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METHODS FOR STUDYING THE NEURAL CONTROL OF NATURAL BEHAVIORS USING UNCONSTRAINED MARMOSETS

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ABSTRACT

There is growing interest in the marmoset as a model species for systems neuroscience inspired by the prospect of genetic tools like those available for mice. Such tools provide opportunities to record, control and modify neural circuits and to model human disease. Phylogenetically situated between key model organisms, the marmoset also provides a unique comparative perspective on the organization of the neural components of voluntary motor control. Methods for behavioral training and neural recording to study motor control with marmosets are not well developed. Early behavioral work with marmosets suggests they may be intolerant of established techniques used with macaques, the most prevalent non-human primate model species in neuroscience research. In chapter 2, I present an approach to voluntary, automated behavioral training of marmosets validated by quantifying upper limb kinematics during foraging and demonstrating abundant voluntary engagement in experimentally useful behavior. Pioneering techniques for performing neural recordings have been developed, but there is still ample opportunity for further refinement. In chapter 3, I describe surgical techniques and a custom configuration of a wireless headstage I developed to record population responses from sensorimotor and premotor cortex. Further I demonstrate that the wireless neural recordings can be done in conjunction with quantification of upper limb kinematics and during unconstrained natural behaviors. Finally, in chapter 4, I summarize the marmoset's unconstrained behavioral repertoire and present preliminary observations of behavioral state specific structure within population responses. These observations suggest that dimensionality of population responses during natural behaviors may be higher than expected from similar results based on more highly constrained behaviors.

CHAPTER 1

THE MARMOSET AS A MODEL SYSTEM FOR STUDYING VOLUNTARY MOTOR CONTROL

1.1 Introduction

The common marmoset offers an opportunity for an integrative study of motor systems. In this review we will demonstrate three ways in which marmosets provide such an opportunity. First, marmosets provide an intermediate point of comparison for understanding principles of motor system organization integrated across species. The marmoset lineage diverged from our own around 33 million years ago (MYA), after that of rodents (~96 MYA), but before that of macaque monkeys (~23 MYA), a few of the most widely used model species in the study of motor systems (Nei and Glazko 2002). While not a simple, strict linear timeline, when considered in the context of the marmoset's basic expression of a primate motor system, comparative corticospinal morphology and cortical field differentiation, these divergence dates hint at an intermediary position for marmosets in the phylogeny of mammalian motor systems organization. Such a position could prove advantageous as the field tries to marry cell-type specific insights available in studies with rodents to the coarser understanding of the primate motor system gained throughout the last five decades from gross anatomical studies and single unit recordings. Second, the marmoset's small size and relatively lissencephalic cerebral cortex allows for recordings of populations of neurons both across multiple cortical areas with multielectrode arrays and within a given area or cortical column by densely sampling populations using optical imaging approaches. The intersection of these neuroanatomical facts

and recording capabilities will allow us to interpolate between multiple cortical fields and the scale of a single cortical column to dramatically enhance our understanding of motor cortical function. The third opportunity offered by the marmoset is to enhance our understanding of the integration of descending signals by spinal interneurons, as the majority of the marmoset's corticospinal terminations ramify in lamina XII of the spinal cord. Moreover these populations of neurons will become identifiable using genetic tools making the marmoset a unique tool to uncover the principles of corticospinal engagement that provide a foundation for voluntary movement.

Goal directed voluntary movements, such as reaching to grasp, depend on coordination of many areas across frontal and parietal cortex (Kalaska, et al. 1997). Only through studying the intracortical communication among these areas as motor behaviors unfold can we hope to gain an integrative understanding of the cortical motor system. Technical challenges often limit the ability to record from populations of individual neurons across many cortical areas in macaques. Due to the relative difference in brain size, the corresponding differences in cortical area size and the differences in encephalization, an electrode array that could only record from a portion of a given area in a macaque could record from multiple areas in a marmoset. The marmoset's relative lack of sulci makes almost the entirety of the cortical motor system accessible to optical imaging techniques that allow for recordings at sampling densities not previously available in the study of primate cortical motor systems. The combination of these neuroanatomical features and technologies for recording single cell activity at different scales and sampling densities promises an unprecedented capacity to bridge our understanding of the cortical motor system across multiple cortical areas with our understanding of cortical computation as implemented at the scale of an individual cortical column. While exciting, a pure focus on the cortical components

of the motor system would neglect fundamental components on which cortical control of movement is built.

The cortical motor system supporting voluntary movement is intimately woven with the subcortical structures like the thalamus (Sherman and Guillery 2013), basal ganglia (Akkal, Dum, and Strick 2007; Hoover and Strick 1999; Miyachi et al. 2006), cerebellum (Akkal, Dum, and Strick 2007; Bostan, Dum, and Strick 2013; Dum and Strick 2002; Kelly and Strick 2003; Kipping et al. 2013; X. Lu et al. 2007; Proville et al. 2014) and brain stem nuclei (Esposito, Capelli, and Arber 2014; H. G. J. M. Kuypers 1981; H. G. Kuypers and Lawrence 1967). Further, without the neural structures of the spinal cord and its descending pathways the brain would have no access to the musculoskeletal system of most of the body and a severely limited capacity to produce motor behaviors. Therefore, any satisfying account of the primate motor system will need to be an integrative account that considers how all of these neural and musculoskeletal structures come together to support skillful motor behavior. In what follows, we will argue that the marmoset presents an interesting model species for studying the primate motor system that has the potential to compel a more integrative view of how the components of the primate motor system come together at multiple spatial and temporal scales to allow for sophisticated and skilled bodily control. I will begin with a description of some peculiarities about marmoset lifestyle and development to suggest that these might reflect inward to the functioning and organization of the marmoset motor system. I will then review what is known about the organization of the marmoset motor system and finish by suggesting that its eccentricities make the marmoset well suited as a model system for providing a more integrative understanding of the motor system.

1.2 Marmoset lifestyle and development

Common marmosets make their living ranging through understory feeding on tree exudates and this lifestyle has shaped their body. Exudates, the gum that exudes from wounds of many tree species, are a major component of the common marmoset diet (Sussman and Kinzey 1984). To exploit this resource a marmoset will spend more than a third of its active hours ranging through trees and vines, foraging and visiting holes gouged in the trunks of trees (Abreu et al. 2016; Francisco et al. 2014). Much of that time is spent clinging to vertical surfaces and using lower anterior teeth to gouge holes and scrape exudate (Sussman and Kinzey 1984). The marmoset masticatory apparatus is well adapted to exudate feeding (Vinyard et al. 2009).

Distribution of enamel on marmoset teeth, with thickening of the labial enamel and an apparent absence of lingual enamel, suggest a condition that could facilitate maintaining sharp edges for gouging as the teeth erode (Vinyard et al. 2009). The fibers of the masseter and temporalis muscles of marmosets are longer, relative to their incisal biting load arms, than those of cotton top tamarins, a closely related species with a similar diet but that does not gouge trees (Eng et al. 2009). This musculoskeletal organization allows those muscles to operate within a more advantageous portion of their length tension curves at wider gapes needed for gouging.

One shouldn't be surprised if a similar combination of effector and musculoskeletal adaptations are manifest in the upper limbs given that they provide support for the head and neck during the almost whole body rocking and stabilization behavior involved in gouging. It seems likely that this lifestyle would also have shaped the structure of the marmoset upper limb and the neural structures that coordinate its control. As anyone that has tried rock climbing can attest, clinging to a vertical surface for any significant duration is challenging. Different than most

other primates, marmosets have claws rather than nails. These certainly go a long way to help anchor the marmoset during gouging. There are likely also adaptations in fiber type distributions throughout the upper limb musculature. For instance, one would expect a greater density of slow oxidative fibers in muscles supporting sustained elbow flexion, like the brachialis, needed for prolonged vertical clinging. The structure of the shoulder likely also manifests adaptations to sustained vertical clinging, leaping and scansorial locomotion (Schmidt and Schilling 2007). The intrinsic properties of the motoneurons both within and across motor pools are in turn likely tuned to optimally and efficiently support their activity regimes (McLean et al. 2007; Tadros et al. 2016). These adaptations almost certainly extend inward to neural control strategies implemented in structures distributed throughout the nervous system, yet we know very little about most.

While a comprehensive description of motor skill development is beyond the scope of this chapter. I would like to mention elements of marmoset behavioral development likely to have a significant reflection in the organization of the marmoset motor system. See Schultz-Darken, Braun, and Emborg (2016) for a recent review of efforts to characterize marmoset development for a more comprehensive account of marmoset behavioral development.

Marmosets live in multigenerational family groups where care of young is shared among family members. For their first five weeks of life, newborns are carried on the bodies of family members as they range through the trees. As of its first week after birth, a newborn marmoset will be able to grasp, hang, and right itself after being placed on its back (Y. Wang, Fang, and Gong 2014). During the next four weeks of its life, it will begin to climb and cling to vertical surfaces. By week eight, the newborn marmoset will essentially have acquired all of the motor behaviors of an adult and begin acquiring food on its own. There seems to be a rostral to caudal

progression in motor development, where facility is gained with upper limbs prior to lower limbs, with a critical period occurring between 2-5 weeks (Y. Wang, Fang, and Gong 2014). This progression very likely parallels the development of the spinal cord and descending pathways such as the corticospinal tract (Porter and Lemon 1993).

A recent assessment of marmoset postnatal development found no significant improvement in the labyrinthian head righting, the tendency to align the head with the vertical plane when the body is tilted, in infant marmosets from two weeks to one month after birth (Braun et al. 2015). Interestingly, they interpret this delayed response as an adaptation to the early period of life when the marmoset had a singular purpose, to hold onto the family member carrying them through the trees. One might expect that this extreme exposure to vestibular sensation during this critical period of motor development would be reflected throughout its motor system (in cortical areas 8c or 3a for instance).

1.3 Current and comparative understanding of the marmoset motor system

Beginning in the early 1990's, Krubitzer and colleagues (Huffman and Krubitzer 2001a, b; Krubitzer and Kaas 1990) began mapping the organization of the somatosensory cortex of marmoset monkeys. These studies were part of a larger effort to understand the principles guiding cortical field organization. This work provides the basis for what we know about marmoset somatosensory cortex and its intracortical and thalamic projections. This work suggests that the cortical fields of the marmoset anterior parietal cortex, namely area 3a, 3b, 1 and 2, are homologous with those of macaques. Further, they are both elaborations on a basic mammalian cortical field organization shaped by strong constraints on mammalian nervous

system evolution dependent on distributions of transcription factors, gene regulatory networks and early experience of developing organisms (Krubitzer and Seelke 2012).

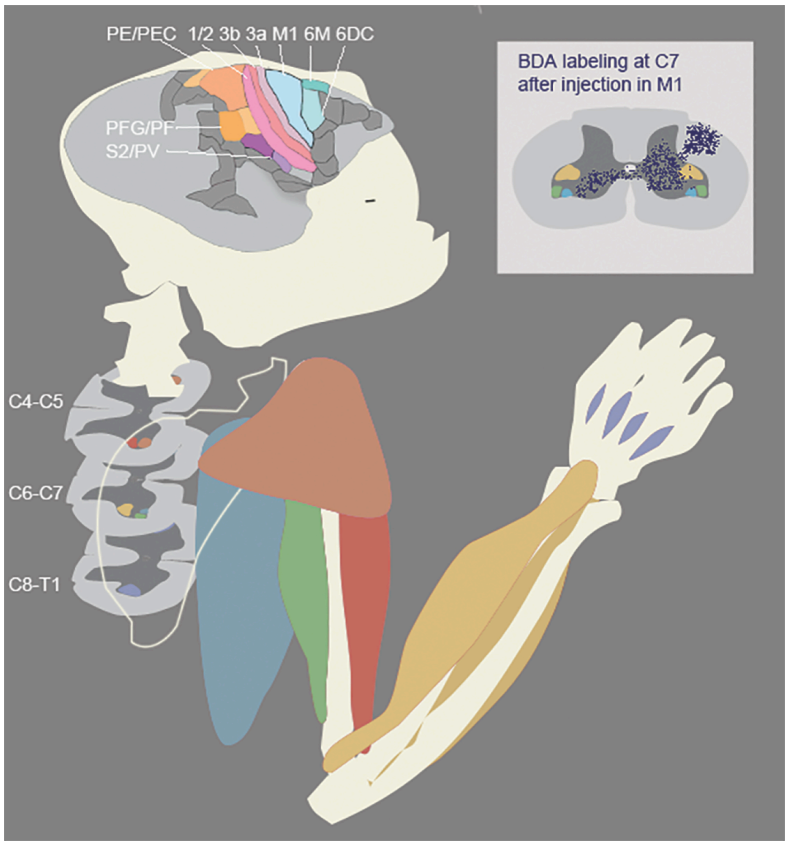

Figure 1 Summary of the current understanding of the corticospinal organization of the marmoset motor system

Colored cortical areas are those that contribute greater than 5% of afferent projections to the upper limb area of primary motor cortex in at least two of the cases investigated by Burman et al. (2014). Dark grey cortical fields represent those areas in which cells projecting to M1 were found in any of the cases described by Burman et al. (2014). Brachial spinal segments illustrated with motor pools colored according to their corresponding upper limb musculature as described by Watson et al. (2015), see text for details. Inset: Illustration of BDA labeling at C7 after injection in M1 as in Kondo et al. (2015).

Since these initial mappings of the marmoset somatosensory cortex, the topography of the marmoset cortical and thalamic motor systems has been mapped by electrical stimulation and tracer studies (Burish, Stepniewska, and Kaas 2008; Burman et al. 2008, [a] 2014, 2015;

Huffman and Krubitzer 2001a, [b] 2001; Krubitzer and Kaas 1990) that have been reviewed by (Bakola, Burman, and Rosa 2015). These studies have found that the gross organization of the cortical motor system of marmosets (Figure 1) is similar to that of both macaques and humans. For instance, as with both humans and macaques, marmosets possess an electrically excitable strip of agranular cortex, the primary motor cortex (M1), with a medial to lateral somatotopic progression of movements elicited by electrical stimulation of hindlimb, torso, upper limb, and face. Differences across species may however arise at a finer scale. For instance, there is evidence for a more fine-grained structure within the upper limb area of M1 in both macaques (Kwan et al. 1978; Park et al. 2001) and humans (Meier et al. 2008) such that distal representations of the wrist and fingers form a central core surrounded by a horse-shoe representation of the proximal segments of the elbow and shoulder. In contrast, in marmosets, there seems to be no clear proximal-distal organization of the upper limb representation at the scales examined so far.

Marmosets generally show less differentiated cortical fields than macaques and humans. In fact, Burman and colleagues (2008) suggest that the marmoset provides a model species with the constituents of anthropoid primate cortex in their simplest form. In macaques, rostral and caudal subdivisions of M1 have been described such that the caudal portion situated within the anterior bank of the central sulcus contains a greater density of corticomotoneurons with projections terminating in the motor pools of the cervical spinal cord (Rathelot and Strick 2009). A rostro-caudal subdivision of M1 has also been suggested on the basis of stronger proprioceptive input to the rostral portion of M1, and stronger cutaneous input to the caudal part of M1 (Picard and Smith 1992; Strick and Preston 1982). Current evidence suggests that corticospinal termination on motoneurons is relatively rare or absent in marmosets (Kondo et al.

2015). So it seems unlikely that there would be great enough variation in the distribution of these characteristics across motor cortex to justify a rostro-caudal differentiation of M1 on such grounds in marmoset.

Within somatosensory cortex (SI) immediately caudal to M1, area 3a receives deep muscle afferent signals conveying proprioceptive information about limb state (Jones and Porter 1980). In their review of area 3a in macaque monkeys, Jones and Porter (1980) question whether 3a projects to area 4. This uncertainty contrasts sharply with recent work done in marmosets that identified significant intracortical projections from 3a to M1 using retrograde tracers (Burman et al. 2014a). Within the cortex of both macaques and humans, area 3a is buried within the central sulcus (Krubitzer et al. 2004). This location has made it relatively difficult for investigators to access area 3a with electrophysiological recordings. In contrast, area 3a sits on the cortical surface of the marmoset brain and is accessible to both single electrode and multielectrode recording approaches (Huffman and Krubitzer 2001a). In fact, the first mapping of the entire somatotopic representation of deep sensory receptors in area 3a of any primate was done in the marmoset due to its lissencephalic cortex (Huffman and Krubitzer 2001a). Both marmosets and macaques also contain area 3b, caudal to area 3a, that responds to cutaneous stimulation and contains a somatotopic organization of the entire body that mirrors that of both M1 and area 3a (Leah A. Krubitzer and Kaas 1990).

The premotor areas of the marmoset have been mapped using cytoarchitectonic characteristics and the afferent projections to areas 6DC, 6DR, 6Va and 8C have been investigated using tracer injections (Bakola, Burman, and Rosa 2014; Burman et al. 2006, [b] 2014, 2015). Areas 6DC and 6DR both receive afferents from the anterior and lateral ventral complexes of the thalamus, and both receive substantial input from the medial premotor cortex

(Burman et al. 2014b). These thalamic connections within the marmoset were found to be largely consistent with those between thalamus and premotor cortex of macaque as described by Matelli and colleagues (1989). Based on their patterns of connections with other cortical and thalamic areas Burman and colleagues (2014b) conclude that there is a strong similarity between dorsal premotor areas 6DC and 6DR with the rostral and caudal subdivisions of dorsal premotor cortex identified in macaques (Barbas and Pandya 1987).

Some interesting differences do appear within marmoset parietal areas (Bakola, Burman, and Rosa 2014). A well-differentiated somatosensory area 2 does not seem to be present in the marmoset. Instead, (Paxinos et al. 2012) have defined an area 1 / 2 in marmosets suggesting that it likely incorporates homologs of both area 1 and area 2 of other primates. In macaques and cebus, a distinct area 2 is present and contains somatotopically-organized sensitivities to both cutaneous and deep muscle stimulation (Padberg et al. 2007). Single and multi-unit recordings in posterior parietal area 5 of macaque monkeys show sensitivity to both cutaneous and deep muscle stimulation of the hand and arm (Padberg et al. 2007) and implicate it in coordinate transformations during reach preparation (Bremner and Andersen 2014). With its prominent projections to M1 and 6DC, posterior parietal area PE should draw greater interest from investigators interested in the cortical contributions to motor control (Bakola, Burman, and Rosa 2014; Burman et al. 2014b).

