Neurobiology: learning from marmosets

Vivien Marx

Many neuroscience labs are intrigued by these social, vocalizing primates.

Marmosets somersault, leap and play-wrestle; they might hang from a branch to reach for the fruit they want or huddle together and groom one another. They communicate with a range of postures, facial expressions and vocalizations such as whirrs, chirps, phees and tsiks. This New World monkey, *Callithrix jacchus*, is native to the forests of eastern Brazil and grows to around 20 centimeters in height with an equally long tail.

Marmosets live in small groups and interact socially in many ways: both parents and other members of the group rear offspring cooperatively, and they learn by watching and imitating others. When marmosets gaze at others or objects, they frequently tilt their head. "The first time I saw marmosets in real life, I was most surprised that they looked me straight in the eye," says neuroscientist Rogier Landman, who is in Guoping Feng's lab at MIT's McGovern Institute for Brain Research. This direct gaze is uncommon among primates, for whom eye contact is considered aggressive behavior, says Atsushi Iriki, who directs the laboratory for symbolic cognitive development at RIKEN Brain Science Institute. This capability to hold eye contact with people suggests that marmosets are a unique primate model of social behaviors, he says.

Marmosets are garnering researchers' attention in the United States, Europe, Japan and China[1–4]. These primates are the focus of a large-scale Japanese project launched in 2014 called Brain Mapping by Integrated Neurotechnologies for Disease Studies (Brain/MINDS) that involves 65 labs at 47 institutions. Some Brain/MINDS teams are mapping the human brain and focusing on human psychiatric disorders and neurodegenerative diseases. These teams collaborate with the majority of the project's researchers, who are exploring what marmosets can teach the research community about the human brain.

L. Serebrennikov/Alamy Stock Photo

Marmosets are getting researchers' attention in the United States, Europe, Japan and China. These monkeys are small, live in small groups, vocalize plenty and show many social behaviors.

Brain/MINDS includes mapping projects at different scales: a 'macroscopic' level, such as with magnetic resonance imaging (MRI)-based diffusion tensor imaging that lets scientists track neural fibers based on the water molecules in the fibers; 'mesoscopic' analysis with light microscopy and tracer injections; and a 'microscopic' level that includes high-resolution structural analysis of fixed brain tissue using electron microscopy. A whole-brain *in situ* hybridization gene atlas is also in the works. Some labs image neuronal activity in behaving animals or gather electrophysiology data with implanted arrays.

Why marmosets?

Marmosets are much smaller than macaques, which are found in many neuroscience labs. As Landman explains, marmosets reproduce quickly and, unlike macaques and people, their brain surface is smooth, which is helpful for imaging and electrophysiology studies. Neuroscientist Hideyuki Okano, dean of Keio University School of Medicine, who co-directs Brain/MINDS, believes marmoset research will help labs obtain and then integrate data across maps of different scales to allow labs to link genes, circuits and behavior in a model organism. Marmosets are not new entrants into neuroscience; labs have been studying brain–behavior links with marmosets for more than 40 years, he says.

Marmoset vocalization and social behavior are promising models, says Kyoto University researcher Katsuki Nakamura, who is part of Brain/MINDS and who studies primate social behavior and evolution. Marmosets, tamarins, gibbons and humans are the only primates with a family unit. Because gibbons cannot be used for experiments, he turned to marmosets. Female marmosets give birth twice a year, probably as an adaptation to predation pressure. The offspring are usually non-identical twins, which presents experimental options, says Nakamura, because the infants have the same parents and grow up in the same family. With same-sex twins, he and his team

Getty Images/iStockphoto Thinkstock Images/Zwilling 330

There are decades of neuroscience research with macaques. Marmosets can expand the variety of questions that can be asked with a primate model.

use one animal as the experimental subject and the other as the control.

As a graduate student, Cory Miller, a neuroscientist at the University of California at San Diego, was intrigued to discover how vocal marmosets are. Their back-and-forth calling probably evolved to maintain social contacts in their dense forest home. Marmosets are not going to help researchers address all neuroscience questions, says Miller, and he hopes that bioengineering labs starting marmoset work or those turning to marmosets from macaques don't have exaggerated expectations. But he likes the breadth of emerging marmoset studies: "you start getting different perspectives, which is actually pretty fun to see."

