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By Kelly Rae Chi

Behavior in Action

Tools and techniques for tracking mammalian behavior.

Even the seemingly simplest mammalian behaviors, such as grooming one's offspring, involve a complex series of tiny movements that may be invisible to the human eye. But in studying those behaviors, how to break them down into reliable, measurable components?

"All of these advances in technology give us data that [weren't] available one to two years ago."

Defining, quantifying and assessing mammalian behavior is a thorny problem. Besides a shift in thinking to incorporate the complexity of animals' actions, "it really takes a great deal of experience and some training to approach behavior work correctly," says Mark Kristal, a psychologist at the University of Buffalo in New York. Scientists come across a number of challenges in imaging and measuring behaviors in the lab and field. For one, testing or observing behavior can interfere with the behavior itself. In studying maternal behaviors in rats, researchers have too much to measure and less than 21 days in which to do it. Assessments of fearful behaviors such as freezing can be variable among different scientists. And researchers who conduct field studies at night may not even be able to see the animals they study.

The Scientist talked to several researchers who have implemented technologies or strategies to capture mammalian behaviors. Here's what they said:

Unhindered Movement

User: Detlef Heck, associate professor of anatomy and neurobiology, University of Tennessee Health Science Center, Memphis

Project goal: Tracking a natural movement in mice

Heck studies how the cerebellum controls movement, and licking is one

movement shown to be regulated by the cerebellum. Standard licking tests involve depriving an animal of water overnight and recording its drinking patterns the next day. The method is not useful for examining spontaneous control of movement because it makes mice anxious, Heck says. He was interested in the natural licking behavior of an undisturbed mouse. "I wanted to be able to get a measure of spontaneous behavior plus a measure of their long-term water consumption," he adds.

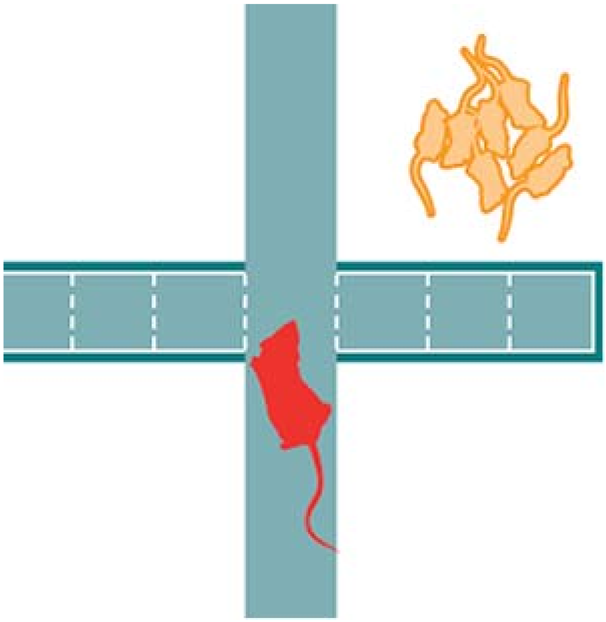
Heck built a set-up in which mice move freely in a cage with constant access to water. When the mouse visits its water spout for a drink, contact between the tongue and spout induces a tiny blip in voltage that is recorded onto a computer. Heck uses software to analyze the number of licks per visit, lick speed, and the frequency of visits, among other things. The system cost him about $10,000; it consists of an analogue digital converter (~$5000), a computer (~$700), data analysis software ($2500), and cage materials.

It also helps to choose the right type of movement. Wayne Aldridge, a psychologist at the University of Michigan in Ann Arbor, studies the link between movement and electrical activity in brain regions such as the basal ganglia. In order to correlate the firing of a neuron to a single movement, that movement must be repeated so that you can create an average representation of the neuron's electrical activity. But, says Aldridge, in movement research, equating one motion with another is tricky. Rodents tend to have consistent patterns of grooming, within and between animals, so he uses grooming as a measure of movement. "We don't have to train the animals or teach them," he says.