The marmoset spinal cord has all of the features typical of a mammalian spinal cord. Watson and colleagues (2015) divided the cord both anatomically and functionally based on identifying the lateral motor column and the preganglionic column by staining for cholinergic neurons using acetylcholinesterase histochemistry and acetyltransferase immunohistochemistry. They found their subdivisions based on the distributions of cholinergic neurons to be in accord

with subdivisions of mouse and chicken spinal cord-based *Hox* gene expression during development (Dasen, Liu, and Jessell 2003). The motor neuron pools of the marmoset spinal cord are arranged in the musculotopic organization characteristic of other primates (Figure 1) (Watson et al. 2015). Though there is some inter- and intra-specific variation in the extent of the brachial spinal cord, specifically whether it spans from C4-C8 or C5-T1, the motor pools are similarly distributed throughout. In the rostral most segments, there are two main motor pools: a ventrolateral pool innervating the deltoids and a dorsolateral pool innervating the biceps. In the more caudal segments of the brachial cord there are four major motor neuron pools: two dorsal groups, one lateral innervating the forearm extensors, and one medial group innervating the forearm flexors; and two ventral groups, one medial innervating the pectoral muscles and one lateral innervating the triceps. In the caudal most segment of the brachial spinal cord, the motor pools come together into a single group of motoneurons innervating the hand. This proximal-distal organization of the motor neuron groups in the brachial spinal cord of the marmoset is very similar to that of the macaque monkey (Jenny and Inukai 1983), cat, rat and mouse (McHanwell and Watson 2009) suggesting that the organization of the motor pools is highly conserved across mammals.

Much of what we know about the contributions of motor cortical areas to upper limb movements comes from work with macaques. A great deal of effort over the last half century has been put toward answering the general question of what do cells in motor cortex encode. This work began in the 1960s with the demonstration of a relationship between the activity of cells recorded from M1 in macaques and the force produced during voluntary movements (Evarts 1968a). This work continued in the 1980s with studies in which macaques made two-dimensional reaching movements while single cells were recorded from the motor cortex (A.

Georgopoulos, Schwartz, and Kettner 1986). Motor cortical cells were found to exhibit directional tuning. These cells had higher firing rates when the animals moved their arms in certain directions rather than others and that the activity of cells within motor cortex could be well modeled by cosine tuning curves. Subsequent to these initial investigations, this basic result was extended to three dimensional reaching (A. P. Georgopoulos, Kettner, and Schwartz 1988), and these functional tuning preferences have been mapped across the motor cortex (A. P. Georgopoulos et al. 2007). In addition to force exerted during voluntary movement (Evarts 1968a) and movement direction (A. Georgopoulos, Schwartz, and Kettner 1986) the activity of motor cortical cells in macaques has been found to correlate to some degree with almost any conceivable movement parameter (Aflalo and Graziano 2007).

We know next to nothing about the cortical basis of upper limb movements in behaving marmosets. While there has been pioneering neurophysiological studies of the auditory and visual systems of behaving marmosets (Eliades and Wang, 2008; Roy and Wang, 2012; Wang et al., 2005; Mitchell and Leopold, 2015; Mitchell et al., 2014)), we have yet to characterize the functional properties of cortical motor areas within the context of actual behavior. Our knowledge of the organization of the motor cortical system contributing to upper limb control has been limited to mapping cortical fields associated with motor control using intracortical microstimulation in anesthetized preparations, tracer injections and cytoarchitectonic characterization (see Bakola et al. (2014) for a review). However, a few studies have assessed motor deficits in stroke or spinal cord injury models (Fujiyoshi et al. 2007; Iwanami et al. 2005). Looking beyond the cortical components of the motor system, a recent electrophysiological and histological study of the marmoset corticospinal system has shown that the marmoset corticospinal system lacks direct corticomotoneuronal projections to the motor pools in lamina

IX of the cervical spinal cord (Figure 1) (Kondo et al. 2015). These findings were anticipated by the consensus of the importance of corticomotoneurons to manual dexterity and the capacity to make relatively independent finger movements (Bortoff and Strick 1993; Lemon and Griffiths 2005).

Corticomotoneuronal projections are believed to play an important role in the ability that both humans and macaques possess for relatively independent finger movements (Porter and Lemon 1993). The majority of marmoset corticospinal projections to the cervical spinal cord were found to terminate in intermediate lamina VII (Kondo et al. 2015). Thus the bulk of cortical influence on spinal resources for muscle activation is mediated by spinal interneurons in the intermediate zone. Although some synaptic boutons from corticospinal fibers could be detected in lamina IX, no post-synaptic effects of medullary stimulation were observed at latencies consistent with monosynaptic corticomotoneuronal connections. Marmosets, therefore, force us to explicitly consider the influence of spinal interneurons on motor control.

1.4 Studying spatiotemporal dynamics of movement coding across multiple scales of motor cortical circuits

Marmosets offer a number of practical advantages as a non-human primate model species for the study of cortical function. Two advantages often mentioned by authors are that the marmoset brain is mostly lissencephalic and that there have been demonstrations of transgenic animals (Belmonte et al. 2015; Jude F. Mitchell and Leopold 2015; E. Sasaki 2015; E. Sasaki et al. 2009). These represent advantages for the study of motor systems as well. The mammalian motor system is distributed throughout the brain and body. Our understanding of the cortical contributions to movement began with single electrode recordings of motor cortical cells. While

much was learned about the functional properties of individual neurons during voluntary movements, any movement is certainly the result of coordinated activity across populations of neurons. With the capability to record from many neurons simultaneously has come the capacity to decode movement intention from neural recordings in a clinically useful way (Hochberg et al. 2006, 2012). These abilities, while certainly impressive, obscure the fact that our understanding of motor cortical functioning contains significant gaps.

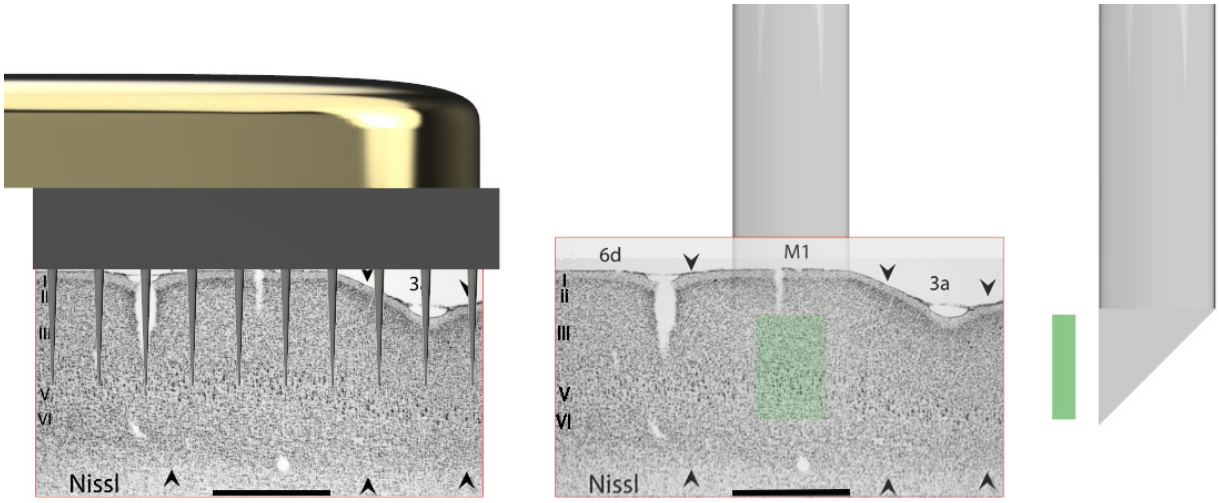

Figure 2 Marmosets offer an opportunity to interpolate across multiple scales of motor cortical functioning

The marmoset's relatively small and smooth brain will allow multielectrode arrays (left panel) to sample neural activity across multiple cortical fields central to the cortical contribution to motor control and calcium imaging techniques (right panel) to sample densely within a given region. Sagittal slices through marmoset sensorimotor cortex adapted from Burish et al. (2008). Scale bar 1 cm.

One gap in our understanding relates to the functional relationships of neural activity within and between cortical areas during ongoing movement. Marmosets offer the potential to build an understanding of how to interpolate the activity of neural populations sparsely sampled across millimeters of cortex using electrophysiological methods with activity sampled more

densely on the scale of a single cortical column using calcium imaging approaches (Figure 2). A second gap is that, based on extracellular recordings, we have little to no access to cell type or identity. This fact severely limits the depth of insight we can gain about a system containing a multitude of cell types. With the combination of its relatively small and lissencephalic cerebral cortex and the prospects for its genetic modification, the marmoset offers the potential to address these gaps in understanding.

The basic organization of the cortical fields are largely conserved across mammals with some species and clade specific adaptations (L. A. Krubitzer and Seelke 2012). However in macaques and humans, central components of the cortical motor system, about (half of M1 and all of 3a) sit within the central sulcus, which makes these areas more difficult to access for neurophysiological recording. As a result, there have been relatively few studies of a significant proportion of the M1 and of the entirety of sensory area 3a (see Krubitzer et al. (2004) Rathelot and Strick (2009) for two exceptions). The marmoset lacks a central sulcus, and as a result the entirety of these key sensorimotor areas is readily accessible to multielectrode array recordings and optical imaging. Furthermore, the small size of the marmoset brain allows for recording across multiple motor cortical areas at once with a typical multi-electrode array. A single well-placed Utah array could potentially record from all of the forelimb area of M1 and from much if not the entire adjacent corresponding upper limb region of sensory area 3a and premotor area 6DC (Figure 2). Simultaneous recordings covering the entirety of these three areas involved in the cortical coordination of the upper limb is unprecedented and will likely lead to insights into how these three areas function together to support skilled upper limb control. With genetic approaches, it should also be possible to untangle the contributions of direct intracortical communications from intracortical communication via the transthalamic pathway (S. M.

Sherman and Guillery 2011). We would predict such contributions to manifest in the distribution of latencies in measures of functional connectivity corresponding to direct cortico-cortical projections and trans-thalamic communication across cortical areas.

As solutions for expressing calcium indicators come online in marmosets, as they have in mice, we will be able to densely sample motor cortical circuits with single cell resolution at the scale of a cortical column (Sadakane et al. 2015). As a result, we will be able to begin bridging two spatial scales at which computations supporting upper limb control unfold: the scale of how multiple areas interact and how computation unfolds within a single cortical column (Figure 2). Additionally, imaging approaches provide the ability to unambiguously track the same neurons over multiple days (Ziv et al. 2013). These factors come together to provide an unprecedented platform for testing multiscale and longitudinal hypotheses in a non-human primate.

Transgenic approaches in mice have proven powerful tools in many areas of study. Genetic access affords cell type specificity, which extracellular multi-unit recording techniques do not provide. The history of our understanding of motor cortex is one of electrical microstimulation and electrophysiological recording. Much of what we know about the organization of the rest of the motor system was learned by electrically stimulating components of the system and recording downstream effects. There are some examples of functionally identifying cell types, such as corticospinal neurons through antidromic pyramidal tract stimulation and corticomotoneuron cells via spike-triggered averaging of muscle activity. While these approaches have been used to gain deeper insight into the nature of the cortical motor system (ex. Griffin et al., (2015); Merchant et al., (2008)), it is difficult to build an understanding of the primate motor system that acknowledges the diversity of cell types we know comprise motor cortical circuits. The ability to target specific genetically accessible classes of cortical

cells, for either recording or manipulation as now available in mice, would allow for a dissection of the primate motor cortical circuits at a resolution not currently available. We would ideally be able to develop cell type specific tools for dissecting the organization of local motor cortical circuits giving rise to the corticospinal tract (premotor, primary motor and sensory areas) and understand their role in the integrating long range inputs from posterior parietal, premotor and somatosensory areas to produce descending commands to the spinal cord.

1.5 Toward an integrative understanding of corticospinal engagement

Without a connection to the motor neurons of the spinal cord, the cortical motor system would have no capacity to move the body. Spinal interneurons are the target of the majority of corticospinal terminations even in animals that possess direct projections from cortex to the motor pools (H. G. J. M. Kuypers 1981; Yoshino-Saito et al. 2010). We know a great deal about the organization of the corticomotoneuronal component of the corticospinal system (Griffin, Hoffman, and Strick 2015; Porter and Lemon 1993). Apart from some very interesting work on the C3-C4 propriospinal system (Bror Alstermark and Isa 2012; B. Alstermark et al. 2007; Kinoshita et al. 2012), and some impressive electrophysiological characterizations of segmental interneuron activity (Fetz et al. 2002; Prut and Perlmutter 2003a, [b] 2003), we know comparatively little about the role of spinal interneuron in mediating corticospinal influence on motoneurons (Harel et al. 2008; Miri, Azim, and Jessell 2013).

Knowledge of the developmental origins of most spinal interneurons is growing and has allowed for the development of tools for genetically targeting the individual components of spinal circuits in the mouse (Catela, Shin, and Dasen 2015). The recent development of genetic approaches in mice has allowed the beginning of a much deeper understanding of the

organization of spinal interneuron circuits (Bikoff et al. 2016; Gabitto et al. 2016). We would like to suggest that the common marmoset offers the opportunity to isolate the cortical contributions to spinal interneuron-mediated motor control in that it has a corticospinal system where corticomotoneuronal projections are sparse or absent.

1.6 Conclusion

This brief review of marmoset motor system anatomy provides a picture of a primate expressing the basic musculoskeletal, cortical and spinal organization we recognize in the motor systems of Old World monkeys and humans. Differences lie in the extent of cortical field differentiation and corticospinal termination. In these differences we can find insights about the principles of motor system organization. Additionally the small size and smoothness of the marmoset brain will allow integration of our understanding of motor cortical functioning at multiple scales using both multielectrode array and imaging techniques in a way that has not been possible so far in a non-human primate. Furthermore, prospects for cell type specific access to interrogate and monitor the nervous system promise to deepen our understanding of motor cortical activity and its integration into the spinal circuitry for movement by spinal interneurons.

CHAPTER 2

DESIGN AND IMPLEMENTATION OF VOLUNTARY BEHAVIORAL TRAINING

2.1 Introduction and Motivation

There is growing interest in the marmoset as a model species for systems neuroscience, but the techniques for working with marmosets in this context are underdeveloped when compared to those used with more standard model species (e.g. rhesus macaques) in neuroscience research. The approach to training a macaque to perform an experimental task has remained unchanged for decades. It generally involves coercing the monkey into some sort of restraint device where it will stay for the next few hours engaging in a task that it has learned to do in exchange for water or juice. Each repetition of the task is rewarded with some quantity of water or juice. This method generally yields hundreds to thousands of repetitions of a given behavior over the course of a training session. It has been a very productive way to work with macaques, yielding many discoveries about neural function across multiple systems and to the development of promising clinical applications such as neuroprosthetics. However, the early behavioral work with marmosets suggests that this approach might be ill suited for working with marmosets (Eliades and Wang 2003, 2008).

Evidence that standard approaches restrict expression of marmoset behavior

Neuroscientists have been recording single neuron responses from macaques since the late 1960's, whereas the first reports of single neuron recordings from awake marmosets are not yet 20 years old (Evarts 1968b; T. Lu, Liang, and Wang 2001). In the early 2000's the lab of

Xiaoqin Wang at Johns Hopkins began developing techniques for performing neural recordings from awake marmosets. This group successfully investigated encoding of auditory stimuli using semi-restraint device adapted from the above described standard approach to working with macaques where the animals body movement is restrained by a chair and the head is fixed using some variety of head mounted post (X. Wang et al. 2005; Bendor and Wang 2005). The Wang group also did a great deal of work to characterize the quantitative structure of the marmosets' vocal behavior (Pistorio, Vintch, and Wang 2006; Miller and Wang 2006; DiMattina and Wang 2006). However, they report that their efforts to combine these to lines of research to ask questions about the sensorimotor cortical activity involved in self-initiated vocalization and processing were limited by the fact that the standard approaches to recording neural responses (i.e. head fixation and body restraint) negatively impact marmosets' expressions of vocal behavior (Eliades and Wang 2008b, 2003, 2005, [a] 2008). Wang (2000) describes, "The main limitation of existing neurophysiological methods is that vocal behaviors of marmosets is substantially restricted or eliminated once an animal is restrained." He concludes by saying the ideal way to do these sorts of neurophysiological recordings is wirelessly.

In a series of papers following these initial assertions Wang and colleagues describe the development of methods that will eventually allow them to study sensorimotor processing in freely vocalizing marmosets. The two main milestones are a technique for recording neural activity from a freely moving marmoset with the design of a tether management system (Eliades and Wang 2008b), and second the development of an approach to wireless neural recordings with marmosets (Roy and Wang 2012). At the time that we were developing our approach, these were the only published efforts for working with marmosets in systems neuroscience and they led to the conclusion that the standard approach to training non-human primates in neuroscience

research might not be optimal for marmosets if we wanted to study the neural control of voluntary movement

In-vivo imaging with voluntary head restraint

Around that time, Scott, Brody, and Tank (2013) published a paper describing a method for performing in vivo calcium imaging utilizing voluntary head restraint with rats. The methods included a custom head-bar to both frame the cranial window for two-photon imaging and provide the kinematic constraints for positioning the cranial window precisely in line with the objective for imaging. The animals were trained to poke their noses into a port for a reward. This nose poke would trigger a clamp to grasp and hold the head-bar. At first, the clamp would hold only briefly, but by end of an acclimation period the rats would tolerate having their heads clamped for upwards of 10 seconds. Once the animal was trained, the process of data collection could proceed without much experimenter intervention. Critically for this voluntary system to work, the rats must not develop an aversion to the recording setup. This sort of voluntary setup, that allowed the animal to engage in the experiment throughout the day as an expression of its normal behavioral repertoire, seemed like a promising approach to behavioral training of marmosets.

Marmoset ethology and its implications for experimental design

Marmosets are obligate gum feeders and prey species. Field studies estimate that marmosets spend about 30 percent of their waking hours feeding on exudates (Maier, Alonso, and Langguth (1982) in Sussman and Kinzey (1984)) and spend 25-30% of its waking time foraging for insects (Stevenson and Rylands 1988; Abreu et al. 2016). In order to feed on exudates, marmosets must gouge wounds into the trunks of trees to access the gum. They gouge

new holes and revisit previously gouged holes to feed on newly accumulated gum (Stevenson and Rylands 1988). These visits generally only last a few seconds (Stevenson and Rylands 1988), a similar duration to what was needed for the data acquisition in Scott, Brody, and Tank (2013) described above. Aside from grooming, the only behavior that have marmosets sitting still for prolonged periods of time is rest, which ones study estimates they spend about 50 percent of there day doing (Stevenson and Rylands 1988). There is nothing in their behavioral repertoire that has them sitting in one place engaging in a single behavior for multiple hours, however, there are reports of them engaging in a single behavior, stealing insects from ants, for multiple 2 to 3 hour periods (Sussman and Kinzey 1984). It is with this in mind that we designed an approach to training marmosets that would allow them to voluntarily engage in experimental behavior throughout their waking hours in short sessions.

