

Nerve fibre trajectories inferred from the tracer fluorescence originating from the prefrontal cortex of a marmoset.

A GAME-CHANGING MARMOSET DATABASE FOR NEUROSCIENCE

A robust, **INTEGRATED DATABASE** consisting of extensive images and gene-expression data from marmosets' brains will fuel many years of neurological research.

Untangling how the human brain functions — and how neurological disorders develop

— remains complex and challenging. While decades of work with animal models have broadened knowledge of the intricacies of animal brains, the obvious inability to conduct invasive tests on humans means we know much less about human brains. Rodent models have yielded valuable information and are useful for trialling theories regarding gene function, but primates remain the most viable — and most similar — models for the human brain.

About a decade ago, Japan became the first country to adopt the common marmoset as a neurological model for a major brain project. A New World primate originating from Brazil, marmosets are easier to look after and cheaper to house than other primate models. They reach sexual maturity within two years and reproduce quickly, giving birth up to twice a year.

WHY MARMOSETS?

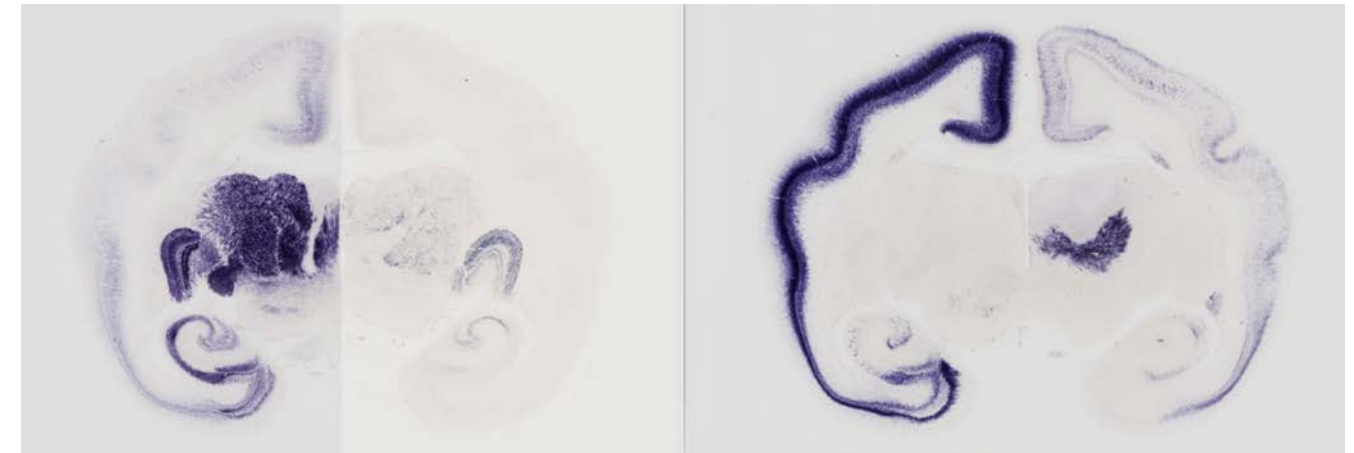
Marmosets share remarkable traits with humans that allow researchers to draw meaningful conclusions about human brain

functionality. "Some key reasons why we began using marmoset models is that they interact in social groups, have close group bonds and use complex vocalizations to communicate," explains neuroscientist Tomomi Shimogori of the RIKEN Center for Brain Science (CBS). "This means that they have much more human-like brain functionality than rodents."

To gain new insights into information processing and diseases of the human brain, the Brain Mapping by Integrated Neurotechnologies for Disease Studies (Brain/MINDS) project,

launched in 2014 in Japan, aims to elucidate the neural circuits underlying higher brain functions using the common marmoset.

A part of the Brain/MINDS project is generating a multimodal database of the marmoset brain, including detailed gene expression, magnetic resonance imaging (MRI) and tracer data (connectomics) — all mapped onto the marmoset brain. Neuroscientist Akiya Watakabe, who is also from the RIKEN CBS, compares the database to a dictionary. "Our database is like a high-quality dictionary



▲ Images of the marmoset brain depict different gene-expression patterns in a common brain image space.

that lists as many words as possible," he explains. "Similarly, we've included numerous genes and connections to create a comprehensive and reliable resource for research¹."