Tips:
• You don't need fancy technology to analyze movement. For example, some researchers analyze a mouse's gait by dipping its paws in ink and watching it walk across a white sheet of paper. "It's more important to spend time to think about ways to quantify natural behaviors," Heck says.
• No two movements are the same, but you can choose movements that are relatively consistent, such as grooming.

Naïve Mothers

User: Joseph Lonstein, associate professor of neuroscience and psychology, Michigan State University, East Lansing



Project goal: Measuring postpartum anxiety in mice

Rats show reduced anxiety after their pups are born, a phenomenon that may be important for the series of chores—such as building nests, retrieving pups, and nursing—that mothers face soon after delivery. Lonstein studies how the brain regulates this reduction in postpartum anxiety. Behavioral differences between anxious and relaxed animals, though, are not very noticeable while moms are tending to their pups.

In order to assess anxiety more directly, Lonstein and his group chose a well-established anxiety task called the elevated plus maze. Parts of this maze are enclosed by walls, while other parts lacking the walls leave the rat exposed to bright light on a platform that's almost two feet off the ground. Compared with relaxed rats, anxious rats will stay in the enclosed portion for longer periods of time.

"One thing that's unique [about studying] postpartum animals is that you have 21 days of lactation," he says. "You've got a narrow window of time to test what you want." That means if you're trying to collect multiple measures on a single animal, such as maternal behaviors, along with anxiety or depression, you have to move fast. What's more, the animal will become more anxious as it repeatedly participates in different tasks— which may make it more difficult to track anxiety in different cohorts, he says. He thus must use more pregnant and lactating rats, but limit his studies to one task per animal.

For those who want to study maternal actions in relaxed animals, new situations—such as a new maze or task—can interfere with measures of maternal behaviors, says psychologist Mark Kristal at the University of Buffalo in New York. "One of the things we emphasize in my lab is to habituate the animals very carefully to the procedures," he adds, for example, by handling them before they become pregnant.

Tips:

• Because maternal behaviors change so rapidly even within the pups' first month of life, use consistent timing when collecting data in different cohorts.
• If you're looking to study anxiety in naïve animals, choose your first task

carefully. If you need to run repeat tests, prioritize the measures that are most important to you.
• To study maternal behaviors in relaxed rather than anxious animals, expose them to new testing situations several times the week before testing.

Night Vision

User: Nickolay Hristov, assistant professor of life sciences, Winston-Salem State University and the Center for Design Innovation, North Carolina

Project goal: Visualizing behaviors that occur in the dark

At 5 p.m. in Carlsbad Caverns National Park in New Mexico, visitors trickle out of the caves that are home to thousands of bats. No one was certain exactly what they did in the dark cavern because light from researchers' headlamps and flashlights would perturb the animals' natural behavior.

Hristov, working as a postdoc with Thomas Kunz and colleagues at Boston University, obtained thermal infrared imaging technology, which was originally developed for military use. Using the technology, they were able to see the bats in total darkness and record their movement—in blobs of color—from just 15 meters away, in the dark. Researchers attempting to study this cave-dwelling species have wondered about the size of the colonies, and how the individuals navigate in the presence of many other bats. Kunz's group found that the number of the bats in caverns fluctuates by as much as a million, depending on the time of year. On a daily basis, the colonies appear to expand and contract to help adjust to ambient temperatures (*Integr Comp Biol*. 48:50–59, 2008).

Much of the work was done using single camera views, but "creative camera placement [of three thermal infrared cameras] can give a lot of information," Hristov says. The researchers are now placing the cameras in specific positions in order to track flight trajectories in three-dimensional space.