2.2 Apparatus Design

Informed by the marmosets' natural behavioral repertoire as estimated by field studies (Sussman and Kinzey 1984; Stevenson and Rylands 1988), early work with marmosets in neuroscience (Eliades and Wang 2008b, 2003, 2005), and the novel approach to training and imaging developed by Scott, Brody, and Tank (2013), I developed a behavioral training apparatus that attaches to the marmosets' home cage. This apparatus allows marmosets to voluntarily engage in behavioral training throughout their waking hours.

Inspired by the gum feeding behavior, the first version of the apparatus trained the marmosets to assume the appropriate posture to receive a small volume of yogurt. This posture placed them in front of a tray that contained foraging substrate. The next major iteration of the apparatus dispensed with the yogurt reward and found that marmosets will still engage in

foraging behavior within the apparatus. The apparatus is subsequently used to record the kinematics of this foraging behavior to study sensorimotor cortical responses related to upper limb movement.

Design criteria

The three primary design criteria for the final apparatus were first that it is mountable to the home cage to allow for voluntary engagement in training throughout the marmosets' waking hours, second that it provides reliable positioning for marmosets and clear views of the upper limbs for capturing the kinematics of reaching movements, and third it needed to provide a flexible way to present different experimental tasks. To validate effectiveness of the apparatus as a training instrument, it also had to have a way to monitor and record the marmosets' behavior within it. And finally, to facilitate training using operant conditioning, the apparatus also had to include a method for precisely timed reward delivery. The three primary design criteria were implemented in all iterations of the apparatus, were as the last two criteria were only implemented in the first few iterations while validating the approach.

Hardware design and iteration

Designs of early versions of the behavioral training apparatus were done with 3D CAD software called SketchUp, while later versions were designed using AutoDesk Fusion 360 (Figure 3). The core of apparatus iterations was constructed using 1/8" or 1/4" clear acrylic sheets (continuous cast, McMaster Carr, Elmhurst, IL) that were cut into interlocking shapes using a laser cutter (Universal Laser Systems VLS4.60). These shapes were then assembled to achieve the form of the apparatus. To monitor the activity of the marmosets within the apparatus, I designed a simple circuit (Figure 4) that included two photocells (CdS -

photoresistor) and one infrared light based switch (IR switch comprising IR phototransistor and IR LED pair), a syringe pump (syringepump.com, NE-500) and a network-connected microcontroller (Arduino YÚN). These sensors acted as triggers to log the marmosets entering and leaving the gate, the belt and the collar of the apparatus. The sensor readings were logged to an SD card within the microcontroller, while the network connection of the microcontroller allowed for remote operation of the apparatus.

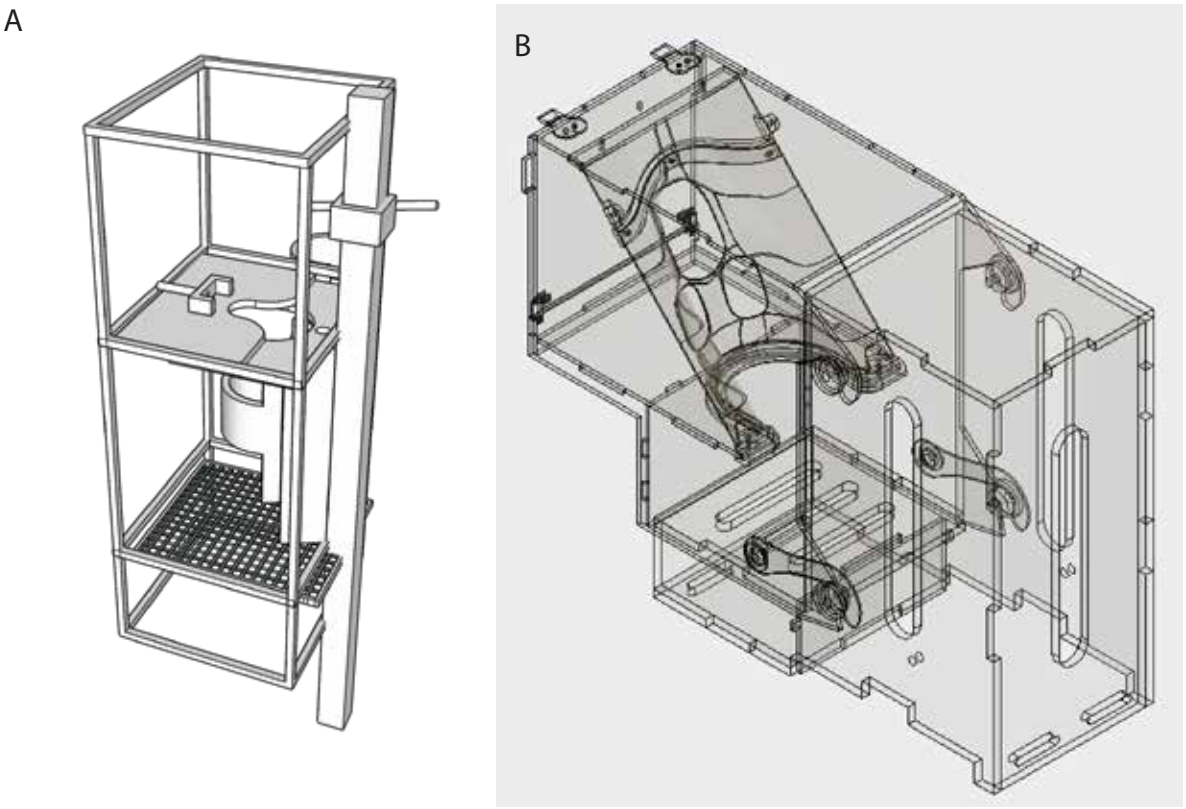

Figure 3 Developing a voluntary in-home cage approach to behavioral training with marmosets
A) Initial sketch of the behavioral training apparatus modeled after the semi-restraint device reported in Remington & Wang 2012, which itself was a miniaturized version of the standard chair used with macaque monkeys. Note the head and torso restraints.
B) Model of current version of behavioral training apparatus that allows for in-home cage voluntary behavioral training.

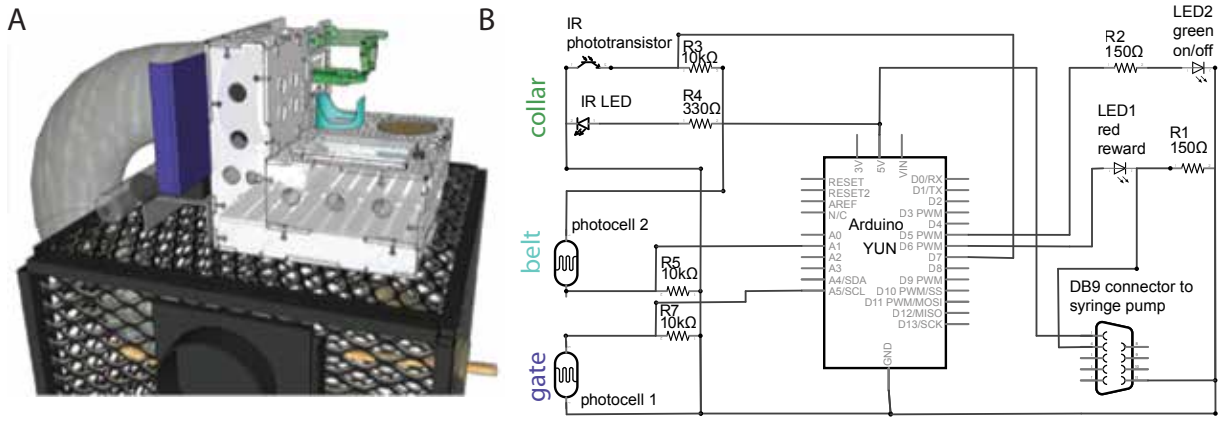

Figure 4 Design of the hardware for monitoring marmoset behavior within the behavioral training apparatus

A) 3D model of intermediate version of the behavioral training apparatus with sensors embedded into the gate (purple), belt (blue) and collar (green) to recording marmoset behavior within the apparatus throughout the day. Foraging tray is placed in front of the belt. Syringe pump connected to deliver reward for marmoset assuming the appropriate posture and placing head within collar. The whole assembly sits on top of the home cage. B) Circuit diagram detailing the circuit coordinating the logging of marmoset behavior and reward delivery.

Software for automating and monitoring training

A custom object-oriented library was written in C++ to coordinate the logging activity within the apparatus, evaluating reward-related contingencies, delivering reward and allowing remote apparatus operation. The object-oriented design of this library is meant to facility integration of experimental tasks that might be developed in the future.

Figure 5 Marmoset foraging within the behavioral training apparatus

2.3 Results

While one of the primary design criteria for the behavioral training apparatus is that it will flexibly support multiple experimental tasks, to start, we limited the experimental task to foraging, a natural behavior for marmosets (Figure 5). In the initial phases of validating the approach, this foraging behavior was coupled with the task of assuming an appropriate posture in exchange for yogurt reward. We found that marmosets will engage in behavior within the training apparatus throughout the day and that they are sensitive to reward availability (Figure 6). As a first step of analysis, we defined each time a marmoset enters the belt of the apparatus as the start of a session, and, each time a marmoset exits the belt, we call that the end of a session. We can then quantify the duration of these sessions as an estimate of how much time marmosets will spend engaging in behavior within the apparatus and how that behavior is distributed throughout their waking hours.

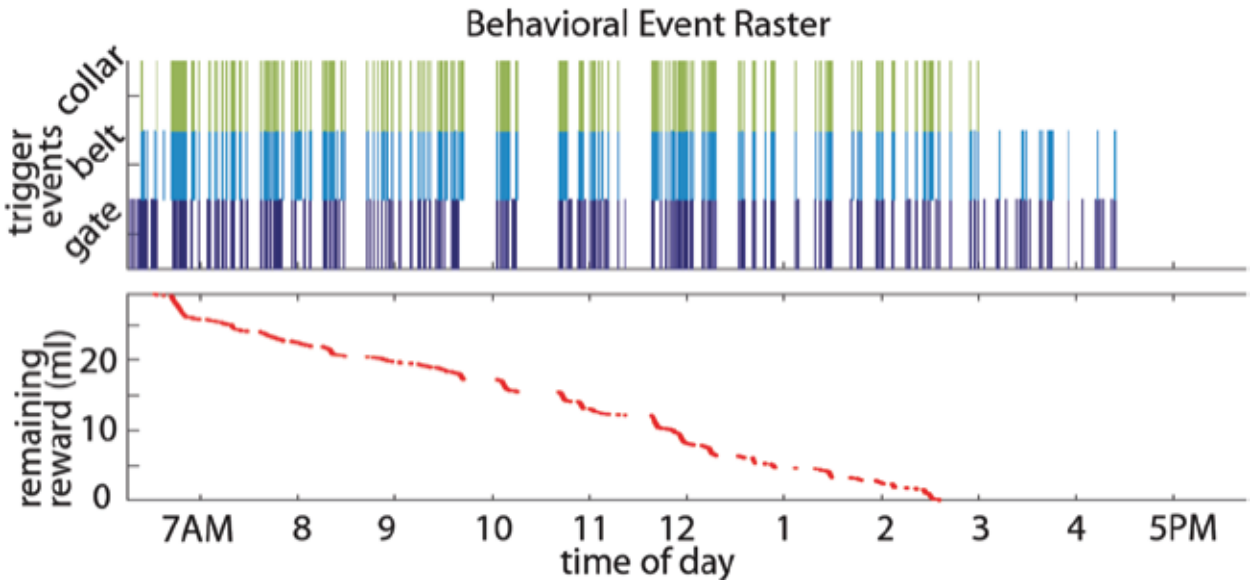

Figure 6 Results for single day of behavior within the apparatus

Vertical ticks indicate the time of trigger events for sensors within the gate, belt and collar. For instance a purple tick indicates the marmoset crossing the gate of the apparatus, a blue tick indicates the marmoset is within the belt of the apparatus and a green tick indicates that the marmoset has its head within the collar of the apparatus. When the marmoset stays within the collar for a few seconds, yogurt is dispensed as positive reinforcement for assuming the appropriate posture. Reward remaining is indicated in the bottom panel.

Over the course multiple days, we found that marmosets would generally spend around 10 seconds at a time engaging in sessions of behavior within the apparatus, but that some of these sessions would last up to five minutes (Figure 7). Scott, Brody, and Tank (2013) found 12 seconds of voluntary head restraint to be an adequate duration for collecting imaging data. I similarly took 12 seconds to be a threshold of adequacy to suggest marmosets would engage in behaviors within the apparatus for durations long enough for data collection. The majority of sessions of marmoset behavior within the apparatus were longer than that 12-second threshold (Figure 7), and again some sessions last up to five minutes. Marmosets' spent up to an hour each day engaging in behavior within the apparatus (Figure 8).

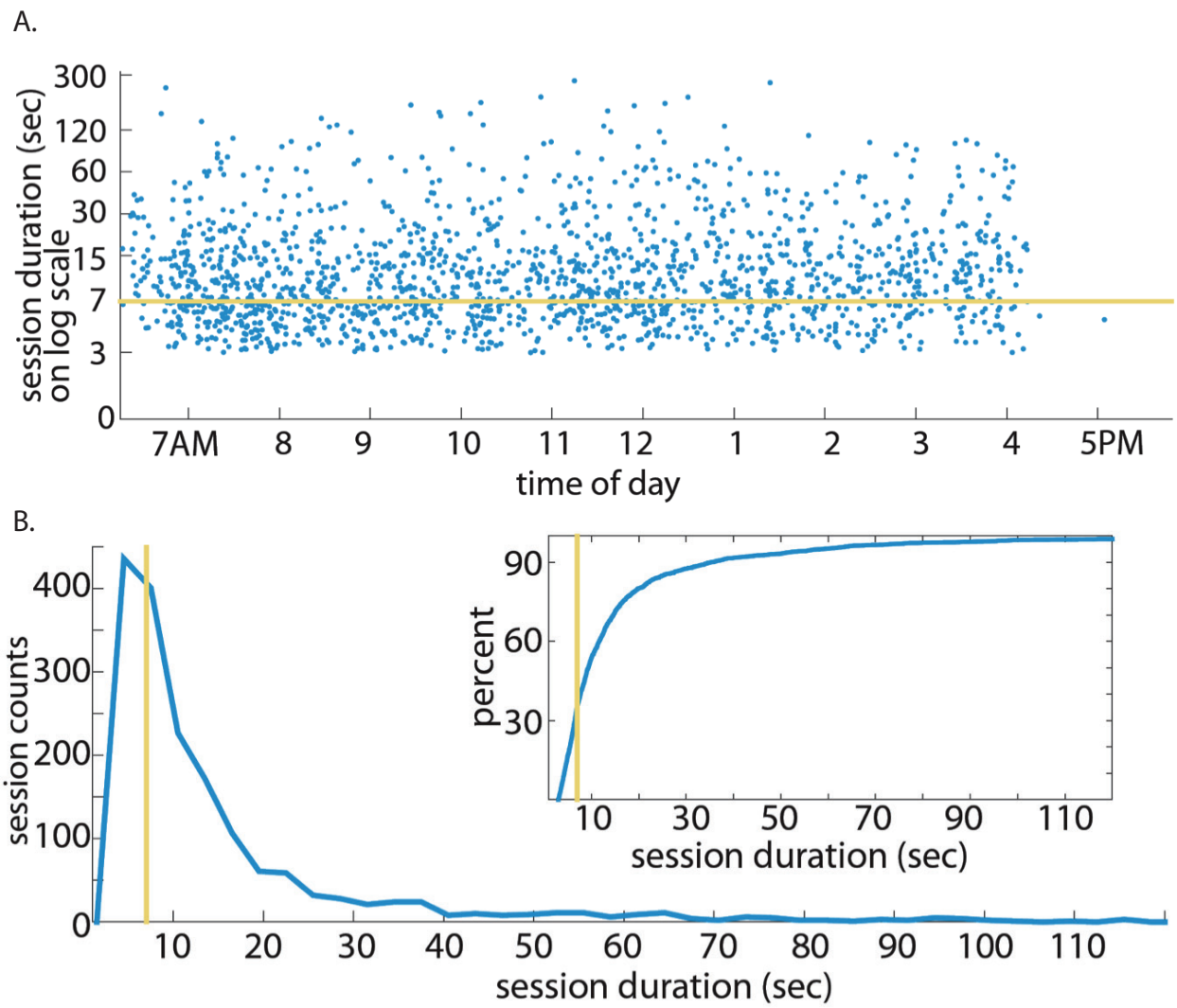

Figure 7 Summary of sessions of behavior within the apparatus pooled across days

A) Sessions pooled across 11 days. Each point represents a single session. B) Distribution of session durations displayed above. Inset: cumulative distribution of session durations. Yellow line in each plot represents the threshold of adequate duration for data collection estimated by Scott et al. (2013).

With the validation that marmosets would engage in this sort of voluntary behavioral training, and a sense of what sort of time we would have to employ experimental tasks, we refined the design of the apparatus to its current form (Figure 3B), and set out to do a basic characterization and optimization of foraging behavior within the apparatus. We used a custom-

written algorithm (Matlab) to define the video frame when the animal started foraging. The reaches were counted manually while watching the videos of these events. The numbers of reaches - for left and right-arm reaches – were entered by the observer after every event into a GUI and collected per hour of the experimental session (12 hours). When the foraging mix is provided in addition to the normal diet provided by husbandry we find that marmosets will perform between 20 and 80 reaches while foraging over the course of a day (Figure 9A). In contrast, if their daily diet is provided within the behavioral training apparatus, they will perform 100-300 reaches per day (Figure 9_B).

