to the human genome," says Mignot. "They really have not been used enough as models."

In 1999, relying on results obtained from the Stanford colony, Mignot and his colleagues discovered that narcolepsy in Dobermans and other breeds results from a mutation in *Hcrtr2*, a gene that encodes a receptor for hypocretin, a neuropeptide produced in two forms in neurons of the dorsolateral hypothalamus and disseminated through the brain stem, spinal cord, and forebrain.

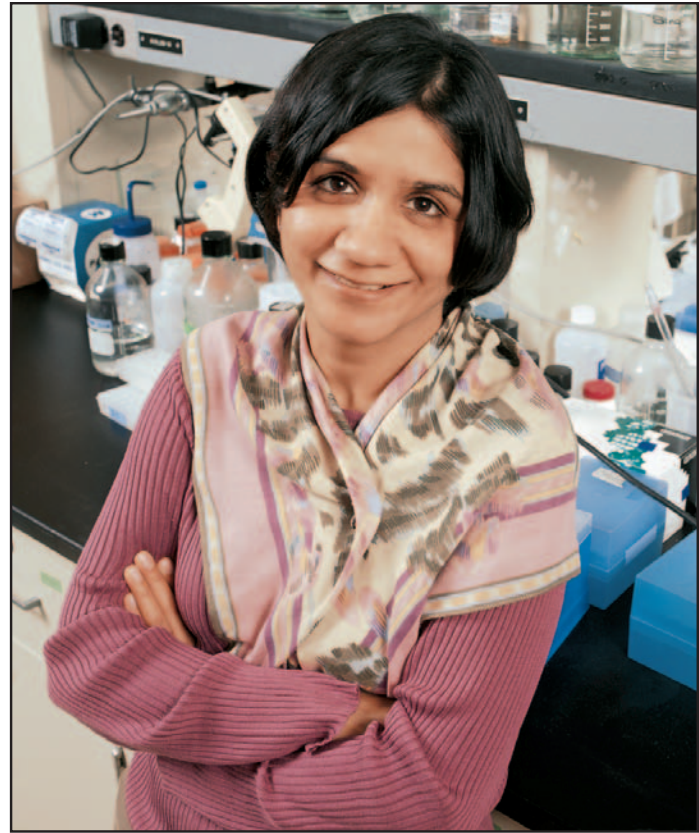
Still, the link from animal model to human behavior is seldom direct. Dobermans and Labradors have slightly different mutations of the *Hcrtr2* gene, Mignot and his colleagues found. And humans appear to develop narcolepsy for a completely different reason: They simply don't produce hypocretins at all.

Interestingly, some dogs, such as Bear, also suffer from this form of narcolepsy. "Bear is especially interesting for us to keep for the future," Mignot says. "Because he lacks hypocretin, like human patients, he could be an ideal model to test a new medication aimed at replacing hypocretins in humans."

Earlier this year, Stanford University began disbanding its renowned colony of narcoleptic dogs, as functional genetic studies are more easily done in other models, and most of the dogs have been adopted. Lately, Mignot has been thinking that Bear will need a very special retirement home: the scientist's own.



Emmanuel Mignot, Stanford University School of Medicine



Amita Sehgal, University of Pennsylvania School of Medicine

Flies Need Sleep Too

Mignot suspects that, in humans with narcolepsy, hypocretin-producing cells are destroyed by an autoimmune process. To examine that possibility, he is isolating genes and proteins expressed in hypocretin-producing cells and testing them as auto-antigens. He is also turning to an entirely new model: zebrafish. They have hypocretin and receptors, and narcoleptic zebrafish demonstrate abnormal sleep patterns similar to those in their human counterparts.

“The decision regarding which animal to use depends on the question you want to address,” says Mignot. “Screening hundreds of thousands of dogs or mice for genetic mutations is just not practical.” But zebrafish, which share a vast number of genes with humans, Mignot notes, can be easily screened in great quantities. Already Mignot has isolated a promoter gene that permits manipulation of zebrafish hypocretin cells in vivo.

That zebrafish doze at all may come as a surprise. But they are not the most esoteric model used to study sleep—that honor likely belongs to the fruit fly. For the last several years, HHMI investigator Amita Sehgal, a neuroscientist at the University of Pennsylvania School of Medicine, has relied on *Drosophila melanogaster* to help define the body’s regulation of sleep.

“Sleep researchers were once of the view that sleep is restricted to mammals and a few birds,” Sehgal says. “But sleep is so important,

you die if you don’t get it. So why should it be restricted to just a few species? Sleep-like states are present in other organisms that we just haven’t looked at yet.”

