NEUROSCIENCE

When rodents run the research

An automated training regimen coaches rats to willingly submit themselves to repeated brain imaging.

Human behavioral research is fraught with complexities, but at least you can give your subjects instructions with the expectation that they will attempt to comply. Laboratory rodents, on the other hand, are generally not 'volunteers' and must be compelled to make their contribution to science. However, an innovative experimental strategy for conducting neuroimaging studies in live rats developed by Princeton University researchers Carlos Brody and David Tank transforms these passive subjects into active participants, giving scientists the potential to run sophisticated studies in a largely 'hands-off' fashion.

Tank's group has long worked at the forefront of cellular-scale brain imaging in live rodents. Earlier studies have employed miniaturized microscopes that animals wear as they interact freely with their surroundings or have used virtual reality simulations that mice 'navigate' on a spherical treadmill while secured to a full-scale microscope. However, Tank was intrigued by the possibility of coupling his imaging approach with computer-controlled systems developed by the Brody lab to automate training of rats for studies related to memory and decision-making behavior. "They have a completely automated facility," says Tank. "There are technicians that basically run the animals, and there is very little experimenter intervention."

The strategy they devised with postdoctoral fellow Ben Scott entails coaxing rats to voluntarily put their heads inside of a 'headport' opening, where the brain is imaged via two-photon microscopy. The rats wear a headplate, which offers access to a window in the skull. A sensor in the headport detects the entry of the rat's head, triggering a system of actuators that precisely repositions the headplate so that the brain is consistently presented to the microscope in the same spatial orientation. "The real innovation here is the fact that on each head insertion, you can see the same neurons," says Tank. When imaging is complete, the rat is released and can return to its business.

Convincing rats to subject themselves to this routine was a challenge. Scott achieved this in a stepwise fashion, using water to reward rats first for simply poking their nose into the headport and eventually for

A voluntary head-clamping system allows brain imaging in rats. Image courtesy of B. Scott.

keeping their head in place long enough to be clamped and microscopically imaged. In some instances the investigators gave the animals a critical degree of control over the terms of their participation. "We had what is basically a 'panic switch,'" says Tank. "If the animal sticks its head in and changes its mind about the trial, there's a switch that they can activate with their paws to release themselves." In the final iteration of their system, rats could be reliably trained within 2–3 weeks.

As an initial imaging demonstration, the researchers used a genetically encoded calcium indicator to monitor selective activation of neurons in the visual cortex in response to differently oriented stimuli. Their auto-adjusting kinematic mount consistently achieved a positional precision of a few micrometers—less than the width of a single cell—and they were therefore able to routinely identify and monitor the same field of labeled neurons over dozens of trials spanning several days.

This voluntary head-clamping system could potentially be used for virtually any brain imaging modality or even combined with head-mounted imaging systems to compare data collected in freely-moving animals relative to more controlled scenarios. The team is also pursuing higher-throughput configurations that could allow scientists to gather both behavioral and imaging data from large numbers of animals with relatively minimal effort. "It's certainly going to be an extra step of development," says Tank, "but it's conceivable."

Michael Eisenstein

RESEARCH PAPERS

Scott, B.B. *et al.* Cellular resolution functional imaging in behaving rats using voluntary head restraint. *Neuron* 80, 371–384 (2013).