Researchers perked up, says Miller, when Keio University researcher Erika Sasaki and colleagues in multiple labs in Japan, including Okano, generated transgenic marmosets[5]. That hinted at the possibility of studying brain and behavior with molecular genetic tools that have not been all that effective in macaques: gene-editing and optogenetic approaches to control neuronal activity with light. At the same time, there are 40 years of visual circuitry research with macaques and "it would take 40 years to replicate that in a marmoset," says Miller, which is among the reasons that marmosets won't replace macaques. Rather, marmosets will be complementary and expand the types of questions that can be asked with a primate model.

For gene-editing experiments, Miller uses viral vectors; he and his team refined an approach that is robust in marmosets, quick and low budget. Creating transgenic marmoset lines takes much institutional support and plenty of resources. Some scientists are having success with CRISPR-based gene editing, he says, "but the reality is we're never going to have 10,000 transgenic lines like we have in mice."

Transgenic marmosets will help scientists parse molecular and genetic mechanisms in comparative primatology, says Iriki, who is also part of Brain/MINDS. Like Nakamura, he sees opportunities to model human vocalization and social behaviors with marmosets.

Being vocal, being social

The ability of monkeys to vocalize was long thought to be fixed at birth, a notion that Princeton University researcher Asif Ghazanfar, who studies vocalization and social behavior, has called into question[6]. Infant marmosets produce "babbling-like sequences of sounds," a mix of mature and immature calls that eventually turns into more mature calls, he says. Infant marmosets move on to mature-sounding contact calls more quickly when they receive more parental feedback, which is also how human infants develop, he says. Developmental stages are comparable between marmosets and humans, and both species rear young cooperatively, with offspring cared for by parents along with older siblings and unrelated group members. Marmoset calls may relate to cooperative breeding in that infants must make themselves heard, he says.

K. Nakamura, Kyoto University/E. Dewalt, A. Arguello, S. Ranade, J.M. Zarate/Nature Publishing Group

Cognitive tests are being adapted for marmosets. In one test, a marmoset is rewarded for selecting a specific image in a pair. As the images change, the marmoset learns to recognize different ones as correct.

To date, no evidence points to semantics in marmoset vocalization, such as an indication that an animal is describing food or a predator. "I think their calls serve social function, much like most of our human conversations do," says Ghazanfar. They regulate relationships. "This is why we often talk about the weather, even though both parties may be standing in the weather," he says. Marmoset vocalization can help researchers study and model aspects of human developmental disorders. With transgenic animals, a lab could apply his findings to study gene-knockout animals and their developmental trajectories. "Then you have much, much greater insights into potential communication disorders and what goes wrong in those disorders," he says.

Ghazanfar uses microstimulation techniques, and he plans to use DREADDS, chemogenic tools in which small molecules are used *in vivo* to activate G-protein-coupled receptors and to turn neurons on or off. He also uses tissue clearing to study behavior-related structural changes in the marmoset brain. Ghazanfar is not affiliated with Brain/MINDS but is forging a link with RIKEN's Iriki to work with data from his lab's marmoset histology atlas.

Vocalization is not just about the brain, says Ghazanfar, which is why recording brain activity is insufficient for studying behavior. A marmoset's brain might be intact but he or she might not vocalize well because something is amiss with the larynx or with respiratory control. That leads to less parental feedback, which affects brain development and vocalization. A brain region is not, in his view, the seat of behavior, nor is the brain "the big controller," he says. Brain, body, biomechanics and the social context all are part of vocalization, and the animal must be seen as a system. This view can help scientists address complex disorders such as autism, he says. But before conclusions can be drawn about human diseases, a model system has to be understood well.

Miller believes the analysis of marmoset vocalization data has just begun. "Studying this the right way is non-trivial, both technically and conceptually," he says. It's also easier to train a monkey to do a task than it is to understand its natural behavior. For example, the way a marmoset positions its tail during its calls might be important for understanding the behavior, or it might just be the animal adjusting its balance on a perch. This research requires extensive observation and neuronal recording.

Miller combines assays to study social behavior and its underlying neurobiology. In one ongoing project, he records electrophysiology data as the animals interact with a 'virtual marmoset' developed in the lab. It is a computer-controlled vocal recording and is similar to tools used with frogs and birds, only, says Miller, "we're creating an individual that they have continued interaction with." During the weeks of the experiment, the animals decide how to interact with this individual as the researchers explore auditory circuitry. Marmosets can remember the virtual monkey they previously 'met'. Preliminary data from this and other work suggest that the state of the animal's prefrontal cortex indicates whether or not an animal will engage in conversational exchanges.