Simply ascertaining that flies sleep was no small task—it’s not as if they close their eyes and snore. But Sehgal notes that fruit flies are immobile for long periods—during that time their threshold for arousal declines, one of the hallmarks of sleep—and these periods fall into regular circadian patterns. Also, like humans, the fly must compensate for any lost rest, which suggests a homeostatic function similar to sleep.

So if fruit flies sleep, what can they tell us of human slumber? In June, Sehgal and her colleagues published two studies demonstrating that a region of the fly brain, called the adult mushroom body, is responsible for regulating the animal’s sleep patterns. Also involved in learning and memory consolidation, the adult mushroom body bears some semblance to both the human hippocampus and thalamus.

Humans spend about a third of their lives asleep, but nobody really knows why. Sehgal’s finding provides evidence for the theory that sleep is necessary for consolidating memory. “In addition to doing other things, like replenishing energy stores and getting rid of the toxic by-products of metabolism, sleep may permit the nervous system to remodel itself and store information,” she says.



Li-Huei Tsai, Massachusetts Institute of Technology

Insights, *Courtesy* of Snails

Findings in animals often lead scientists to reconsider not just basic human functions like sleep but also traits once considered quintessentially human, such as how we learn and store what we have learned. Columbia's Kandel asserts that animal-based molecular biology soon will affect psychiatry as profoundly as it has neurology.

Nearly 50 years ago, against the advice of his mentors, Kandel elected to study fear and learned memory of fear in a highly unlikely subject: a giant marine snail called *Aplysia californica*, which grows up to a foot long and can weigh several pounds. (Kandel nonetheless refers to *Aplysia* as "beautiful.")

Many scientists of the day thought the neurobiology of an invertebrate could have little relevance to the mental functions of humans. Chief among the doubters, Kandel recalls, were students of behavior: psychiatrists, psychoanalysts, and psychologists. At that time only two scientists in the world bothered to study this snail at all.

But in a body of work spanning decades, Kandel and other researchers used *Aplysia*, and later mice, to establish a molecular basis for memory storage and learning in the brain. Scientists working with rats eventually traced the memory of fear to the amygdala and the body's expression of fear to the hypothalamus. Using these findings to explore the molecular underpinnings for

MODELING EMOTIONAL COMPLEXITY

Of all the human pathologies that scientists have attempted to model in animals, perhaps none is as difficult as schizophrenia. Striking about 1 percent of the population, the disease is marked by three broad types of symptoms: positive (delusions, hallucinations), negative (withdrawal, lack of expression), and cognitive (poor working memory, short attention span).

It's a formidable recipe. Against these considerable odds, however, two HHMI investigators have had success in approximating certain aspects of the disease in animal models.

Over the years several studies have reported that antipsychotic drugs ameliorate schizophrenia by blocking excessive dopamine transmission in the brain. In 2004, researchers learned that these patients often have large numbers of D2 dopamine receptors in the striatum, an area deep within the brain that controls movement and balance.

In an attempt to extend this finding, Eric R. Kandel's Columbia University lab created mice that also over-expressed these receptors, and his team discovered that the mice suffered a hallmark of schizophrenia: impaired working memory. This deficit persisted even when the researchers allowed production of D2 receptors to return to normal.

Early in an animal's development, Kandel reasoned, the damage caused by increased dopamine transmission may produce irreversible changes in other parts of the brain.

But dopamine may not be the only culprit. Calcineurin is an enzyme involved in the regulation of the immune response and in learning. In 2001, HHMI investigator Susumu Tonegawa and his colleagues at the Massachusetts Institute of Technology found that knock-out mice missing calcineurin in the fore-brain also suffer impaired working memory. And the mutants display other symptoms reminiscent of schizophrenia: They are hyperactive and withdrawn from other animals, for example, and they are more easily startled than normal mice.

"Based on our mouse study, we were able to go on to obtain direct genetic evidence that variation in a human calcineurin gene is associated with schizophrenia susceptibility in humans," says Tonegawa. "These data provide strong support for the involvement of calcineurin-related genes in schizophrenia etiology."

Precisely which of these are involved is still a mystery, and in any event the disease is likely to depend on interaction with the environment. Adds Tonegawa, "In recent years, real progress has been made in identifying disease genes. We hope that advances like our calcineurin findings will ultimately lead to better therapies." — M.M.

fear in mice, Kandel discovered that they bear great resemblance to the mechanisms observed in *Aplysia*.

