National Institute of Health BRAIN Initiative

2021 Marmoset Community White Paper

Central to the mission of the BRAIN initiative is the creation and application of innovative technologies and gathering of information for understanding how individual cell types and complex neural circuits interact in space and time to generate normal brain function and dysfunction in the diseased brain. Priority research areas of the BRAIN Initiative include the generation of brain cell atlases, understanding circuit wiring, the function of specific cell types and circuits via causal manipulations, large scale multi-area interactions, and the link between brain function and behavior. <u>Marmosets offer unique advantages for both developing new technologies and expanding our knowledge of the brain</u>. As non-human primates, this New World species is within the same taxonomic family as humans (Order: Primates) and share the core brain architecture and broad behavioral repertoire. For example, <u>marmosets have an extensively developed prefrontal cortex</u>, and their motor control, sensory perception and social cognition is very similar to those of humans. In contrast to the more commonly used macaque monkey, however, they offer the advantage of a much smaller body (300-400gr in weight), a small lissencephalic brain, a shorter gestational period (5-6 months) and life span (8-12 yrs of age), a faster maturational period (sexual maturity is reached around 1.5 yrs of age), and high fecundity (producing litters of 2-3 offspring). <u>These advantages make marmosets an ideal non-human primate species to address many goals of the BRAIN Initiative</u>. For example, the fast maturation and high fecundity of the marmoset is a great advantage for the generation of transgenic lines. Indeed, recent advances in genetic engineering in marmosets have opened new pathways to study the brain, allowing modeling of disorders with a genetic component, such as Alzheimer's disease, Schizophrenia, Autism and Huntington's disease, in which mouse models have so far been unsuccessful in translation to humans. Moreover, <u>the marmoset small brain size is ideal for studying circuit wiring and connectomics in a complex non-human primate brain</u> that is several orders of magnitudes smaller than the macaque brain, whose large brain size still poses a big data challenge for computational tools. Additionally, in contrast to the large and convoluted macaque brain, the marmoset's small lissencephalic brain allows the accessibility needed for brain-wide, high resolution *in vivo* imaging techniques, such as two-photon microscopy.

Breadth of Current Research. There are several research questions within the BRAIN Initiative mission currently being addressed using the marmoset as a model species. Following the initial development of calcium imaging in the marmoset brain (23, 61, 62), several laboratories are applying this technique to image network dynamics in real time in behaving marmosets (63-65). Similarly, following the initial development of *in vivo* optogenetics in the marmoset (19), reports are rapidly accumulating on its application to study marmoset cortical function and behavior (66-69). These approaches will advance our knowledge of the neural basis of cognition and behavior, a major goal of the BRAIN Initiative. Studies are currently underway to produce a spatially specific catalog of cell types in the marmoset brain, using single-cell RNA sequencing; using this approach a recent study has profiled RNA expression in a large number of inhibitory neurons across several species, including marmosets (70). These approaches will pave the way for future studies of primate genetics and circuits. BRAIN Initiative funds have been, and continue to be, used successfully for the development of new viral tools for targeting specific cell types in the non-human primate brain. For example, novel recombinant adeno-associated virus (rAAV) vectors that restrict gene expression to GABAergic interneurons in many vertebrate species including marmosets using the mDlx enhancer (16) or the h56D promoter (71) have recently been developed. Moreover, over the past year, two laboratories have developed viral vectors for selective transgene expression in specific inhibitory neuron subtypes (parvalbumin and somatostatin-positive interneurons) in marmoset cortex (71, 72). Studies of the auditory system have successfully leveraged the aforementioned advantages of marmosets to pioneer numerous neural recording and behavioral techniques to make new discoveries about the physiological mechanisms underlying sensory perception and social communication in the primate brain (73-86). More recently, researchers have also begun to take advantage of the marmoset natural tendency to orient towards visual stimuli, perform visual tasks, and the accessibility of the middle temporal (MT) visual area and frontal eye field on the cortical surface of this species, to study a diverse range of visual behaviors in marmosets (26, 87-93).

The Future. The marmoset is a unique model to investigate the non-human primate brain in ways that are not allowed by other primate species. Promising future research areas are briefly discussed below. First, the marmoset small lissencephalic brain is ideally suited for the development of wide-field calcium imaging to enable imaging of millions of neurons across cortical layers and multiple brain areas. Future efforts are directed towards increasing the width and depth capabilities of imaging in this primate species Second, the marmoset small brain size is also ideal for the development of large-scale manipulations of cortical circuit activity, to understand interareal interactions. Future efforts are directed towards developing large-area manipulations throughout the cortical depth, and performing spatiotemporally patterned photostimulation to mimic the spatiotemporal patterns of neuronal activity. Third, efforts are under way to couple single-cell RNA sequencing with behavioral studies in marmoset (as previously done in mouse (94)), to establish computational tools that allow linking gene expression in specific cell types to behaviorally relevant circuits in a primate. Fourth, current and future efforts are under way for further development of viral tools for cell specific targeting in non-human primate brains; for example, rAAV vectors that can specifically infect additional subtypes of inhibitory neurons in marmoset cortex, beyond those recently reported. Finally, a revolution in understanding the human brain in health and disease will require non-invasive real-time mapping of neurotransmitter and calcium signaling. New vasoactive imaging probes with high sensitivity and resolution have been developed in rodents (95, 96) and are currently being developed for marmosets.

Authors.
Alessandra Angelucci
Rogier Landman
John Reynolds