The scientists can switch the virtual marmoset's character, says Miller: "they're humming along in their conversation and then something changes." As the animal adjusts to the new conversant, the scientists can observe and record changed neuronal firing patterns. The system helps them track long-term social interactions and many behavioral facets and

C. Heise, Alamy Stock Photo

Marmoset vocalization involves the brain, body, biomechanics and the social context, says Princeton University researcher Asif Ghazanfar.

is unlike the rigid approach taken in many primate research labs, he says, where a reaction to a presented stimulus is recorded. This is "real-time social cognition as it unfolds."

Miller and other researchers, including National Institutes of Health researcher David Leopold and Xiaoqin Wang at Johns Hopkins University, are also building an automated system to track many animals over long periods as they record data and manipulate circuitry to then merge behavioral and neural data. "It's in some ways a massive engineering project," he says. "But it's what we need, I think, to understand the primate social brain."

Modeling disease

Large-scale human genome sequencing helps researchers search for genes involved in disorders such as autism, depression, developmental delay, and neurodegenerative diseases such as Alzheimer's and Parkinson's. The search matters especially because of the continued dearth of drugs for treating these conditions and insufficient understanding of the mechanisms underlying these disorders, which is why better models are needed, says Steven Hyman of the Broad Institute of Harvard and MIT, who is a former director of the US National Institute of Mental Health.

Research with mice has, says MIT's Landman, "tremendously increased knowledge because of being able to connect the molecular level all the way up to the system and behavioral level, and connect genetics to behavior." Some knowledge gained from rodents does not translate well to humans partially because some brain structures and functions differ greatly between rodents and humans. "That gap, I hope, can be closed with marmoset models so therapy for humans can be improved," he says.

Mice can help researchers study fundamental mechanisms; for example, says Hyman, optogenetic techniques can be used to study the reward and fear circuitry that is evolutionarily conserved between humans and rodents. But, he says, many human disease conditions are not just fear circuitry or reward circuitry run amok; rather, they involve cognitive controls that are not implemented in the brains of rodents as they are in people. Although mice have a prefrontal cortex, they lack the dorsolateral prefrontal cortex that humans and primates share and that is involved in cognition, learning and memory. "Rodents are basically solitary, nocturnal and olfactory and we're social, diurnal and visual," he says. These are some of the other differences between mouse and human brains, which is why, he says, "we really do need primate models."

Humans parted phylogenetic ways with chimpanzees around 7 million years ago, rhesus macaques 25 million years ago and marmosets around 35 million years ago. The human brain is larger and more complex than the primate brain, says Nakamura. The parallels to be drawn to humans won't be perfect, but he believes some basic functions of the marmoset brain are similar to those of the human brain.

Marmosets read visual cues in the faces and postures of others and react to them. This gaze can help scientists parse social interactions in families and explore learning behavior, aggression and peacemaking. Studying marmosets requires behavioral observation, cognitive testing and patience, says Nakamura. Marmosets are harder to train than macaque monkeys, and marmoset-specific cognitive tests are needed. Landman and his team have also discovered how hard it is to train marmosets, which is why they tailor experiments to the animals' natural behavior and environment as much as possible. "Luckily, advances in technology such as telemetry, computer-aided tracking and machine-learning make that feasible," he says.

Marmosets may seem harder to train because tasks being tested have been developed and refined relative to macaques, with whom neuroscientists are much more familiar, says Iriki. Labs will need to keep in mind that although the tasks do not necessarily exactly represent human traits, there are tasks to which marmosets are better suited than macaques, and behaviors in which they are more similar to people, such as vocal communication, social cooperation and altruism. More tests are needed to assess these behaviors.

For cognitive tests with marmosets, which the animals perform well, Nakamura and his team have developed an apparatus with a small computer and a touch-sensitive screen similar to systems used with other monkeys but suited for marmoset paws[7]. It is set up at the animal's home cage so it does not have to be moved for testing. One type of exercise involves showing the animals pairs of graphical patterns with a multitude of shapes and colors. One image in a pair is associated with a reward, and the marmoset has to touch that 'baited pattern' on the screen to get the reward, says Nakamura. Then comes reversal, and the marmoset has to give up the former rule and learn the new one, says Nakamura. This kind of test is one that human subjects fail if they have prefrontal cortex damage, he says.