The database is open to all, allowing researchers to delve into developmental and neurodegenerative disorders and other important areas. "The integrated database will fuel many years of ground-breaking research, bringing us closer to unravelling the complexities of the human brain and discovering new ways to tackle neurological disorders," says Watakabe.

REVEALING GENES' SECRETS

Shimogori's team has analysed the expression patterns of more than 2,700 genes in the marmoset brain — around 10% of all its genes². These genes are primarily associated with human mental and neurological conditions. "When there's any kind of defect in the human body, it is often down to a fault in the genes," says Shimogori. "By revealing exactly where genes are expressed in the brain at different times, in specific areas and circuits, we can understand how each gene malfunctions, providing valuable information for behavioural problems."

When comparing the gene-

expression data of human and marmoset brains, Shimogori's team discovered striking similarities in gene-expression patterns³. Their research has also provided detailed insights into how genes are expressed in marmosets throughout their life cycle. Such comprehensive gene-expression data is challenging to obtain in the human brain due to limited sample availability.

"OUR DATABASE IS LIKE A HIGH-QUALITY DICTIONARY THAT LISTS AS MANY WORDS AS POSSIBLE."

"When a particular messenger RNA (mRNA) is expressed, it suggests that gene products such as proteins likely exist in that brain region," says Shimogori. "We detect this mRNA expression, using probes for each specific mRNA that light up when they meet the correct mRNA molecules in the brain. We then know which mRNAs are expressed in that region, allowing us to deduce gene-expression patterns."

Interestingly, many genes associated with a high risk of neurological and mental

disorders are expressed in different regions from rodent brains, suggesting that rodents may not be the most suitable models for studying these disorders. However, to study the effects of these genes, researchers can manipulate them in rodents, which are cost effective and readily available as model animals. This enables scientists to uncover gene function at the cellular level and gain valuable insights. They can subsequently validate their findings by manipulating the genes in the primate model.

Having a database that tracks gene expression is extremely valuable in deepening our knowledge of distinct cell types, as well as neuronal function and dysfunction. However, solely examining gene expression is inadequate for obtaining a thorough grasp of how genes influence brain function. Gene expression affects the formation and operation of neural circuits and their connectivity. "By studying both gene expression and connectomics, we can augment the information we acquire and attain valuable understandings into how neurons communicate, interact, process information, create memories and generate behaviour," says Shimogori.

PREFRONTAL CONNECTIONS

Studying marmosets allows researchers to employ brain analysis techniques that are not feasible in humans. One example is tracer experiments, where tracing substances are directly injected into the brain to examine the neural connectivity. When combined with serial two-photon tomography imaging, high-resolution 3D images of neuronal structures within the brain can be obtained.

Leading the way in pioneering this technique for marmosets is Watakabe, who recently unveiled the organization of prefrontal cortex circuits in marmosets for the first time⁴. "The tracers label the axons that extend from the neurons at each injection site. The fluorescent tracer patterns then show up in images," says Watakabe. "Here, you label tens of thousands of neurons simultaneously. The patterns of axon projections coming from these neuronal populations were very exciting."

The team revealed two distinctive patterns of axon projections from the marmoset prefrontal cortex. They found that many prefrontal cortex neurons had axons converging into narrow, well-defined columns, and these 'patchy' projections appear to be

topographically organized and interconnected. On the other hand, Watakabe's group also found axons extending into wide areas in low densities, resulting in a diffuse pattern of projections.