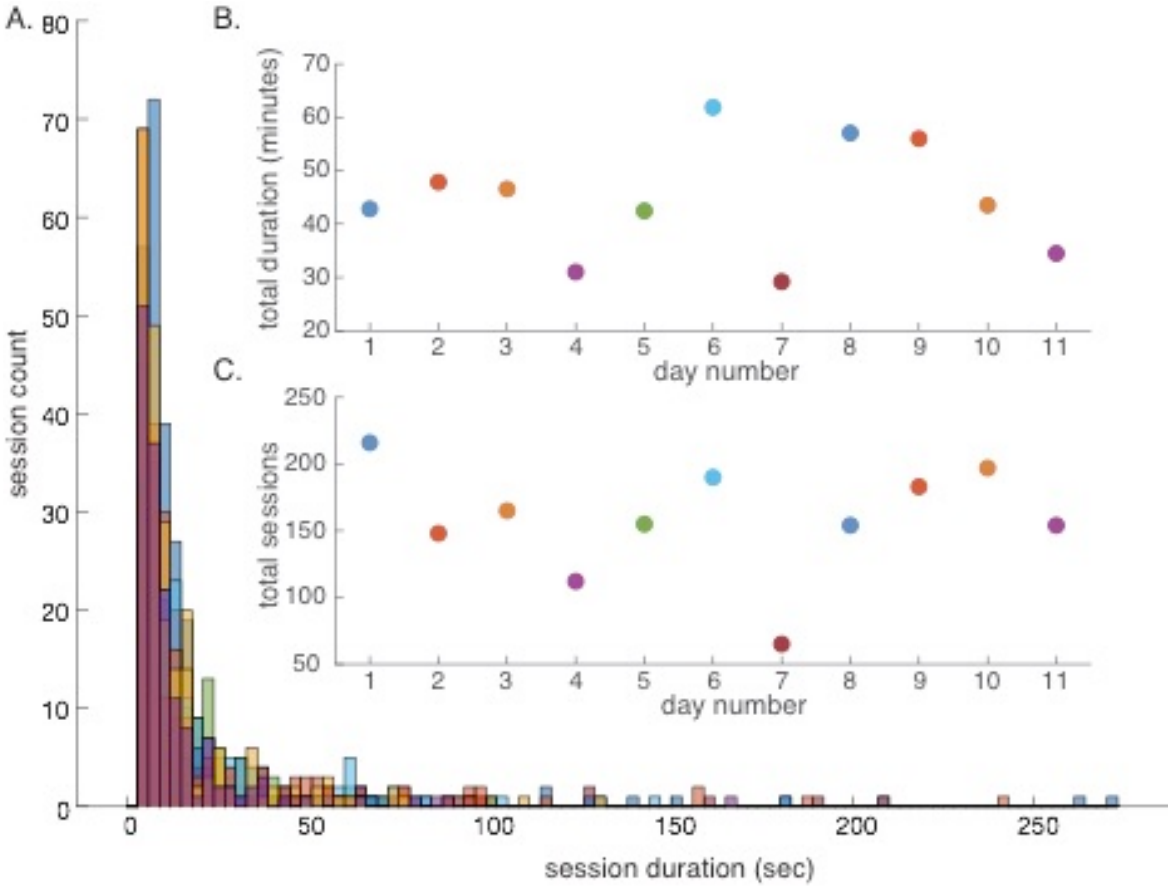

Figure 8 Summary of sessions of behavior within the apparatus broken down by day
A) Distribution of session durations for each of 11 days (same as in Figure 5, but not pooled). B) Total duration of all sessions for each day. C) Number of sessions of behavior for each day.

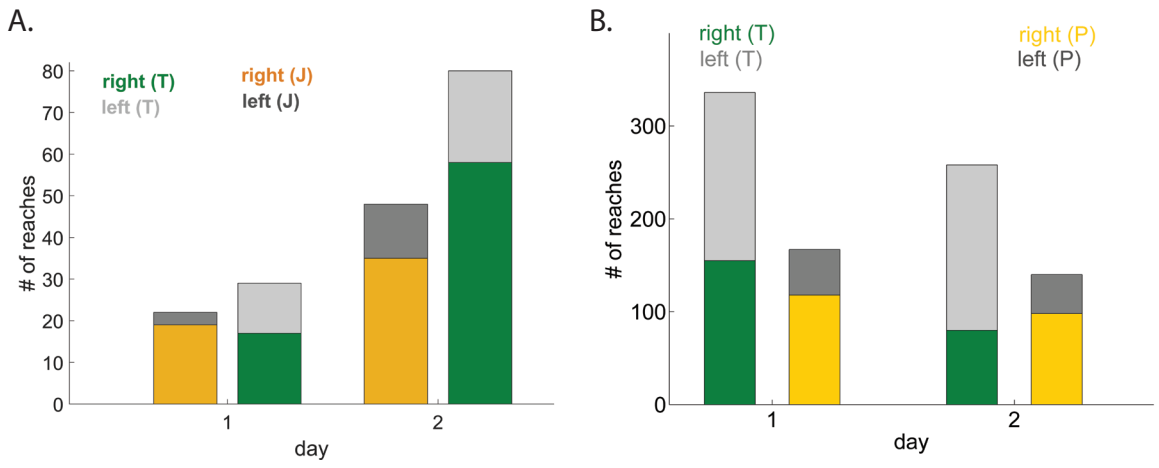

Figure 9 Counting of reaches during foraging behavior within the apparatus

A) Counts of reaches for two marmosets (T and J) across two days when foraging mix was provided in addition to daily diet. B) Counts of reaches for two marmosets (T and P) across two days when daily diet was provided within the training apparatus in conjunction with foraging mix. Note the different range for the y-axis of A) and B).

Recording upper limb kinematics during foraging with XROMM

With this behavioral training apparatus that allows us to elicit substantial quantities of self-initiated natural reaching behavior, we then developed an approach to record the kinematics of the upper limb during foraging. Our first efforts to do this were with the VICON system which tracks the 3D motion of retro-reflective markers placed on the surface of the skin. I designed and printed a bracelet that incorporated the retro-reflective markers needed for this method, and trained a marmoset to provide me her arm so that I could put on this bracelet using positive reinforcement (using methods similar to those for voluntary venipuncture used with macaques (Coleman et al. 2008)). Unfortunately, I was not able to train her not to remove the retro-reflective tape on the markers once the bracelet was on her wrist. With this suggestion that marmosets would not tolerate the markers needed for the VICON system, and with similar reports of this from other groups (Young, Stricklen, and Chadwell 2016; Takemi et al. 2014), we moved to using a bi-planar x-ray based system called XROMM, or X-Ray Reconstruction of

Moving Morphology (Brainerd et al. 2010; NSF MRI 1338066). This system allows us to reconstruct time varying joint angles by tracking the 3D position of radio-opaque tantalum beads (.5-1 mm, Bal-tec) placed within either bones or soft tissue. Using a set of tools developed at Brown University (Brainerd et al. 2010; Miranda et al. 2011; Knörlein et al. 2016) we can translate the position of these markers into the movement of rigid bodies and joint coordinate systems which allows highly precise analysis of joint kinematics (Knörlein et al. 2016).

We placed markers in the torso and upper limb subcutaneously using angiocatheters (16G, Becton, Dickinson and Company) (Figure 10B). The marker set illustrated allowed reconstruction of the seven degrees of freedom of the shoulder, elbow and wrist (Figure 10C). While the XROMM is most accurate when markers are placed in the bone, we did not place any markers in the bone due size constraints. For instance, the head of the ulna is about 1mm in diameter and clearly would not accommodate a 1mm marker. We thus did not consider scapula-thoracic movement. We found the following parameters to be adequate for XROMM data collection: 90 kV, 25 mA at 200 frames per second.

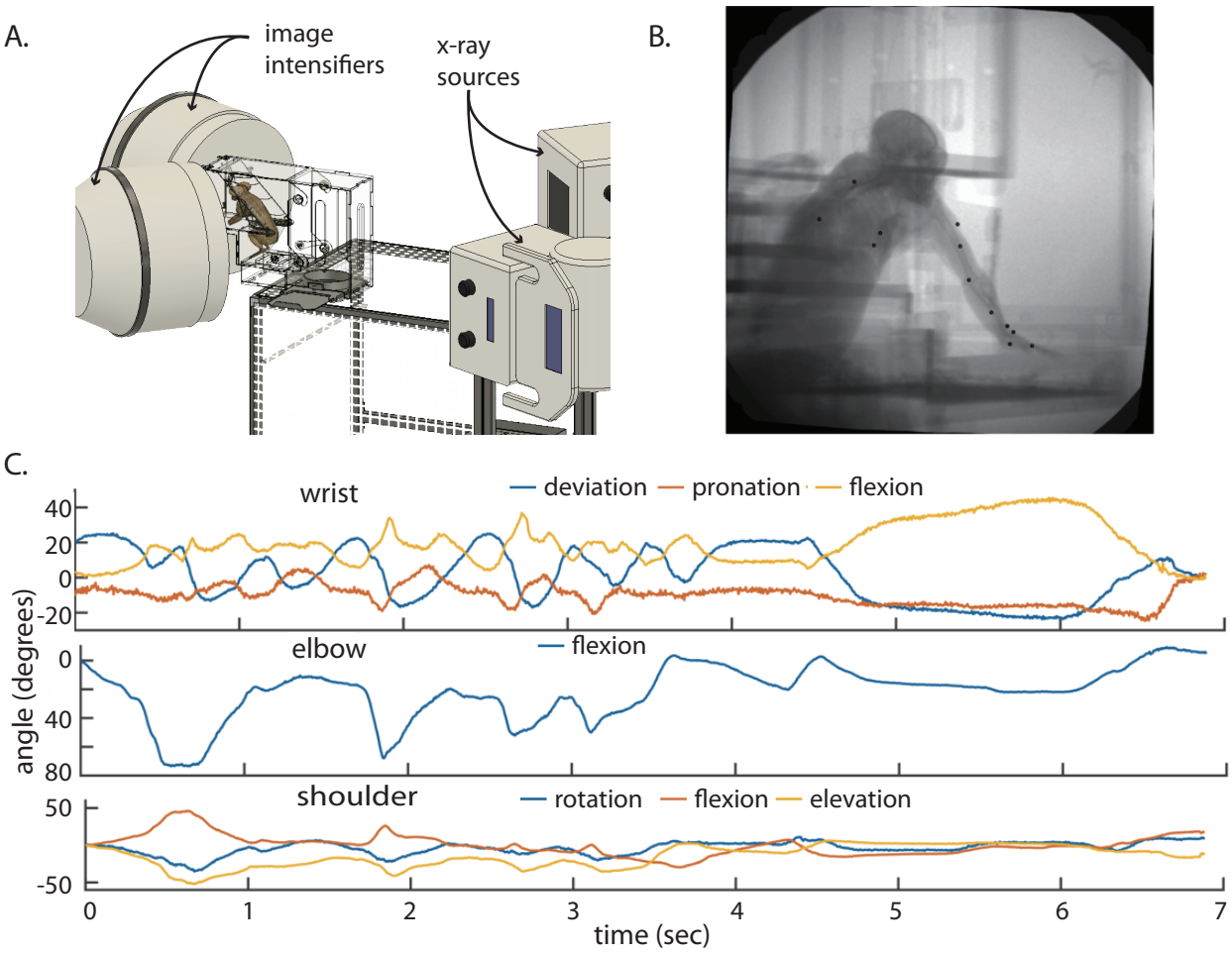

Figure 10 Capturing the kinematics of upper limb movements with XROMM

A) Illustration of XROMM bi-planar x-ray motion capture system with behavioral training apparatus and marmoset in the capture volume. B) Single frame of x-ray video of marmoset foraging within the apparatus. Note radio-opaque markers placed within the marmoset's torso and upper limb. C) Seven degrees of freedom of upper limb movement reconstructed by tracking the movement of the radio-opaque markers seen in B).

2.4 Discussion

Since the completion of this work, a few more studies have been published where marmosets were trained to perform some sort of experimental task. Mitchel and colleagues (Mitchell, Reynolds, and Miller 2014; Mitchell, Priebe, and Miller 2015) trained marmosets to perform eye fixation and a smooth pursuit eye movement task. Further, Prins et al. (2017)

trained marmosets to perform basic reaching tasks with, and Pohlmeyer et al. (2012, 2014) trained marmosets to perform a reaching task with a robotic arm as part of a study of neuroprosthetic skill acquisition. In each of these studies, standard approaches to training non-human primates in neuroscience were employed and yielded a modest amount of data. While Mitchel and colleagues (Mitchell, Reynolds, and Miller 2014; Mitchell, Priebe, and Miller 2015) found that marmosets would perform hundreds of trials of eye fixation in a given experimental session, when asked to perform a smooth pursuit task, only on average 50 trials were performed successfully. This estimate of the quantities of experimental trials performed per session is in line with a recent study reporting marmosets trained to perform reaching tasks using the standard approach adapted from work with macaques (Prins et al. 2017). In these studies, marmosets would perform about 50 trials over the course of experimental sessions that were around 50 minutes when sessions would be ended due to marmosets' declining levels of cooperation.

It is clear that marmosets do have the capacity to make a contribution to our understanding of systems neuroscience; they show sensitivity to reward contingency (Figure 4) and the receptivity to operant conditioning (Remington, Osmanski, and Wang 2012) and a strong potential for targeted circuit manipulations (E. Sasaki et al. 2009; Belmonte et al. 2015). They do however also demonstrate aversion to the traditional constraints placed on non-human primates in neuroscience (Eliades and Wang 2008b, 2005, 2003). While the standard approach does yield modest quantities of experimentally useful behavior as described above, the potential of this approach is limited by the extent to which an experimenter can make a marmoset comfortable with the constraints. That limit seems to be about 50 trials over about 50 minutes. In contrast, the techniques we designed sought to increase that limit by eliminating the use of restraint and making the experimental training apparatus available to the marmosets throughout

their waking hours. With this paradigm, our initial estimates suggest that we can at least double in not quadruple the quantity of experimentally useful behavior with the added benefit that this behavior is self-initiated rather then generated through restriction.

While it is promising that, within the apparatus, marmosets will voluntarily produce greater quantities of experimentally useful behavior than they would with the standard approach. We would very much like a solution to quantify the kinematics of behavior that could be implemented in the home-cage as well. We are able to reconstruct seven degrees of freedom of the shoulder, elbow and wrist during foraging behavior with the XROMM, but unfortunately we cannot use the XROMM in the home cage and we cannot practically use it throughout the marmosets' waking hours. To take full advantage of the quantity of experimentally useful behavior produced within the apparatus, we need a solution for capturing kinematics within the home-cage. We are currently working to develop a computer vision based motion capture solution that could be implemented using visible light cameras in the home-cage setting. We have captured datasets of simultaneous x-ray and visible light video so that we can use the accurate kinematic results of XROMM to validate and quantify the error of the approach developed with the visible light cameras.

2.5 Conclusion

The marmoset continues to gain popularity as a model species for systems neurosciences and those working with them are beginning to establish behavioral training techniques. Many of these methods are essentially miniaturized versions of the techniques that have become standard for working with macaque monkeys in neuroscience over the last half-century. There is some suggestion that these techniques might not be optimally suited for marmosets. We presented a

method for in home-cage, semi-automated, and voluntary behavioral training of marmosets that is possibly twice as productive as adaptations of the more traditional approaches. The method presented allows training multiple marmosets in parallel. It provides a flexible platform for a variety of experimental tasks and liberates the animals from excessive restraint and the trainer from tedious hands on training involvement. It additionally provides a platform for marmosets to self-initiate natural behavior in addition to engaging in more traditional operant paradigms. This flexible approach should allow us to contextualize results from constrained and over-trained experimental tasks within the space of the marmoset's natural behavioral repertoire.

CHAPTER 3

REFINING APPROACHES TO RECORDING NEURAL POPULATION ACTIVITY IN COMMON MARMOSETS

3.3 Introduction

Background and Motivation

The marmoset represents a promising model species for systems neuroscience (Miller 2017; Miller et al. 2016; Jude F. Mitchell and Leopold 2015; Walker, MacLean, and Hatsopoulos 2017; Hagan, Rosa, and Lui 2017; Oikonomidis et al. 2017). While there are studies of the marmoset brain dating back to the 1900s (Peden and Bonin 1947), we found only a single report of electrophysiology performed with an awake behaving marmoset prior to the year 2000. It described the chronic implantation of stimulation electrodes into the hypothalamus to elicit and study fight or flight responses (Lipp and Hunsperger 1978). Methods for recording neural activity from awake behaving marmosets have only been in development since the early 2000s (T. Lu, Liang, and Wang 2001). As a field, we are only beginning to develop the methodologies needed to work with these animals, and as such we have a unique opportunity to establish refined techniques for neural recording that could set the precedent for many years to come. For this reason, we chose to put a great deal of effort into developing a refined approach to performing neural recordings with marmosets. This technique involves the design and fabrication of a custom titanium pedestal for mounting the connector of the electrode array and a custom configuration of a wireless transmitter for transferring neural signals from the animal's head to the neural recording equipment. In what follows I will briefly describe current trends in cranial

implant approaches across species and current approaches to neural recordings with marmosets to provide context for the choices we made in the design of our approach. I will then detail the surgical planning and methodology design. Finally, I will present some preliminary results that our methods have yielded and discuss further plans for refinement.

Cranial implants in rodents, non-human primates and humans

Custom titanium implants have become the norm for human surgical procedures, such as cranioplasties and bone anchored prosthesis installations, where there is a need to attach a prosthetic to the bone (van Eck and McGough 2015; Cabraja, Klein, and Lehman 2009). The combination of CT scanning, 3D modeling and digital fabrication allows implants to be designed and fabricated to fit precisely a patient's anatomy (Parthasarathy 2014). This approach, in combination with surface preparations designed to promote osseointegration, yields favorable surgical outcomes with relatively few post-operative complications. In the time since the introduction, by Evarts (1968), of the cranial chamber technique for in-vivo single electrode recording with macaque monkeys, methyl methacrylate bone cements has become a fixture in the implantation of both recording chambers and head fixation equipment. However, the use of such bone cements has been shown to lead to implant instability and bacterial colonization (Johnston et al. 2016). Over the past 10 years, researchers working with macaques have begun to adapt the approaches used with humans to design and fabricate custom titanium cranial implants as an alternative to using methyl methacrylate based bone cements (Adams et al. 2007; Hacking et al. 2012; Lanz et al. 2013). Efforts to improve osseointegration through implant surface preparation have also yielded positive results for long term implant maintenance and animal health (Hacking et al. 2012; Adams et al. 2007). While it does seem that there may be movement toward higher standards of care for macaque cranial implants, the approach typical taken with rodent and avian

models, in contrast, is still to use bone cement to anchor neural recording and head fixation equipment (e.g. Dzirasa et al. 2011). As the field is establishing the marmoset as a model it seems sensible to draw from the higher standard of care when designing approaches to neural recordings that may set precedents for future work with this developing model species.