To examine wider (kilometer-scale) distances of movement in the field, researchers might consider global positioning system telemetry tags that can accurately trace a bat's flight route, allowing researchers to infer when it is eating or drinking. In some cases the data are overlaid onto geographic information system maps so that researchers can see the environmental context of the behavior. "In our studies, we know the specific tree branch the bat was using throughout the night," says Ran Nathan, professor of ecology at the Hebrew University of Jerusalem. "All of these advances in technology give us data that [weren't] available one to two years ago." The tags run Nathan about $900 to $1500, and depending on species and application, are good for a few days when researchers sample the animal's location once per second. Unfortunately, many of the units cannot be recovered.

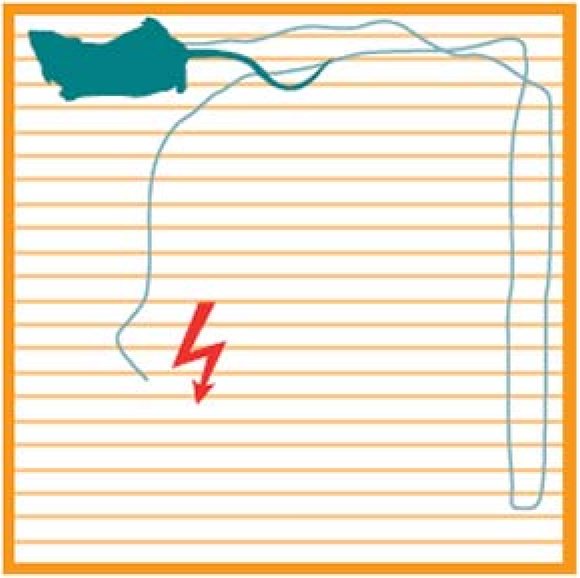
Tips:
• Thermal infrared imaging and other imaging methods can be combined with 3D analysis to study collective group behavior not only in bats but in species ranging from butterflies to elephants, Hristov says.
• When considering telemetry and other field studies that capture aerial movement, don't forget to account for the properties and dynamics of the atmosphere; consult an atmospheric scientist. Similarly for biologists studying sea animals—consult an oceanographer.

Computerizing Fear Factors

User: Marcelo Wood, assistant professor of neurobiology and behavior, University of California, Irvine

Project goal: Standardizing a measure of learning and memory

Fear conditioning—a technique in which researchers teach animals to connect a neutral stimulus, such as a tone, with an aversive stimulus, such as a foot shock—is a well-established way to investigate the mechanisms of learning and memory.



As a postdoctoral researcher studying learning and memory in mice, Wood noticed a lot of variability in the field among scientists' assessments of fearful behaviors, such as freezing. "Scoring freezing or other fear-related behaviors is very subjective, and each person will do it differently," he says. "The other thing is that it's extremely time consuming." Wood started his own lab in 2006, and he wanted to prevent such variability.

Wood researched several different companies who sold automated systems for tracking motion, ultimately settling on Ethovision software (Nolus Information Technology, Wageningen, the Netherlands) because it can be directly connected to test chambers that allow the user to control when to deliver electrical shocks to the bottoms of the cages. The software can also be used to study other learning and memory paradigms. The system samples the cage every few seconds, and at each time point, tells researchers whether the animal is moving. The set-up cost Wood about $15,000 in 2006.

Even with the sophisticated set-up, Wood's group still had to spend 3 months tweaking variables because the software is designed to analyze movement, not learning or fear. "The main problem is, how do you tell a computer what a freezing mouse looks like?" he says. "It seems like a simple thing, but the computer can mistake a mouse that's relaxed and not moving for one that's scared." In order to make it work, the group took the same dataset and repeatedly ran it through Ethovision, adjusting sampling rates, pixel thresholds, and other settings, until the software spit out data statistically indistinguishable from those collected by a human observer (*J Neurosci Methods* 178:323–26, 2009).

Tips:

• When you first start using motion-tracking systems, expect to spend at least a month tweaking settings to fit your experimental paradigms.

• Talk to researchers who have worked out these specifics before you. Many labs have published papers on automated scoring techniques, Wood says.