Separately the team, in collaboration with a company, built and used an apparatus for studying a social cognitive task called joint attention. A marmoset has to select which box, in an assembly of specially stacked boxes, contains food. During training for the task, the boxes have transparent doors, and the experimenters point to where the food is. Then in the experiment they point to baited boxes with opaque doors. If the animals reach for the right box, they get the food it contains. The scientists repeated the test with marmosets that had received drugs that hindered their ability to follow the experimenter's pointing hand and gaze, which mimics autism-associated behavior. Nakamura and his team are developing new types of tests to study social behavior and memory tests to work on models of Alzheimer's. For now, he says, there is a lack of good testing tasks for memory assessment in marmosets that are akin to the types of tests run with people with Alzheimer's or amnesia.

The Brain/MINDS teams are also developing automated testing systems to integrate molecular and anatomical data, electrophysiology recordings and behavioral analyses, says Okano. Passive video- and audio-taping removes the bias an observer can cause and lets labs capture behaviors and development over extended periods of time in one animal or a group. Although such systems exist, work is under way to modify them for use with marmosets, says Landman, which means collaboration between hardware and software developers, scientists and veterinary care staff.

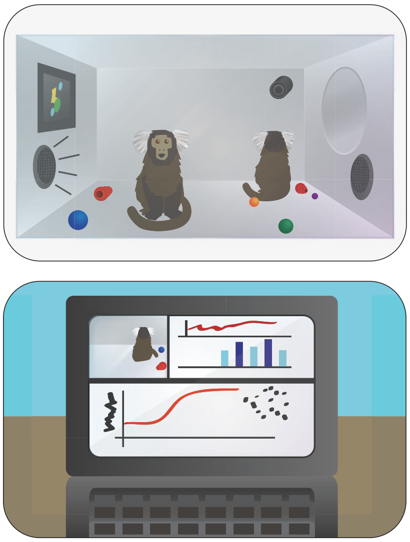
Iriki sees great value in using molecular imaging with probes and modalities such as MRI or ultrasound to obtain repeated longitudinal data points over long periods and from the same maturing, interacting animals. For disease modeling, automated data analysis might allow early detection of disorder symptoms, which would address ethical issues because animal suffering could be avoided, he says.

With a group of marmosets, it might be possible to detect 'spontaneous induction of mental disorders', says Iriki. "This is a reverse way of research in contrast to genetically manipulated disease studies," he says. Along with colleagues across Japan, he recently co-authored a study in which a macaque showed symptoms of autism, which they then analyzed further. But, he says, this work was difficult and took much luck to complete. "Marmosets should have a great advantage in these kinds of studies, when using the automated system in a relatively large colony of small animals."

For basic and applied research, Iriki and his team have built a prototype of a system to fully automate primate behavior collection and analysis[8]. Integrating data of various types and from different sensors is challenging, he says. Electrophysiology or motion data and cognitive test results need to be transformed to allow their analysis as a dynamic time series. Next, behavior patterns must be extracted. This approach takes a more holistic view of the animals, he says, and it converts behavior analysis into more of a data-driven science.

The Broad Institute's Hyman believes that genome-scale analysis of many individuals will help to pinpoint afflicted neural functions or brain regions and molecular mechanisms that play a role in neurological disorders. Analysis of rodent behavior has, in his view, neither delivered enough insight into the mechanism of disorders such as depression nor brought greatly improved drugs[9]. The inability to accurately capture intricate molecular facets of human disease such as autism or depression with rodents has contributed to failed drug candidates and the exit of pharmaceutical and biotech companies from research on neurological disorders, he says.

Changes in an animal's social behavior are not always indications that a complex



A. Iriki, RIKEN/E. Dewalt, S. Ranade/Nature Publishing Group

RIKEN's Atsushi Iriki and his team are developing an automated system, now a prototype, to automatically record, analyze and manipulate marmoset behavior.

disorder such as autism has been well modeled, says Miller. Autism is more complicated than a mouse shunning other mice, he says. Exploring how genetic changes alter primate behaviors can show scientists how well they are modeling human sociality. With transgenic marmosets, neuroscientists hope they can study conditions such as autism spectrum disorders and link genes, anatomy, neural circuits and behaviors in ways that are more similar to the human condition than the mouse models.