Neural recordings with marmosets

The history of neural recording with marmosets parallels the history of behavioral training described in the previous chapter in that it follows methods developed with macaques and was pioneered in the Wang lab at Johns Hopkins University. While anesthetized recordings had been done with marmosets since the 1980's (Aitkin et al. 1986), it wasn't until the early 2000's that methods were developed to perform recordings with awake marmosets (T. Lu, Liang, and Wang 2001). In this study, Lu and colleagues targeted auditory cortex around the lateral sulcus. For this, skin would be removed and temporalis muscle resected to expose the bone over the auditory cortex. In an initial surgery two posts would be cemented to the skull for later use in head fixation. In a second procedure, to prepare the skull for single unit recordings, the bone over auditory cortex would be covered in a thin layer of bone cement. In early studies, small holes would be drilled in this surface for single electrode penetrations (T. Lu, Liang, and Wang 2001). In later studies, these single electrode penetrations are replaced by an 4x4 array of movable electrodes, specifically the Warp 16 drive (NeuraLynx, Inc.) (Eliades and Wang 2008b). This electrode array assembly would then be cemented to the skull (Figure 14D). These techniques successfully afforded opportunities to ask questions about encoding of auditory stimuli (T. Lu, Liang, and Wang 2001; Bendor and Wang 2005; Barbour and Wang 2003). However, when they were applied toward addressing complementary questions about sensorimotor interactions during vocal production, these methods severely limited or eliminated

the self-initiated voluntary production of vocal behavior (Eliades and Wang 2003). Responding to this limitation, Wang and colleagues developed methods for recording from marmosets without head fixation normally imposed on non-human primates in neuroscience research. The first iteration of this process resulted in a tethered neural recording setup similar to those employed in work with rodent and avian models (Eliades and Wang 2008b). Though see Lipp and Hunsperger (1978) for a singular early example of this approach. While this method did increase the occurrence of vocalization, it was complicated by the fact that marmosets behave in three dimensions and have hands that can reach up to the tether. The second iteration of these methods, responding to marmosets' tendency to climb up and get tangled in the tether, dispensed with the tether in favor of a wireless system (Roy and Wang 2012).

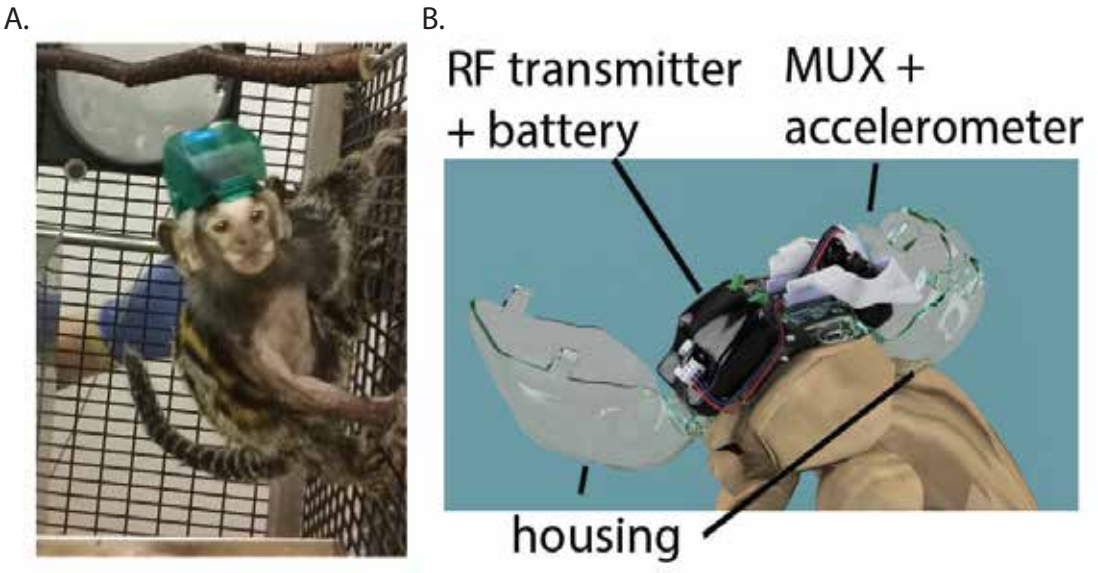

Figure 11 Modular wireless headstage for recording neural population responses from freely moving marmosets

A) Photograph of freely moving marmoset wearing wireless headstage during neural recording. B) Rendering of custom headstage configuration. 3D printed housing unfolded to illustrate headstage components within.

While both of these iterations represent improvements, we tried to carry these innovations forward with further refinements. In what follows, we will describe our efforts to further refine approaches to recording neural population responses with marmosets by developing 1) a modular configuration of a wireless headstage that allows us to minimize both handling and package size on the head of the marmoset (Figure 11) and 2) a potentially less disruptive cranial implant approach with improved potential for healing (Figure 12).

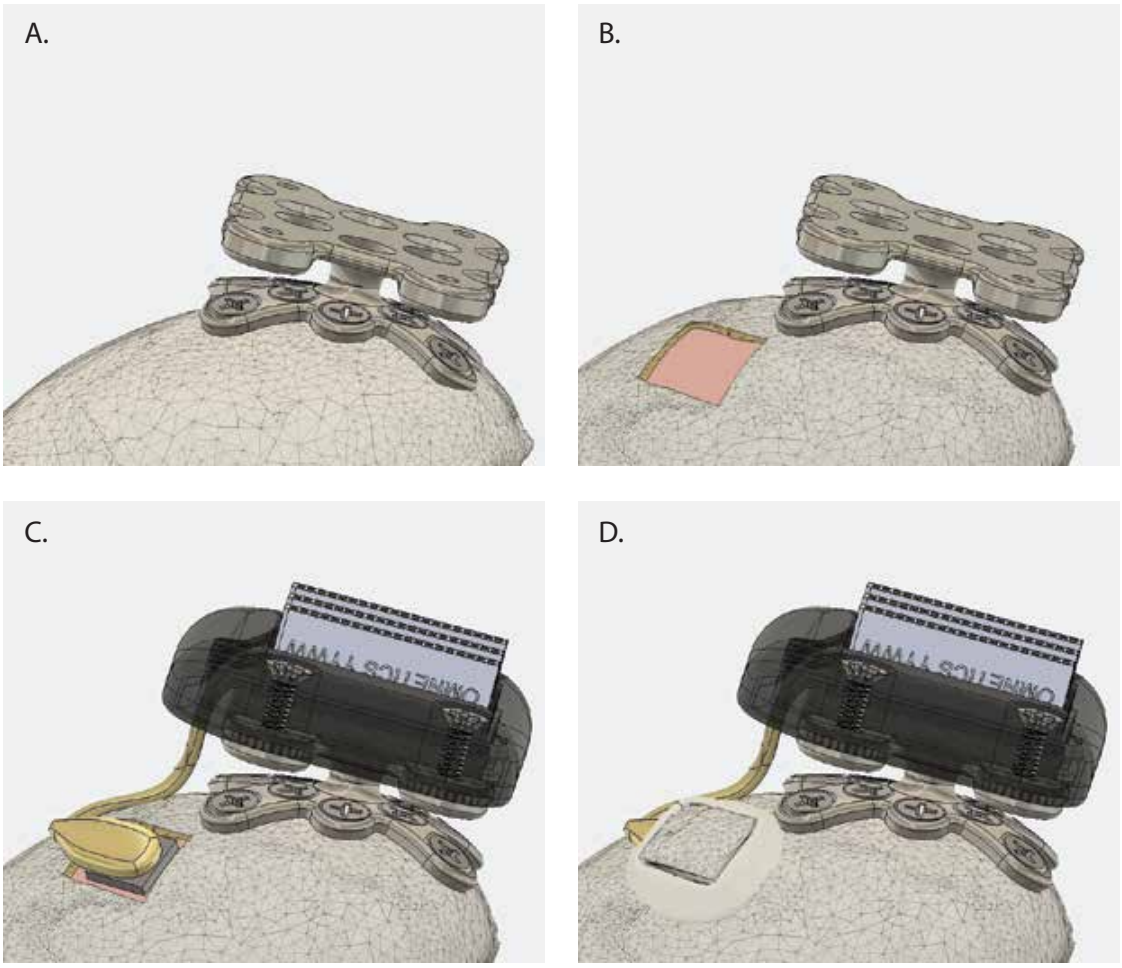

Figure 12 Illustration of proposed approach to implanting a Utah Array in marmoset sensorimotor and premotor cortex

Steps include: A) Install custom titanium pedestal. B) Perform craniotomy over sensorimotor and premotor cortex. C) Insert electrode array and fix array connector to pedestal. D) Close craniotomy with autologous bone flap, dural and bone substitutes.

3.2 Methods

Electrode Array

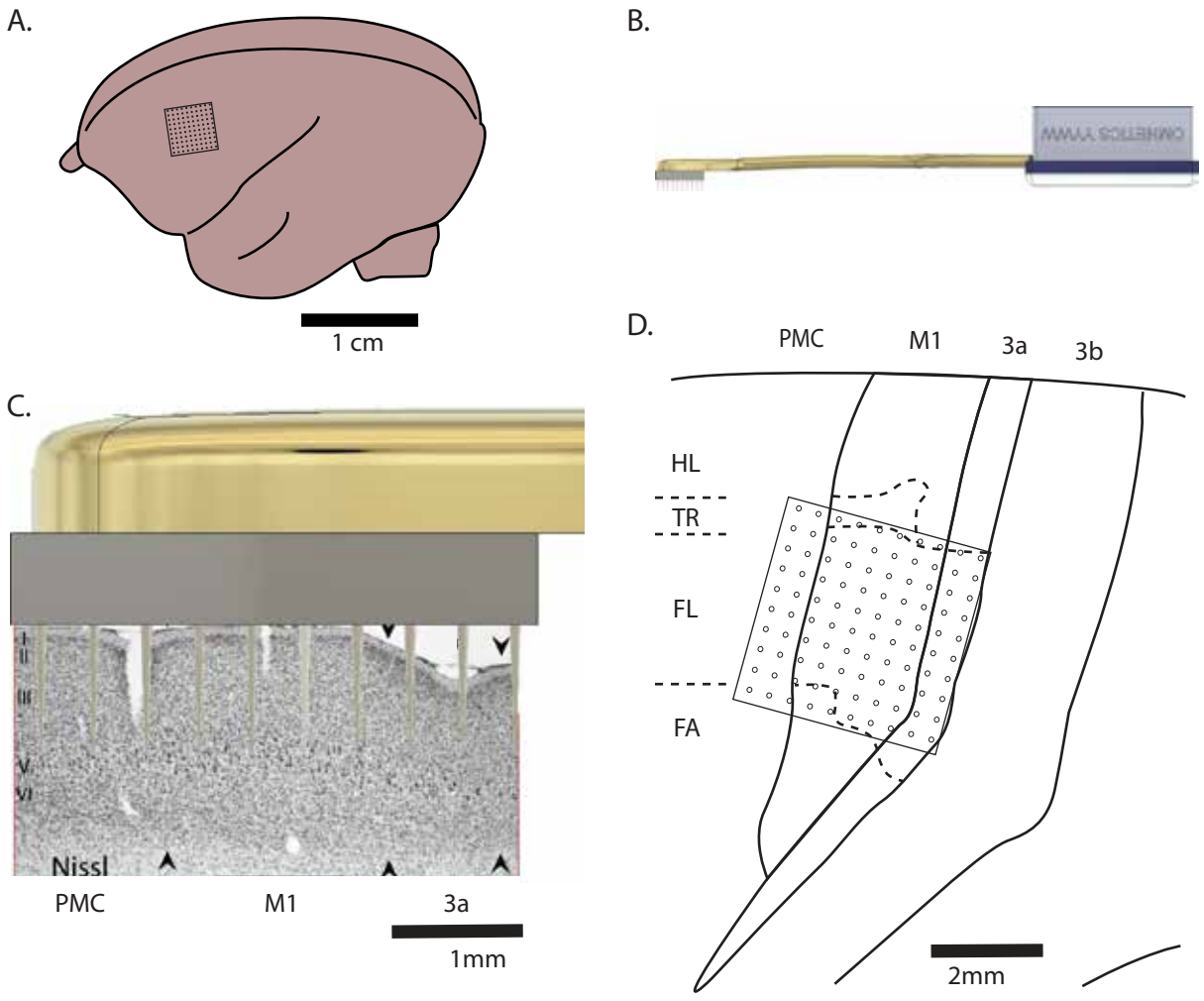

Figure 13 Relevant dimensions of the Utah array and marmoset sensorimotor and premotor cortices

A) Illustration of the marmoset brain with footprint of the electrode array targeting forelimb primary motor cortex (M1). B) Model of Utah array, wire bundle and Omnetics connector same scale as in panel A. C) Utah array with 1 mm electrodes placed within sagittal section of marmoset sensorimotor and premotor cortices. Sagittal section from Burish et al. (2008). D) Outlines of somatotopic organization of marmoset sensorimotor and premotor cortex with footprint of array targeting forelimb M1. Cortical area outlines redrawn from case 06-27 of Burish et al. (2008).

To target marmoset sensorimotor cortex for population recordings we chose to use the Utah Array (Blackrock Microsystems). This is a 10 x 10 grid of 1.5 mm long electrodes with an inter- electrode spacing of 400 microns allowing it to span a 4 mm by 4 mm section of cortical surface. The forelimb area of the marmoset primary motor cortex (fl-M1) is about 2 mm by 2 mm (Burman et al. 2008). Thus, if the center of the array is centered on fl-M1, the array will also span portions of adjacent primary sensory area 3a and premotor cortical area 6Dc (Figure 13).

Surgical Planning

While the Hatsopoulos lab has more than 20 years of experience using Utah arrays with macaques, this would be the first array implantation we would do with a marmoset. Further, there are no published reports of Utah array implantations with marmosets. We used three methods to gain some insight into marmoset specific considerations before performing an array implantation with one of our marmosets. First we arranged to perform a practice procedure with a marmoset that had been scheduled for euthanasia at the Wisconsin National Primate Research Center (WNPRC), second we performed CT scans of marmoset cadaver material obtained from the University of Illinois Urbana Champaign Department of Anthropology and the WNPRC to prepare surgical planning models, and third, we practiced the surgical plan on this cadaver material before the actual surgery.

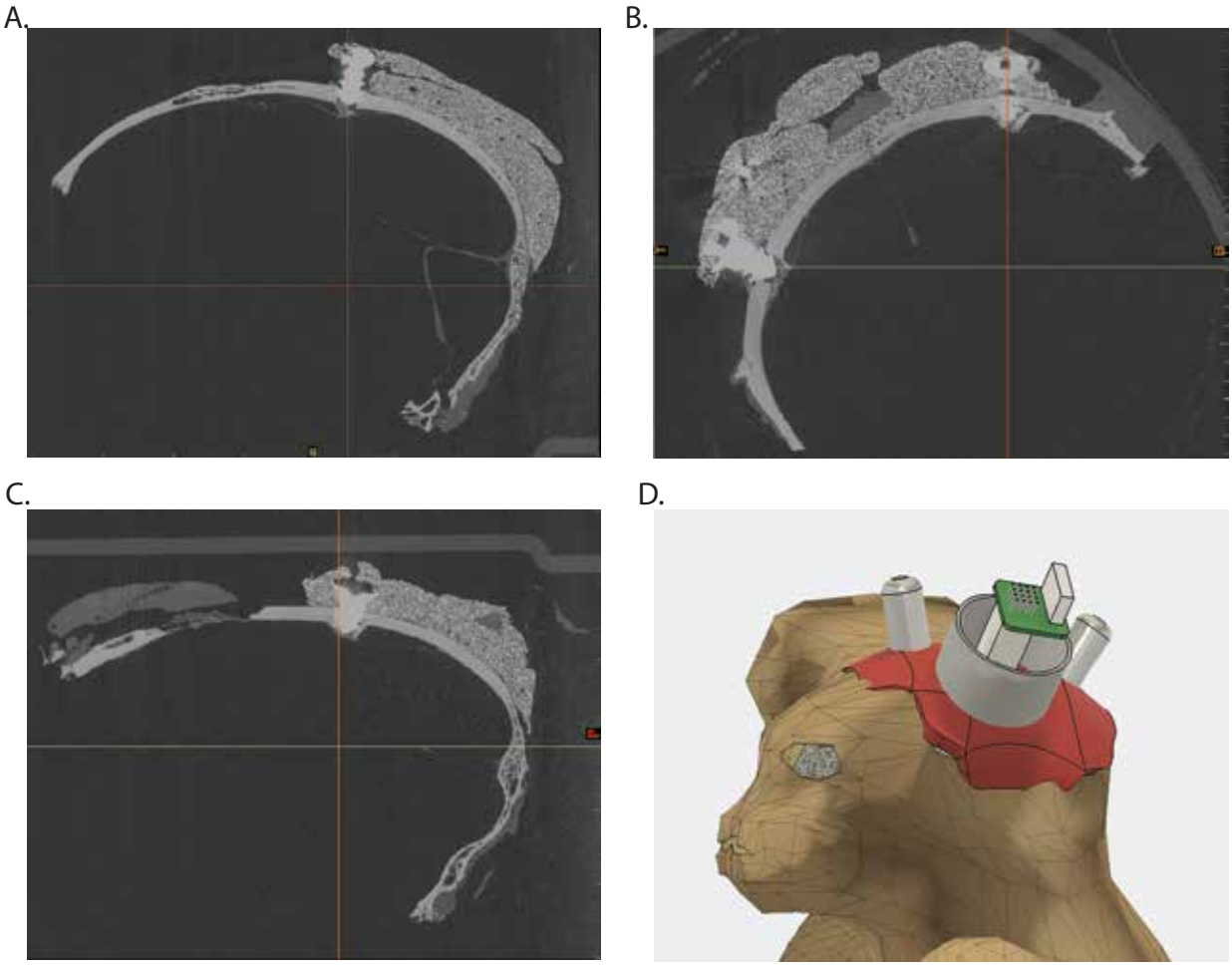

Figure 14 Standard approach to mounting neural recording equipment to the head of marmosets A-C) Sections through micro-CT scan of a marmoset calvarium with bone screws and cement used to anchor the connector for an electrode array. D) Rendering of multielectrode array recording preparation described by Eliades & Wang (2008).