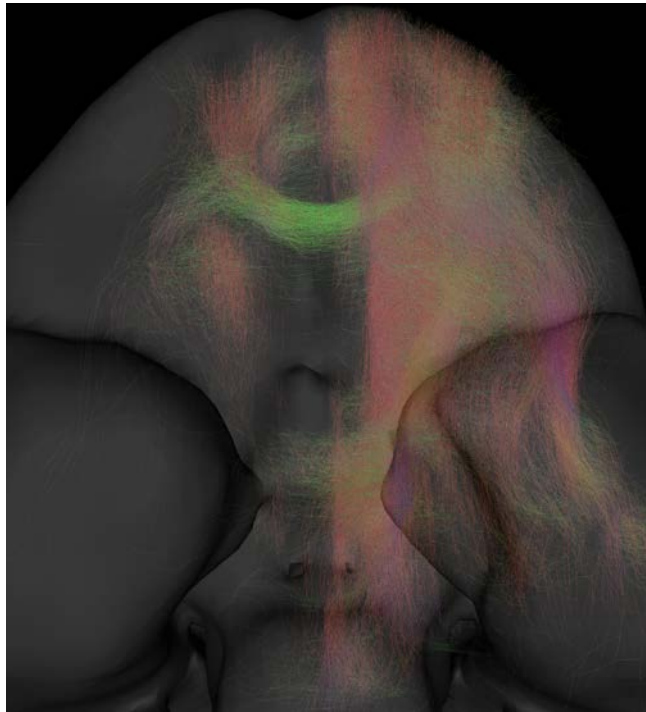
"These two patterns, diffuse and patchy, don't co-exist in rodent brains. We believe this could be an important property of the primate prefrontal cortex," says Watakabe. "We're continuing injections into other cortical regions to obtain a fuller view. These patchy projections are interesting from both functional and developmental perspectives."

MULTIDIMENSIONAL MAP

The integration of diverse data types, including gene-expression data and connectivity data derived from modalities like microscopy and MRI, holds immense promise for neuroscience. Gene-expression data provides insights into the molecular and genetic underpinnings of brain function, while connectivity data reveals the intricate network of connections between brain regions. Integrating these datasets enables scientists to explore how genes interact within specific circuits or brain regions and how these interactions influence overall brain function.

However, it is challenging to integrate data from different modalities into a comprehensive 3D map of an entire primate brain since it requires ensuring that every gene and its associated connections are accurately positioned within this intricate map. This is where the expertise of image analyst and computer scientist, Henrik Skibbe of the RIKEN CBS, comes in.

"Working with brain images is fascinating, not to mention beautiful," says Skibbe, who has



▲ A high-resolution reconstruction of nerve fibres at the single-axon level from a marmoset's prefrontal cortex.

been working at RIKEN since 2019. "Mapping the complexities of the marmoset brain so that it can be compared with human datasets requires a meticulous and methodical approach⁵."

While MRI images from humans and animal models are generated in three dimensions, the gene-expression data collected by Shimogori's group is currently analysed in two dimensions using thin brain slices.

"Our goal is to reconstruct the brain slices into 3D data and develop techniques to directly compare the gene-expression data with tracer and MRI images," says Skibbe. This is complicated by the variability in gene-expression patterns and the uniqueness of each marmoset's brain, akin to the uniqueness in human brains. Helpfully, structural correspondences can be used to compare across individuals. Skibbe's team are developing deep-learning algorithms to

optimize the processing and analysis of these images.

However, a key challenge remains: AI requires immense amounts of training data to learn how to identify and analyse components accurately. For example, AI can stumble on subtle differences between images, such as the tonal colour of individual pixels, which can lead algorithms to misinterpret structures. These neurological datasets from marmosets are very new to science, so Skibbe is breaking new ground in developing techniques to analyse them.

"It's vital that we make our integrated database accessible, automating it and bringing in AI capabilities that can systematically explore the data and find connections far quicker than humans can," Skibbe emphasizes. "To achieve this, we're investing heavily in engineering to create extensive training data — it sets up a cycle of improvement. We begin

with hand-crafted algorithms, and then we input the data to improve the machine-learning algorithm, which in turn improves the datasets."

ONGOING IMPROVEMENTS

The RIKEN team is continuously enhancing their accessible database and encouraging scientists worldwide to use it and contribute to its growth. "We'll continue expanding the database, refining the data and interpretations, and incorporating comparative information between species, including human data," says Watakabe.

A leading aim for the near future is to facilitate cross-species comparisons and the examination of conserved genes and connectivity patterns. "We hope our database will be used daily by scientists across the world — just like an internet search engine. For instance, you can simply enter a gene name and access all the available information about it from the database," explains Shimogori. "Eventually, we will incorporate all crossovers between species." "We hope our database will help answer questions that haven't even been thought of yet," adds Skibbe. ■

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