Now it appears that happiness may be similarly discernible in the brain. In a recently published study, Kandel and Columbia colleague Michael Rogan showed that mice conditioned to associate a sound with safety showed reduced electrophysiological activity in the amygdala, perhaps representing a decrease in fear. At the same time, they showed increased activity in parts of the dorsal striatum involved in positive emotion and reward. “These mice just walk around unafraid, as if they own the place,” says Kandel.

Nearly 30 percent of Americans suffer an anxiety disorder at some point in their lives, and the finding has made Kandel wonder if certain of these disorders might result from defects in the neural systems that communicate security.

Depressed Mice

Fortunately, the gap between human anxiety and animal fear may prove smaller than once thought. Scientists have yet to discover an *Aplysia* with full-blown panic disorder, of course, but rodent models of depression have led directly to a number of useful medications. Better models, though, may soon bring better therapies.

Scientists studying depression, along with drugs to reduce it, have relied largely on wild-type mice that were “socially defeated.” Submissive mice were housed in containers with dominant mice, and the behavior developing in the submissives was judged to be as close to depression as researchers could get.

But last year HHMI investigator Li-Huei Tsai, a neurobiologist now at the Massachusetts Institute of Technology, led a team that developed mutant mice whose disease may more closely mimic that of humans.

Tsai and her colleagues found that a protein called Par4 binds to a type of dopamine receptor and that mice with mutated Par4 demonstrate something akin to depression. Dopamine is a neurotransmitter active in the brain pathways governing mood, reward, and motivation.

In one standard test, mutant mice placed in a clear plastic container filled with water stopped swimming long before wild-type mice. “They are fully conscious, but they quickly become immobile,” says Tsai. “They don’t struggle. There is a loss of motivation.”

In another experiment, hungry mutant and wild-type mice are placed in an open environment with food at the far end. Mice are averse to open spaces, so this set-up presumably creates some anxiety. Wild-type mice are quick to surmount their discomfort and find the food. But the depressed mice take a very long time to follow suit.

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“The mutant animals fail to do what they need to do,” says Tsai. “They find it more difficult to overcome their anxiety and fear.” Most current medications for depression, the SSRIs (selective serotonin reuptake inhibitors) and MAOIs (monoamine oxidase inhibitors), modify synaptic levels of serotonin or norepinephrine. Tsai’s experiments with depressed mice suggest that dopamine may also provide an excellent target for intervention.

Pheromone-Driven Humans?

Closer examination of animal neurobiology doesn’t just tell us about disease—it tells us much about normal human behavior, too. “If you want to study behavioral genetics,” says HHMI investigator Catherine Dulac, a molecular biologist at Harvard University, “you need something robust so that the changes you introduce will be observable.”

Few behaviors are more robust and observable than reproduction, Dulac adds. In mice, as in many animals, certain behaviors hard-wired into the brain—mating and aggression, for example—are strongly influenced by airborne molecules, called pheromones, released by one animal and perceived by another.

Although the vomeronasal organ (VNO), the structure a mouse uses to sense pheromones, resides in the nasal cavity, researchers have long believed that the animal’s pheromone-sensing apparatus is completely separate from the olfactory system, which regulates smell. (continued on page 60)

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Dulac and her colleagues discovered last year that the conventional view is likely to be wrong. The researchers used a modified virus to trace the connections from the VNO and the olfactory system to neurons producing luteinizing hormone-releasing hormone (LHRH), thought to be activated by pheromones.

The neural input into the LHRH neurons did not come primarily from the VNO, as they'd expected; instead, LHRH neurons seemed to receive input from the olfactory epithelium—a sheet of cells inside the nasal cavity that are involved in smell. Mutant male mice without VNOs are able to mate, Dulac has found, but do so indiscriminately with both males and females. Mutant males without functional olfactory epithelia, however, no longer respond to sexual stimuli such as female urine.

The findings raise some interesting questions about human sexual behavior. Despite oft-cited anecdotal evidence, the fact that humans have no VNO has long argued against the possibility that their reproductive habits are influenced by pheromones. Now, it appears, a VNO may not be necessary for pheromones to work in humans.

“Mating and reproduction are animal behaviors, and humans are animals too,” says Dulac. She hears an instinctive confirmation of the evolutionary link every time she gives a talk about her work. “It’s very striking to me that, even though I am showing pictures of male and female mice, there are instantly giggles in the room,” she says. “Somehow, everyone senses that this behavior raises questions about human behavior.” ■

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