For the practice surgery, the marmoset's skull was fixed within a stereotaxic frame and maintained at a surgical level of anesthesia by veterinary staff. All procedures described below were approved by the IACUCs on the University of Chicago or the WNPRC. We made an incision along the midline of the skull, located the site for the craniotomy using stereotaxic coordinates from both Paxinos et al. (2012) and Burman et al. (2014), and positioned the connector for the Utah array approximately where it was to be cemented to the skull. We then

installed two bone screws (Veterinary Orthopedic Implants) to anchor the cement for the connector. After applying the cement to the skull around the bone screws, we cemented the connector to the skull (Figure 14A-C). We then prepared a 6mm x 7mm craniotomy centered at 9.8 mm AP and 4.7 mm ML. After completing the craniotomy, we removed the dura within the craniotomy before inserting the Utah array with a pneumatic inserter (Blackrock Microsystems). We took a few lessons away from this practice surgery. First, using bone cement to connect the pedestal to the skull in this way is far from ideal: it leaves a sizable wound open to infection; there is significant potential for damaging the brain when installing the screws needed for this approach (Figure 14A-C); and there is no potential for cleanly removing the array should the need to de-instrument the animal arise. In addition, during this procedure, we experienced significant problems with swelling of the brain. This brain swelling will be discussed further below.

To prepare 3D models for surgical planning to address some the issues we uncovered during the practice procedure, we CT scanned post mortem marmoset material using the PaleoCT microCT scanner at the University of Chicago. These specimens were scanned with the 240kV tube at 140 kV and 50uA with a voxel size of 65.374 um. CT scans were segmented using Amira (ThermoFisher Scientific) on the Midway Computing Cluster of the University of Chicago Research Computing Center. The results of segmentation were exported as .stl files, which were then brought into Blender (Blender Foundation) for mesh post processing. Blender was used to establish the reference frame described by Paxinos et al. (2012) to locate and perform a virtual craniotomy for surgical planning. The post-processed .stl file was imported into Fusion 360 (Autodesk), where all subsequent designing took place. This workflow allowed

us to precisely define marmoset specific anatomical considerations, to plan the mechanics of the surgical procedure virtually and to design all of the tools necessary for the array implantation.

Anatomical considerations

A few features of marmoset cranial anatomy where key to surgical planning: skull thickness, lack of appreciable anatomical space between the brain and skull, and the geometry of temporalis muscle insertion. First, the dorsal aspect of the parietal bone and the posterior aspect of the frontal bone, the relevant cranial bones to these procedures, are about 1 mm thick (Figure 14A-C). This is relevant for pedestal installation, craniotomy preparation and array insertion. Second, there is little to no anatomical space between the internal surface of the skull and surface of the brain as you might find in a human or macaque. This means that the electrode array (Figure 13C), which would fit well within subdural space of a macaque, will bulge beyond the contour of the skull after it has been inserted in the marmoset. As such the standard approach to closing the dura over the array and replacing the bone flap used with macaques or humans is complicated (Fellows and Suner 2009). Additionally, due to the thickness of the skull, and the lack of anatomical space between the brain and skull, we had to take extra care not to damage the brain when installing the screws to attach the titanium pedestal to the skull (Figure 12A; Figure 14-C). To address these issues, we chose to use bone and dural substitutes to facilitate craniotomy closure and also developed a system for precisely installing screws into the parietal bone. Lastly, the forelimb area of primary motor cortex is about 5mm lateral to the midline in the marmoset. This meant that it might not be necessary to resect the temporalis muscle to access motor cortex and doing so just to expose enough bone for the cementing of the array connector seemed excessive and undesirable. These considerations further motivated the design of a titanium pedestal as an alternative to cement.

Surgical Approach

While the methods developed by Wang and colleagues to record population responses were certainly pioneering (Roy and Wang 2012; T. Lu, Liang, and Wang 2001; Eliades and Wang 2008b), we saw a few opportunities for further refinements. The choice of the Utah Array offers the potential of a much smaller footprint than the single electrode penetrations or the Warp 16 array used in the Wang lab (Figure 14D) both of which involve essentially replacing half the animal's scalp with a layer of acrylic. There are two connector options provided by Blackrock Microsystems for the Utah Array: the Cereport, which is intended for large primates, and a small animal connector comprising a PCB with three 32-channel Omnetics nano connectors (Figure 3, B). The first option has a transcutaneous diameter of 12mm, but the curvature of the skull-contacting surface would not permit adequate contact with the marmoset skull as it was designed to fit the much larger skulls of macaques or humans. The second option, as mentioned above, is meant to be installed by cementing the PCB to the skull of the animal (Figure 14A-C). This is the approach we took for the practice procedure, but, like the method developed by Wang and colleagues, it required us to remove most of the animal's scalp leaving a large wound open for infection and with little chance of healing. We chose to minimize surgical impact in subsequent procedures by designing a custom titanium pedestal to replace the bone cement and a surgical guide for carefully mounting this pedestal to the skull. Additionally, this design maximizes biocompatibility by employing hydroxyapatite preparations to promote osseointegration (Lanz et al. 2013). These three components, the titanium pedestal, the surgical guide and the hydroxyapatite use, are described in greater detail below.

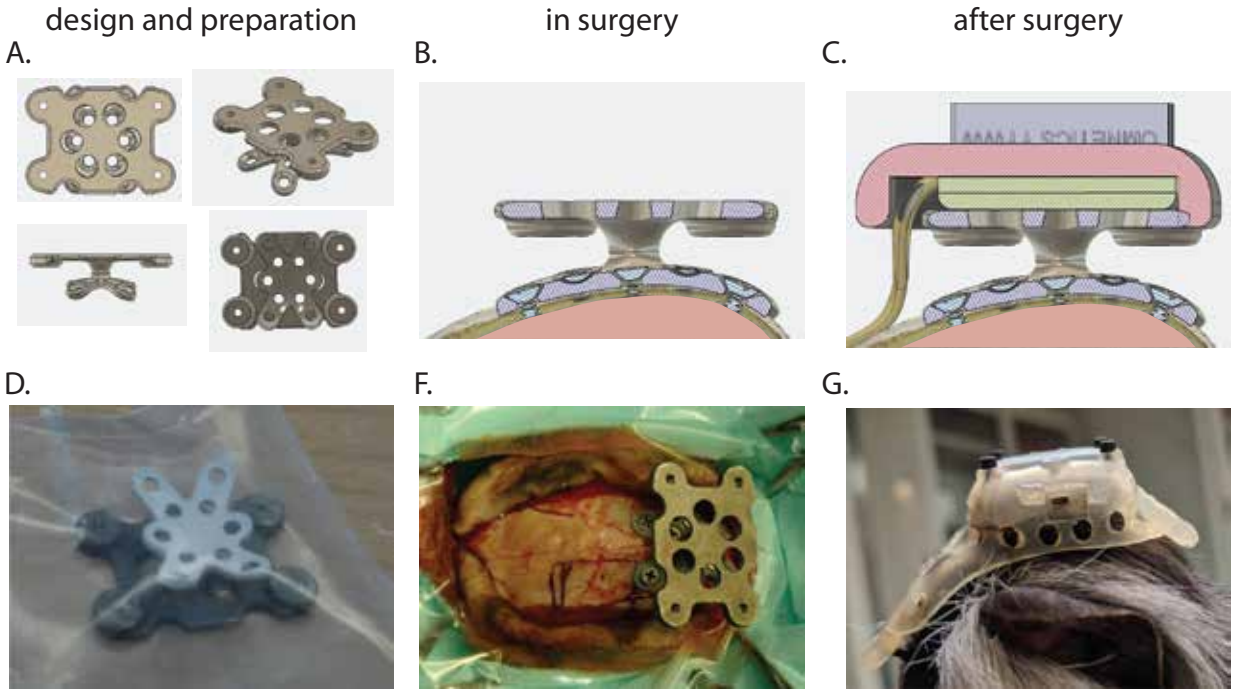

Figure 15 Pedestal design, implantation and housing

Top row (A-C) illustrates the design of the pedestal and 3D printed components for mounting the array connector. Bottom row (D-G) contains photos of pedestal and connector configuration corresponding to the illustrations above. A) Multiple views of pedestal model. Note 3 mm diameter stem connecting feet to platform and geometry of feet designed to respect temporalis muscle insertion. B) Cutaway view of pedestal anchored to skull with screws. Note thickness of the skull and screw penetration into bone. C) Cutaway view of pedestal with array connector and its fixture. D) Fabricated pedestal with feet coated with hydroxyapatite. F) Surgical photograph taken after installation of the pedestal. G) Photograph taken of the pedestal and helmet on the head of a marmoset.

The design of a titanium pedestal for mounting the array connector to the skull was motivated by a desire for an alternative to using bone cement for that purpose. We designed the pedestal such that its feet conformed to the contour of a reference marmoset skull and that the feet arrangement respected the insertion of the temporalis (Figure 15). While it perhaps would be useful to make custom contoured feet for each marmoset, at the time we did not have access

to a CT scanner appropriate for live marmosets, but we found that the contours of the parietal bones did not vary greatly across three reference skulls obtained from UIUC. We did have to do modest bending of the feet during surgery. In the future, it would be worth using the newly installed small animal CT scanner at the University of Chicago to make marmoset specific pedestals. In the cadaveric samples we observed, there was variability in the lateral distance of temporalis muscle insertions off the midline with most/all inserting more than 5mm lateral to the midline. The arrangement of the pedestal feet was designed as a compromise between providing a broad base for the array connector and avoiding the insertion of the temporalis muscles to reduce the chances of needing to retract these muscles in surgery.

The feet of the pedestal are connected to the platform for the array connector by a short stem just 3 mm in diameter. As a key part of the design, this stalk allows for a minimal diameter of the transcutaneous portion of the pedestal, which should reduce the chance of infection and skin retraction. Above the stalk is the platform on which the array connector sits. There are two sets of holes in the platform: one central set of six unthreaded holes for accessing the cranial screws and a second set of four threaded 0-80 holes for mounting a custom 3D printed fixture for the array fixing the array connector onto the platform.

Once the pedestal design was complete, it was sent out to be fabricated in titanium (Ti64) using direct metal laser sintering (DMLS) (by Proto3000, Ontario, Canada). This process uses a laser to melt, or sinter, powdered metal one layer at a time to fabricate metal parts with complex geometry that would otherwise be difficult to fabricate using traditional machining techniques. After fabrication, the resulting DMLS produced pedestal surface was mostly smooth aside from roughness where touch-points of supports had been during fabrication. These rough points happened to be on the dorsal surface of the feet, precisely where they might irritate or abrade the

skin above the feet. As such, I hand filed this surface until smooth. The only other machining we needed to do by hand after DMLS production of the pedestal was to thread the holes (0-80) for mounting the fixture for the array connector. After filing the top surface of the feet and threading the screw holes for mounting the fixture, the pedestal was sent out to have the feet coated with hydroxyapatite using plasma spraying (APS Materials, Inc., Dayton, Ohio) (Figure 15D). Hydroxyapatite coating of custom titanium implants has become a common approach to promoting osseointegration of human orthopedic implants (Tsikandylakis, Berlin, and Brånemark 2014) and has recently been adapted for use with macaque cranial implants (Chen et al. 2017; Adams et al. 2007; Lanz et al. 2013), but to our knowledge we are the first to adapt this approach for marmosets.

Surgical guide design and bone screw installation

As the marmoset skull is just 1 mm thick and there is no appreciable space between the skull and the brain, we took care to minimize the chance of damage to the brain when installing the cranial screws necessary to mount the pedestal. The feet of the pedestal are 1 mm thick and we wanted the head of the screws to sit flush with the dorsal surface of these feet to minimize the chance of skin erosion. As such we had about 2 mm of working distance assuming the pedestal feet are flush with the skull. We chose 1.4mm diameter and 2 mm long titanium screws (grade 2, DIN 965) (ChinaTiScrews, Jiangsu, China). These screws have a thread pitch of 0.3 mm, which should allow three threads to implant in bone. To create a set of surgical screws we manually filed the tips of these screws to obtain a finer range of lengths from 1.6 – 2.4 mm. We also prepared some 1.6 mm diameter screws as potential rescue screws. While the options available for bone screws of this size were not plentiful, the screws described above did seem

appropriate, the challenge then was to develop a method for installing them without damaging the underlying cortex.

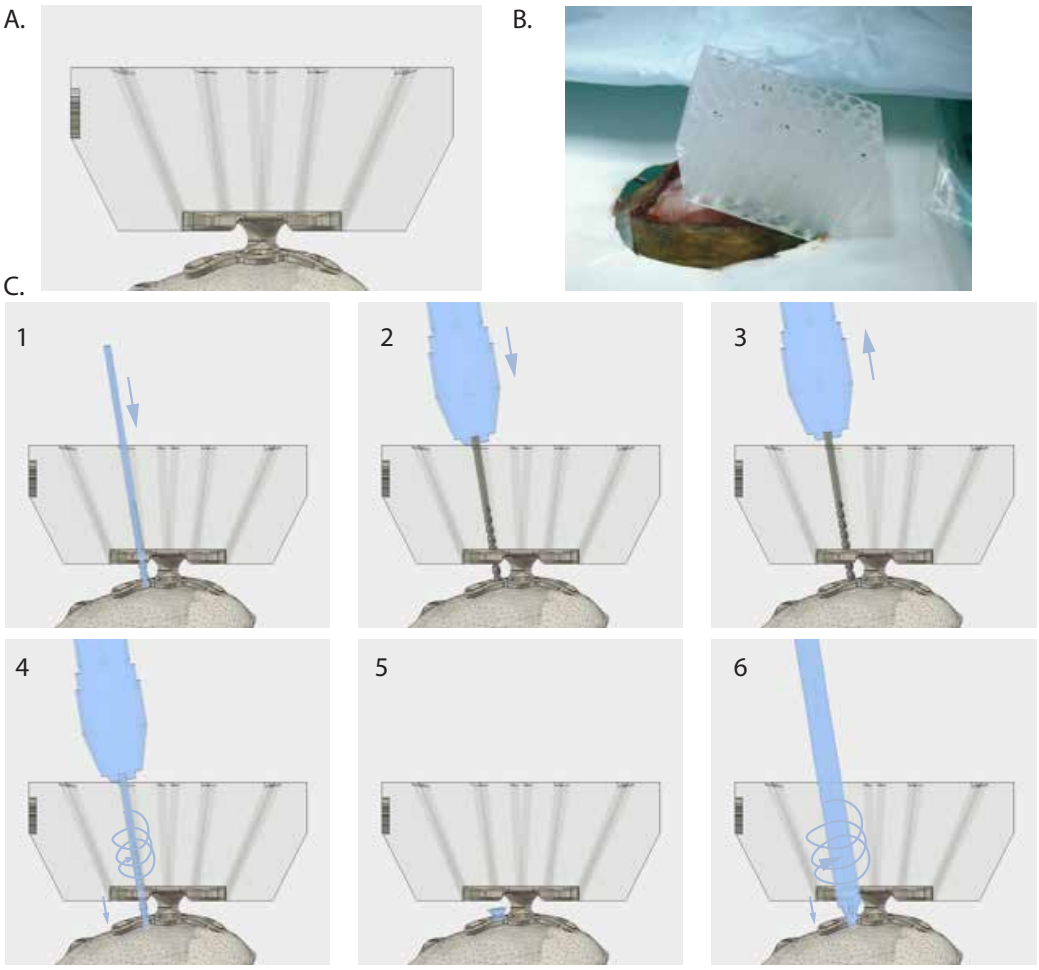

Figure 16 Surgical guide and bone screw placement for pedestal implantation

A) Model of surgical guide mounted on top of the pedestal. Tool paths passing diagonally through the body of the guide allow alignment of drill and screwdriver with the holes in the feet of the pedestal such that they are perpendicular to the skull at the point of contact. B) Surgical photograph taken after the surgical guide was placed onto the pedestal. C) Sequence of steps used to install each bone screw: 1) Insert drill bit into tool path of guide. 2) Grab bit with pin vise such that the jaws of the pin vice are flush with the top of the bit and the tip of the bit is contacting the bone. 3) Remove the bit from the tool path with the pin vice. Loosen the jaws of the vice, extend the bit just under 1mm and retighten the jaws of the pin vice. 4) Replace the bit into the same tool path. While applying constant, but not excessive downward force, rotate pin vice to drill a hole. 5) Place screw above the hole. 6) Insert screw driver into tool path and, while applying constant downward pressure, rotate screw driver about three turns such that the head of the screw is flush with the dorsal aspect of the pedestal foot.

The approach we took was to design a surgical guide that would allow us to hold a drill bit and screwdriver at an appropriate and constant angle and that would limit the depth of the hole drilled to less than 1 mm (Figure 16). This surgical guide was designed to sit on top of the pedestal and align these hand tools with the holes in the feet of the pedestal. After mounting the guide to the top of the pedestal, to prepare each hole for subsequent bone screw placement, we did the following: 1) We inserted a 1.2 mm drill bit (# 0565A215, McMaster Carr) into one of the holes in the guide such that the tip of the bit contacted the surface of the skull and the other end of the bit extended beyond the top surface of the guide. 2) We placed this end of the drill bit into a pin vice such that the jaws of the pin vice made contact with the top surface of the surgical guide and closed the jaws to clamp the drill bit. 3) We lifted the pin vice with the bit clamped out of the surgical guide, and loosened the jaws of the pin vice enough to be able to extend the bit just under 1 mm out beyond where it was clamped. We replaced the pin vice and bit back into the hole of the surgical guide from which it had just been removed such that the jaws of the pin vice now sat just less than 1 mm above the top surface of the surgical guide. 4) We then used the pin vise to drill a hole of a depth limited by the distance between the jaws of the pin vice and the top of the surgical guide. 5) With the hole prepared at to the appropriate depth, we removed the drill bit, placed a screw at the opening of the hole. 6). Lastly we use a screw driver (#261, Wiha tools, MN, USA), taking care to apply constant but not excessive force while turning the screw driver no more than three turns, to install the screw three threads deep.