Even for highly penetrant genes, human genetics researchers are discovering the large role genetic background plays, says Hyman. In work with transgenic or gene-edited macaques or marmosets, this genetic background should be part of the experimental design in order to shed light on how these genes affect an organism. For example, 22q11.2 microdeletion syndrome can affect around 40 genes on chromosome 22 and leads to conditions in people such as cardiac defects, dysmorphic facial features and psychiatric symptoms. The degree and type of these symptoms vary considerably, he says, and this variation is probably related to genetic background. A quarter of affected children develop schizophrenia, one-fifth are autistic, and others have a variety of issues such as attention deficit disorder. With *SHANK3*, a gene linked to autism and rarely to schizophrenia, a genetic background is also likely interacting with this penetrant gene, he says. When researchers engineer primates, they need to explore ways to introduce penetrant genes plus a reasonable number of genes known to interact with the penetrant gene.

Ethics

"Understanding the evolution of social behavior is fantastically important," says Hyman. For the labs intent on translational efforts, he says, "the behavioral analysis has to be complemented by a neurobiological analysis." To know how a potential drug will act on a nervous system, it's critical for researchers to connect behavior and neurobiology, and they must be sure that the same cells, synapses and circuits are affected. The behavior in the animal model does not have to be identical to the human behavior, but, he says, "what we don't want to be studying is phenocopies." Behavior can seem similar but differ mechanistically and from an evolutionary perspective. Especially with a model that is as costly, time-consuming and ethically fraught as primates, he says, "we really have to make sure we have thought about it in a very systematic way."

Brain/MINDS labs breed marmosets and do not remove animals from the wild, says Okano, and the scientists embrace the four R's of replacement, reduction, refinement and rehabilitation—that is, they strive to replace or avoid the use of animals when possible, use as few animals as possible, refine experimental methods to minimize potential pain or suffering, and commit to rehabilitate animals.

As scientists from the United States and China point out in a recent review, disease models necessarily involve "a risk of suffering," and labs working with primates including marmosets must ensure that animals receive careful veterinary care and that both their behavioral and physical well-being are tracked and addressed. These check-ups should happen for all animals, especially those in which genes have been altered, says Landman. When genes are knocked in, they are usually placed in so-called safe-harbor zones to minimize interactions, and therefore such experiments might not elevate an existing risk of harm. Risks might be greater in experiments that disrupt genes involved in brain functions. As they consider risks, labs must also strike a thoughtful balance, the researchers say, between using too many animals and having insufficient numbers for statistically valid conclusions.

The MIT team began building their marmoset colony two years ago and the animals are now successfully breeding, an indicator that the animals are happy and healthy, says Landman. Besides specialized veterinary care, the animals have a stable environment, plenty of room, a varied diet, toys and entertainment, he says. When some animals don't get along, they are shielded from one another, and the marmosets are given places to huddle together and rest. Nakamura says he and his group have altered the design and size of cages for marmosets and they explore many kinds of enrichment. Enrichment strategies include toys for climbing and chewing and puzzles of various sorts.

Work with marmosets is important for medical advances, says Hyman, but "it's also ethically incumbent on us to take really good care of these animals and to never think of them as a routine preparation." Given that marmosets are more like people than mice, scientists must reserve the use of genetically engineered animals for important experiments that cannot be done in other ways. And, he says, "we have to be sensible to the concerns that we would cause undue suffering."

1. Okano, H., Miyawaki, A. & Kasai, K. *Phil. Trans. R. Soc. Lond. B Biol. Sci.* **370**, 20140310 (2015).
2. Miller, C.T. *et al. Neuron* **90**, 219–233 (2016).
3. Jennings, C.G. *et al. Nat. Neurosci.* **19**, 1123–1130 (2016).
4. Grillner, S. *et al. Nat. Neurosci.* **19**, 1118–1122 (2016).
5. Sasaki, E. *et al. Nature* **459**, 523–527 (2009).
6. Takahashi, D.Y. *et al. Science* **349**, 734–738 (2015).
7. Takemoto, A. *et al. Neurosci. Res.* **93**, 136–143 (2015).
8. Yoshida, K. *et al. Sci. Adv.* **2**, e1600558 (2016).
9. Hyman, S.E. *Sci. Transl. Med.* **4**, 155cm11 (2012).

Vivien Marx is technology editor for *Nature Methods* (v.marx@us.nature.com).