Bone substitute for craniotomy closure

As the pad supporting the electrodes of the Utah array is about 2 mm thick, once implanted, this pad will extend about 1mm beyond the outer surface of the skull (Figure 12C). As a result, any attempt to replace the bone flap removed during craniotomy preparation is

complicated by a gap of at least 1 mm between the skull and the bone flap sitting on top of the array pad. To address this issue, we chose a hydroxyapatite based bone substitute, HydroSet Injectable HA Bone Substitute (Stryker, MI, USA), to bridge that gap (Figure 12D). Our results were mixed due to a number of factors that we will discuss in the surgical outcomes below. In the future, we may use the same material with an updated application method sold as DirectInject (Stryker, MI, USA).

Custom helmet

We designed and 3D printed a helmet to protect both the craniotomy site and the sutures during the postoperative period (Figure 15G). The helmet sits on top of the fixture for the array connector and allows access to the array connector while preventing the marmoset from manipulating the craniotomy site, the wire bundle for the electrode array and the sutures used for closing the initial midline incision in the weeks after surgery. The helmet is perforated to promote airflow and wound healing. In addition to protecting the surgical site, this helmet provides a surface for subsequent mounting of the wireless headstage components for neural recordings.

Wireless recording approach

With the design of the wireless headstage setup we wanted to minimize firstly, the height of the assembly on the marmoset's head as well as the amount of handling necessary to mount the headstage onto the array connector. Three 32-channel Omnetics nano connectors comprise the connector for the Utah array (Figure 13B). Mating with this connector requires aligning many small pins and then applying a fair amount of force. As such we sought a headstage configuration that did not necessitate that we mate and un-mate this connector before and after

each recording session. To achieve these aims, we worked with a company called Triangle Biosystems to arrive at a custom modular configuration of their W64 headstage, capable of transmitting 63 channels of neural signals (Figure 11). We separated the headstage into two packages. The first package contains the multiplexer and accelerometer boards. This package attaches to the array connector with two 32-channel ribbon cables, and sits on the back of the marmoset's head during periods when we are performing neural recordings. For instance, if we are planning to do recordings each day of a given week, we will attach these ribbon cables to connect the array connector to the multiplexer/accelerometer package and mount this package to the helmet at the beginning of the week. This package will then remain on the head of the animal throughout the week. After we are finished with the final recording session, we will remove the multiplexer/accelerometer package from the marmoset's head. The second package contains the wireless transmitter board and a 180 mAh battery. This battery provides power for about four hours of recording after which it needs to be recharged. We configured the headstage such that the multiplexer/accelerometer package connects to the transmitter/battery package with a single three-pin (power, ground and signal) cable. When we need to recharge the battery, we only need to mate this three-pin connector, which requires considerably less force and precision than mating the 32 pin Omnetics nano connectors.

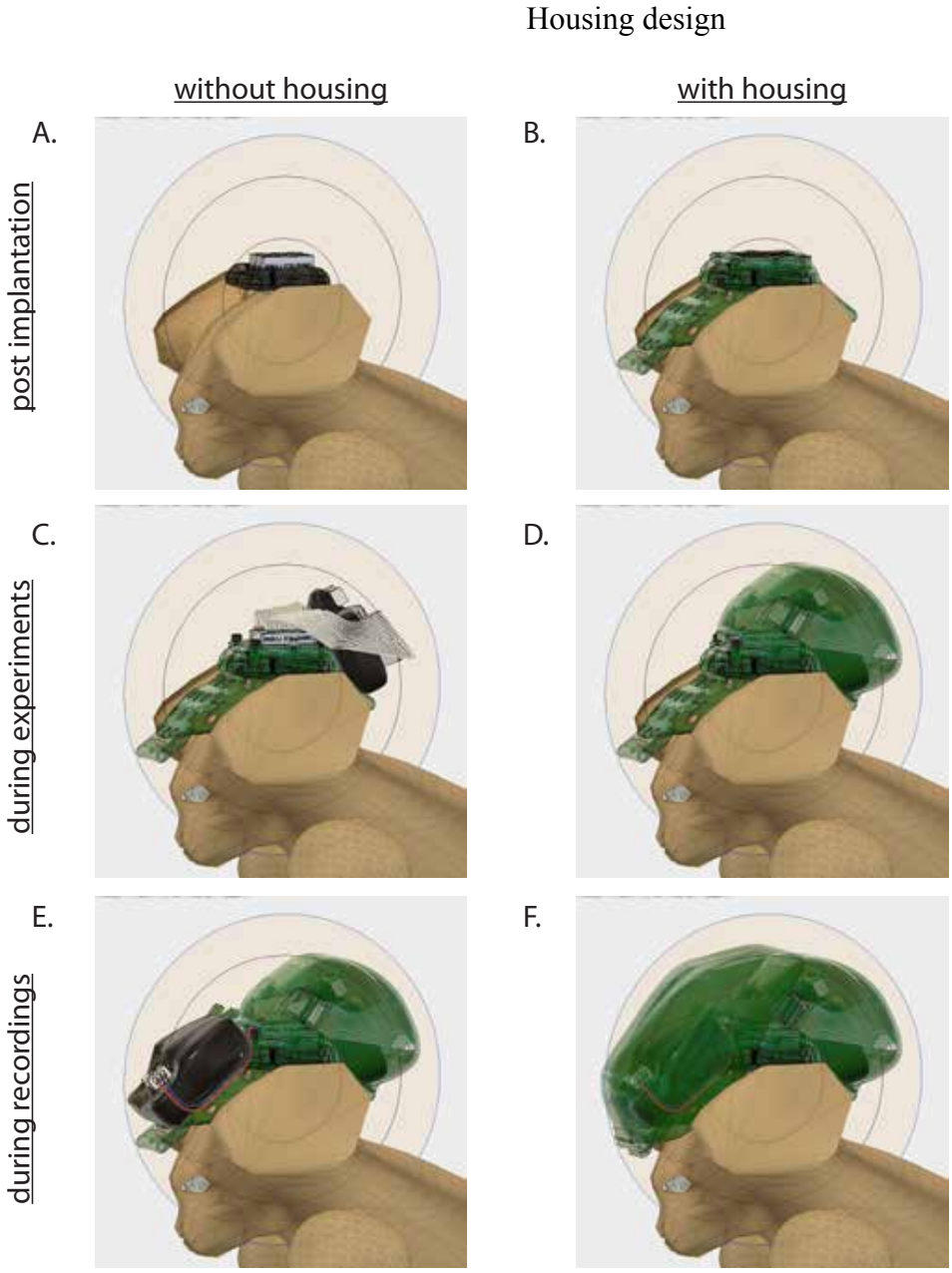

Figure 17 Modular wireless headstage and housing design

A-B) Postoperative assembly with array connector fixed to pedestal (A) and helmet (B) protecting connector and craniotomy site. (C-D) Head mounted assembly during experiments (i.e. when we are performing neural recordings daily) with MUX/accelerometer package connected to array with 32 channel Omnetics ribbon cables (C) and 3D-printed snap-fit housing mounted to protect MUX package. (E-F) Assembly during recordings with RF transmitter/battery packages attached to MUX package with 3-pin connector (E) and snap-fit housing for transmitter/battery package. Circle sketches indicate 15, 30 and 40mm radius from the center of the pedestal feet.

We designed a nested modular housing to protect the two packages that comprise the wireless headstage (Figure 17). The housing for the multiplexer/accelerometer package attaches to a hinge in the back of the helmet and closes to the helmet with a pair of snap-fits on either side of the helmet. When the multiplexer/accelerometer package is mounted to the helmet, but we are not recording, a low profile housing attaches to a hinge in the front of the helmet to protect the cable that allows signal and power transmission between the two headstage packages (Figure 17C-D). While recording, this low profile housing is replaced with the transmitter/battery package, which is protected by its own 3D printed housing that attaches to the helmet with a hinge and snap-fits (Figure 17E-F). This modular configuration allows the headstage assembly to be low profile and easily attachable and removable.

3.3 Results

Surgical Notes

Thus far, we have done array implants with two marmosets. The first procedure went well until the point when we removed the dura to insert the array. At this point the brain began to swell to where it was expanding out of the skull. We ultimately did insert the array, after significant time and effort put toward reducing cerebral edema. However, we chose not to close the craniotomy to avoid the build-up of intracranial pressure. This marmoset recovered from the surgery. She did however present with subtle unilateral motor deficits initially that resolved after a few weeks. While we did see single unit activity on the array during one recording session seven months after the implantation, the array did not consistently yield usable neural signals. After eight months we noticed that the pedestal was able to slightly rotate independent of the skull suggesting that some of the screws anchoring the pedestal to the skull may have come

loose. We decided to remove the pedestal rather than risk it becoming progressively looser. During the removal procedure it was clear that the bone around the screws had become soft and the screws had been ejected. There was however significant soft tissue grown, perhaps periosteum, around to the feet of the pedestal. This tissue was strongly holding the pedestal onto the skull, and we had to slowly cut this tissue off of the feet in order to disconnect the pedestal. After this procedure, we determined that despite the screws being ejected from the bone, the pedestal was not in danger of being accidentally detached given the amount of effort it took to remove deliberately. After detaching the pedestal, we were able to suture the skin and it healed well. The appearance of this marmoset today, gives little to no indication that she ever had any cranial implant (Figure 18). It is worth noting that this would not have been the case if the array connecter had been cemented to the skull. That is, we would not have been able to suture the skin due to the size of the wound imposed by the cement, and we would likely have had to euthanize the marmoset.

Figure 18 Marmoset after titanium pedestal explant
Note healthy scalp.

After the brain swelling we encountered during this first procedure, we clearly needed to understand how to avoid this swelling in the future. To gain additional insight into what might be the problem, we conducted a survey of other investigators that have done marmoset cranial procedures. The details of surgical approaches varied considerably to the point that, even after conducting the survey, it was not entirely clear what the root of the problem might be. However, some investigators, though not all, mentioned taking care to minimize the buildup of heat during the preparation of the craniotomy. This was not something we had explicitly done during either the practice procedure at the WNPRC or during our first array implantation at the University of Chicago.

For the second array implantation we took several steps to minimize the accumulation of heat during the preparation of the craniotomy. First, we used a smaller drill bit (0.5 mm rose burr rather than the 2 mm rose burr used in both of the previous procedures). A smaller bit

would produce less friction during drilling, which will reduce heat buildup. Second, we kept a bowl of sterile saline on ice and used this to frequently quench the drill bit to disperse any heat that might build up as we were drilling. Third, we were careful not to make repeated passes over the same section of bone. And lastly, we irrigated the craniotomy site with cold saline as we were drilling. These efforts successfully minimized brain swelling during this second procedure. After removing the dura, we stimulated the cortical surface with a ball electrode to localize the forelimb area of motor cortex. Modest cerebral edema did occur during our effort to elicit forelimb muscle responses through surface stimulation. Deciding to move forward without stimulation effects, we inserted the electrode array based on stereotaxic coordinates. With the array inserted, we draped the dural substitute over the array and any exposed brain tissue. With the dural substitute in place, we dripped HydroSet Hydroxyapatite Bone Substitute to close the craniotomy site. It was not possible to seal the craniotomy site as illustrated in (Figure 12D) because we did not have a reliable method to restrict application of the bone substitute to the gap between the craniotomy margins and the bone flap. The marmoset recovered from this surgery and showed no apparent motor deficits.

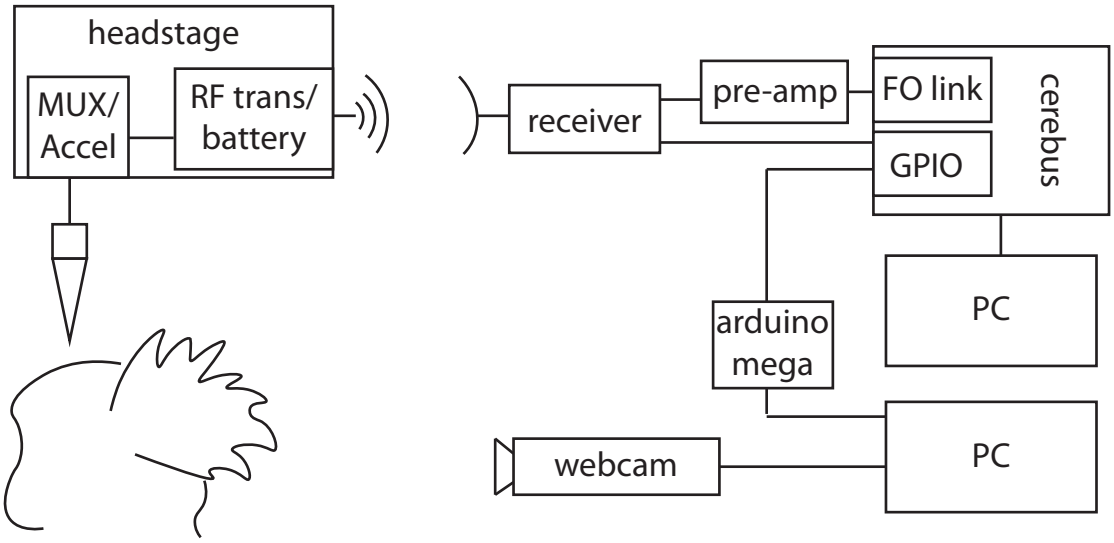

Figure 19 Wireless neural recording system diagram
System comprises 64-channel W64 wireless headstage and receiver from Triangle Biosystems International connected to the front-end amplifier of the Cerebus Neural Data Acquisition System from Blackrock Microsystems. Neural recordings were synchronized with RGB video recordings through an Arduino Mega.

The success of this second procedure allowed us to record population activity from the sensorimotor cortex of this marmoset. Neural signals are amplified with a gain of 800 across the wireless system, band pass filtered from 0.8 Hz to 7 kHz and transmitted by a 3GHz carrier frequency up to 4 meters. The receiver of the TBSI system is connected to the front-end amplifier of the Cerebus neural data acquisition system (Blackrock Microsystems) and sampled at 30 kHz (Figure 19). Putative single unit spike waveforms were extracted around threshold crossings -6.25x below the RMS noise floor. These extracted spikes were sorted offline to assign waveforms to single neurons. The full 30kHz recordings will be used in later analyses of local field potentials, but will not be discussed further.

Spike sorting and approach to handling noise

Spike sorting was performed using Plexon Offline Sorter (Plexon Inc., TX, USA). Threshold crossings were imported into offline sorter. After importing the recording, all artifacts that appeared simultaneously across more than 80% of channel were removed. To eliminate further noise from the recording, the waveforms for each channel were viewed in the energy vs. nonlinear energy feature. We found that this feature space provided a spread of waveforms that allowed for convenient manual identification and invalidation of noise waveforms. After rejecting cross channel artifacts and a large portion of the noise waveforms using the energy vs. nonlinear energy feature space, we recomputed the principal components of the remaining waveforms. At this point, it was generally fairly easy to discriminate clusters of waveforms corresponding to single units apart from another cluster of noise waveforms. In all, about 25 well-isolated units with signal to noise ratios (SNR) greater than or equal to 2.8 were present on the array (Figure 20). SNR for each unit was computed as the peak minus the trough of mean waveform divided by two times the standard deviation of the waveforms across time. In the process of spike sorting, we also found some spike waveforms that were not associated with the clusters of any well-isolated units. We could see similarity in these waveforms to suggest up to about 25 more single units within the recording, but none of these units had many spikes and were not included in any subsequent analyses.

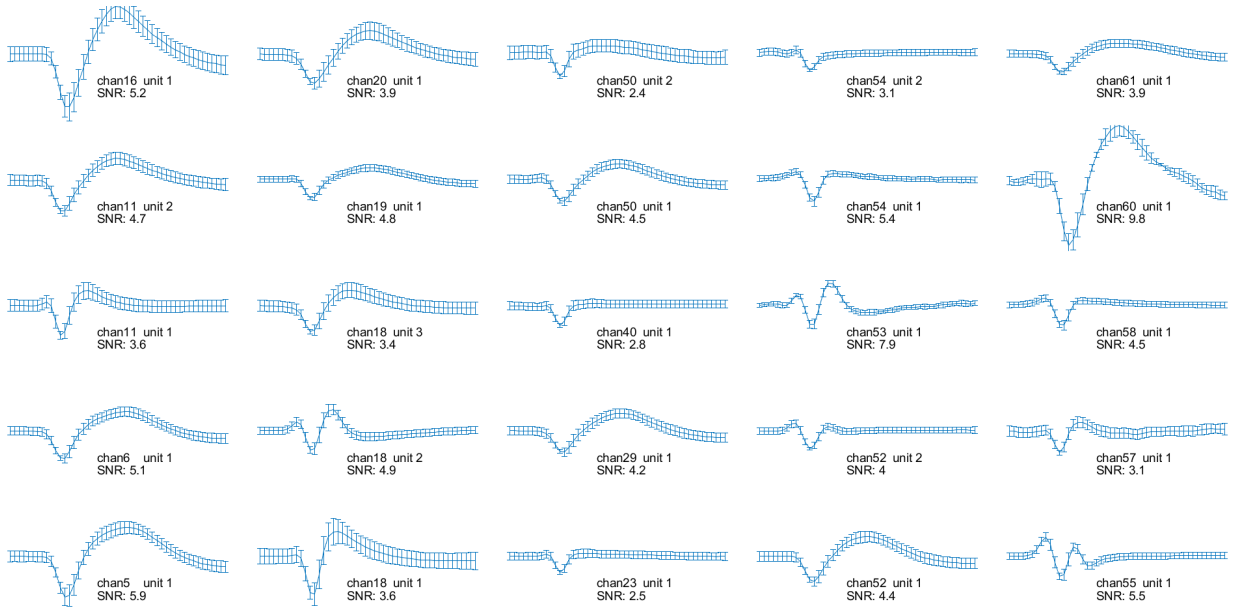

Figure 20 Mean waveforms from 25 neurons recorded from marmoset sensorimotor and premotor cortex with a single Utah array and wireless headstage
Recording TY20170512.

Testing neural recording system with simultaneous kinematic recordings

Our current method for recording upper limb kinematics is X-Ray Reconstruction of Moving Morphology (XROMM) (Brainerd et al. 2010; Knörlein et al. 2016). It is described in greater detail within the previous chapter, but briefly it used bi-planar x-rays and calibrated cameras to track the movement of radio-opaque markers placed in the marmoset's arm and torso. To use the XROMM with the wireless neural recording system, we needed to establish that the x-ray generation for the XROMM would not introduce excessive noise into the neural recordings. Conventional approaches to electromagnetic shielding would not be feasible due the size of the XROMM system and due to the need for radio transparent line of sight between the x-ray generators and the image intensifiers of the system. We found that such shielding would not

be necessary as we could easily identify single unit spiking above the noise floor of neural recordings even during x-ray generation (Figure 21).

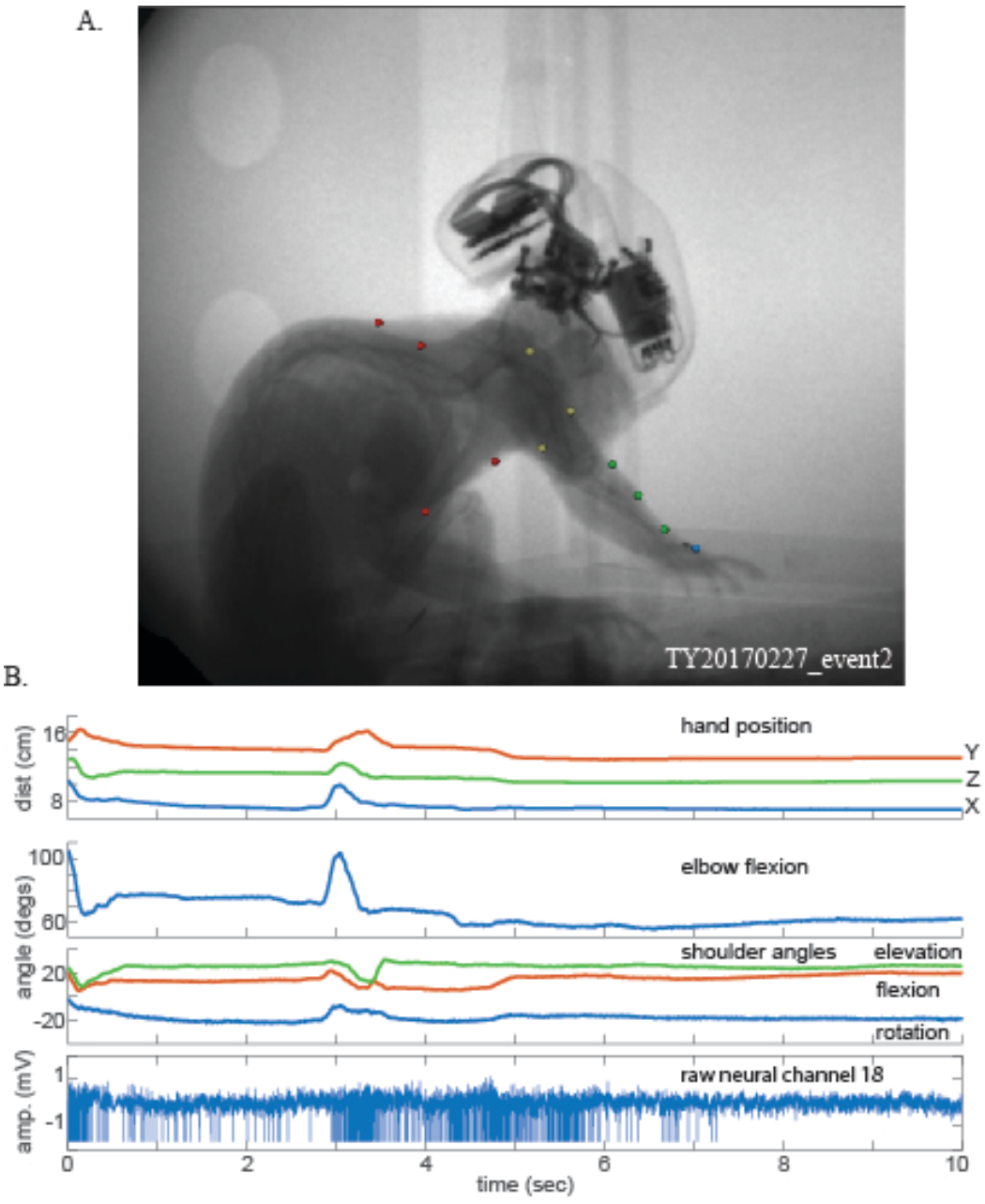

Figure 21 Wireless neural population recording with simultaneous kinematic recordings with XROMM

A) Single frame of x-ray video captured with the XROMM system. Note radio-opaque markers highlighted in red (torso), yellow (upper arm), green (forearm) and blue (hand).
B) Simultaneously recorded kinematic and neural data.

Testing in home-cage with no effort to manage RF atmosphere

In the wild, the marmoset's natural motor repertoire would unfold across 50,000 square meters (Pinheiro and Mendes Pontes 2015). It would include climbing and leaping through trees while foraging for fruits, insects and small animals, vertically clinging to the trunks of trees to feed on exudate, social activities like grooming, play, resting and infant care. While we cannot provide 50,000 square meters for our marmosets, we are able to observe much of this behavior within their home-cages. These cages are 2'x2'x6' in size and framed with extruded aluminum with joints of plastic (Esto connectors). The floor of the cages is a panel of stainless steel. The walls of the cages are polyvinyl chloride coated galvanized wire mesh with a spacing of 1" x 0.5". The home-cages are furnished with a stainless steel sheet metal nestbox, a foraging shelf also made of stainless steel sheet metal and many perches made of both dowels and branches from tree pruning. This is hardly the ideal EM environment for performing wireless signal transmission. Roy and Wang (2012) performed their recordings within a 60x41x30 cm recording cage within an EM shielded recording room. To give marmosets the space to engage in a wide range of natural behaviors, we chose to perform these recordings within the larger home cage and we successfully recorded population activity without abundant noise corruption (Figure 22). There were however brief instances during these recordings when amplifiers would be saturated and neural signals were lost within the noise. These periods were dealt with by removing cross-channel artifacts during spike sorting as these saturation events occurred across all channels.

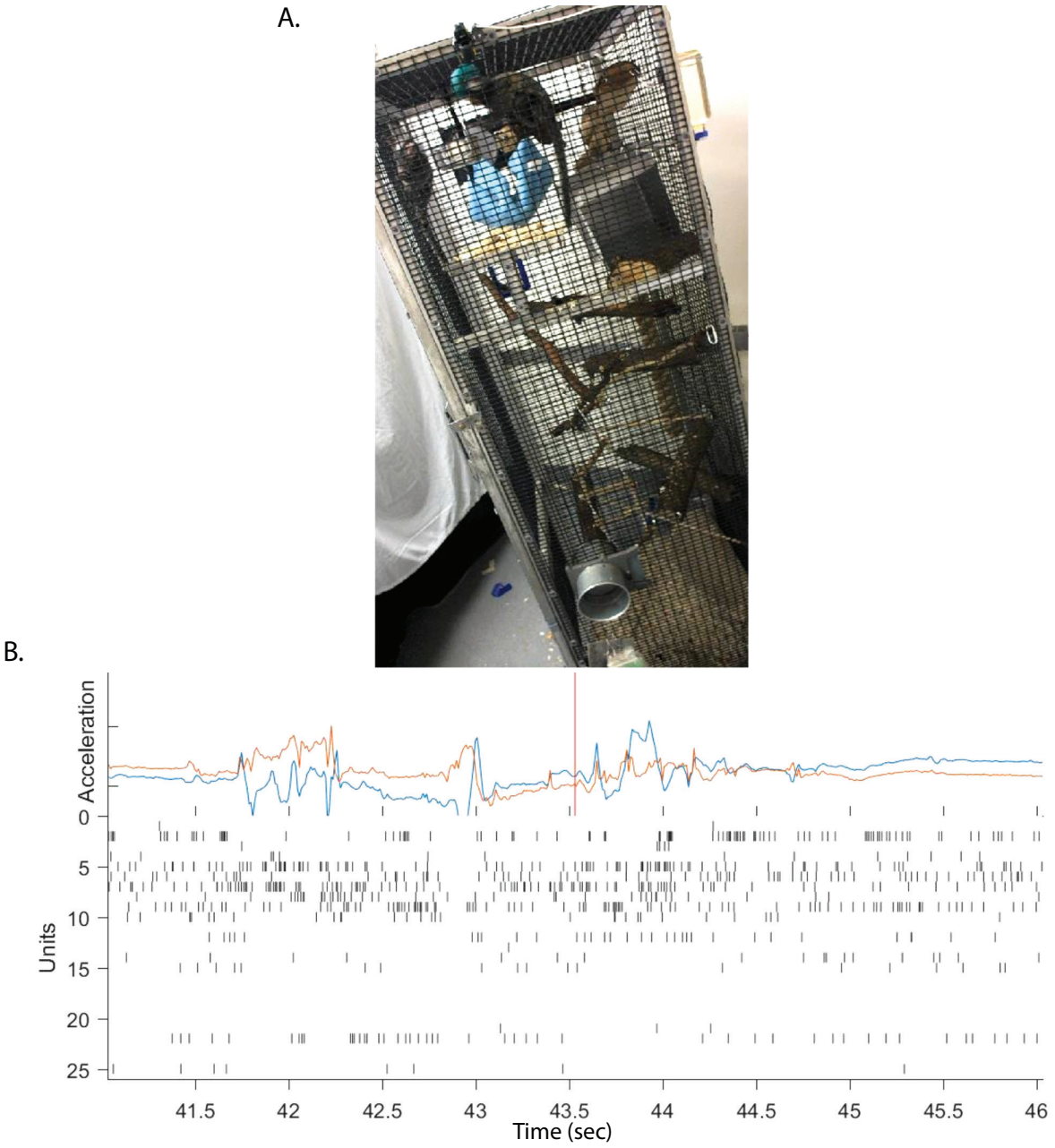

Figure 22 Wireless neural population recordings during unconstrained movement across the marmoset's natural behavioral repertoire

A) Single frame of video recording of marmoset engaging in unconstrained natural behavior within his homecage. Marmoset can be seen engaging in food manipulation at the top of the frame. Note green headstage housing. B) Simultaneously recorded accelerometer data and neural population data transmitted from the wireless headstage within the green housing on the marmoset's head. Recording TY20170512.

3.4 Discussion

As the use of marmosets in neuroscience continues to grow, we are presented with the opportunity to set precedents for their care and use. We have detailed our efforts to develop and refine methods for implanting multielectrode arrays and mounting neural recording equipment to the head of marmosets. We also demonstrated the successful use of these techniques to record population responses from freely moving marmosets engaging in natural behaviors and while recording upper limb kinematics during foraging with the XROMM system. While we do believe these approaches represent improvements and hope they will inspire further refinements, they are still certainly a work in progress. Below we reflect on the results obtained with our surgical approach and wireless headstage configuration and suggest avenues for further enhancements.

Surgical outcomes

With our surgical approach design, we sought to maximize the healing potential of the array implantation and minimize the need for postoperative maintenance of the surgical site. To actualize these goals we designed a custom titanium pedestal to fix the connector for the electrode array to the head of the marmoset, we designed a surgical guide to precisely install bone screws to anchor this pedestal, and coated the feet of this pedestal with hydroxyapatite to promote osseointegration. So far we have done array implantations with two marmosets.

In both procedures, we encountered some degree of brain swelling. After the first procedure, we surveyed other investigators that have performed cranial procedures with marmosets and decided to do all that we could to minimize heat buildup while performing the craniotomy. These efforts seemed effective during the second procedure, as there was no

noticeable swelling after the craniotomy. We did however encounter swelling in the course of stimulating with the surface electrode to localize M1. In the future, we may consider alternative methods for localizing M1 that may prove more reliable (e.g. intracortical microstimulation with a sharp electrode, or possibly an optogenetic approach).

It is possible the some of the swelling we experienced in both procedures was due to cortical micro trauma induced by our method of removing the dura. The tools and approach used to remove the dura during these procedures were the same as those used in both human and macaque. It may be the case that commonly approaches used with rodent or avian models might more successfully allow us to remove the dura with minimal trauma.

The ideal solution for closing the craniotomy without disturbing the array or compressing the brain would be to replace the autologous bone flap over the array and seal the space between the craniotomy boundaries and the edges of the bone flap with precise application of HA bone substitute. We were unable to do this in either of these procedures due to 1) premature curing of the HA bone substitute, and 2) contamination of the autologous bone flap in surgery. In future procedures we intend to close the craniotomy in this way.

Both during surgery and after surgery, the wire bundle from the array to the connector caused complications. During surgery we found it difficult to bend the wire bundle in such a way that it did not raise up off of the skull where it might cause post-operative skin erosion. Additionally, after the first procedure, post-operative complications arose when a thread that became wrapped around the pedestal stalk disturbed the wire bundle. Both of these issues might have been avoided if the wire bundle had been enclosed in the stalk of the pedestal. We are considering a refinement of the pedestal design that would allow this sort of protection.

Eight of months after the first procedure, we noticed some movement of the pedestal in relation to the skull and decided to remove the pedestal. During this procedure it became clear the screws used to anchor the pedestal to the parietal bones had been ejected and the bone around them had become soft. Despite this fact, the pedestal still remained held to the skull with clear thin soft (perhaps periosteal) tissue, to such an extent that it was difficult to remove this pedestal. This tissue had to be carefully dissected away with a scalpel. The pedestal implanted during the second procedure remains firmly anchored to the skull more than a year after it was implanted. It is not clear exactly why the screws from the first procedure were rejected and those from the second remain firmly implanted more than a year after the surgery. Unfortunately, it seems that this sort of screw rejection is not uncommon in cranial procedures of this sort. In the future, we could think about using the Sheatz method that was more common in early examples of fixing hardware to the skulls of non-human primates in neuroscience (Evarts 1968a; Lipp and Hunsperger 1978). This would involve the design of a custom fastener and could be improved by using HA bone substitute to fill the keyholes used to insert the fastener. We could also consider designing a custom screw with deeper threads modeled after the cortical bone screws used by orthopedic surgeons in larger animals. An additional improvement we could implement that might help with chronic pedestal anchoring would be customizing the feet of each pedestal to match exactly the curvature of each marmoset's skull rather than basing the feet for each pedestal on a single common reference marmoset skull. This would eliminate any space between the pedestal feet and the skull, and would ensure there would be no need to bend the feet in surgery. Lastly, we could split the pedestal into two components: the feet and the platform for the array connector. The feet implantation procedure could be performed as a non-major surgery some number of weeks or months before the array implantation to allow for osseointegration

without the additional potential torques imposed by the higher profile platform, array connector and helmet.

Headstage setup

Our wireless headstage configuration has allowed us to successfully record population activity with minimal handling and head-mounted package size in freely moving animals. We plan to implement two further refinements to optimize battery life and almost eliminate handling when replacing the battery during daily use of the headstage. The first improvement, to address battery life, will be to integrate a low power radio board to remotely and programmatically control when the headstage is turned on and off. Currently, the experimenter has to turn the headstage on with a magnet close to the animal's head when the battery is attached to the headstage. This is a clever solution in that it allows one to operate the headstage without manipulating the animal. However, it requires the experimenter to wave a magnet by the head of the animal to operate the switch.

We have developed a behavioral training apparatus that marmosets can use throughout their waking hours with little experimenter intervention. They tend to engage in brief bouts of behavior within this apparatus (average ~10 second duration) throughout the day, usually totaling more than an hour per day. Using the triggers within this apparatus and a headstage that we can control remotely, we plan to build a system where the headstage is recording only while marmosets are within the apparatus engaging in some experimentally interesting behavior. This will allow us to target headstage power usage only to epochs when the marmoset is engaging in behaviors of interest.

The remote operation solution described above would provide the flexibility to choose whether we change the battery after a four hour session comprising all behaviors or after roughly

four full days of recording only during specific behaviors of interest. To facilitate battery changing, whenever it is needed, we are planning one final improvement on the current headstage configuration: a quick connect option that would allow us to remove and replace the battery/transmitter package without handling the marmoset at all. Currently, any time we need to change the battery, one experimenter will hold the marmoset while the other experimenter first attaches the transmitter/battery package to the helmet, then mates the 3-pin connector that allows signal, power and ground transmission between the MUX/accelerometer package and the transmitter/battery package, and finally mounts the transmitter/battery housing using the hinge and snap fits. We plan to redesign the headstage package housings such that the transmitter/battery package is mounted within its housing, and the mating of that three pin connector is accomplish automatically when closing the housing's hinge and snap-fits.

Recording setup

While we have successfully used this wireless neural recording system to record population activity during unconstrained natural behavior of a freely moving marmoset and in conjunction with the XROMM to simultaneously capture forelimb kinematics, there are still short periods of our recordings during which neural signals are overwhelmed by noise. We have recently installed a fixture for the receiver antennas that should provide better signal coverage. We are also working with TBSI to record an indicator of the wireless signal quality so that we can more easily identify the best antenna configuration in a cluttered EM environment.

Lastly, we plan to realize the full potential of the approach we have described above by recording from two marmosets in parallel. We have two recording systems that operate on separate frequencies (F1 at 3GHz and F2 at 3.2GHz). Experiments with awake, behaving marmosets do seem to yield data more slowly than similar experiments with macaques. The

ability to record population responses throughout the day from multiple marmosets, complemented by the approach to automated, voluntary in-home cage behavioral training described in the previous chapter, promises to favorably and effectively address data acquisition difficulties for future experiments.

3.5 Conclusion

We presented our efforts to develop and refine approaches to performing neural recordings with marmosets. While still a work in progress, they are an improvement of previously reported techniques. As the use of marmosets in behavioral neuroscience continues to grow, we hope other groups will adopt and refine further these techniques such that thoughtful precedents will be set for the care and use of these animals that promise to contribute significantly to our understanding of the nervous system in health and disease.

CHAPTER 4

Preliminary observations of sensorimotor cortical population responses across the marmoset natural behavioral repertoire

4.1 Introduction

Theoretical and ethological perspectives suggest investigating cortical population responses during complex, natural behaviors will be key to understanding the cortical population code (Krakauer et al. 2017). Multiple groups have found surprisingly low dimensional structure in population responses while studying several cortical areas during highly trained and constrained behaviors (Gao and Ganguli 2015). Evidence for low dimensional structure of neural population activity in macaque motor cortex across different tasks has recently been reported, but it remains unclear if such structure persists across naturalistic behaviors (Gallego et al. 2017). Models of motor cortical tuning applied to highly constrained movements can effectively account for significant fractions of variance in neural activity, and as a result, such models have been applied to implement impressive neuroprosthetic control (Collinger et al. 2013; Hochberg et al. 2012). However, there is evidence that these same models account for only a small portion of the variance in neural firing when applied to unconstrained arm movements (Aflalo and Graziano 2007). More recently, the structure of the space spanned by neural population responses during controlled reaching tasks has been shown to limit the capacity for neuroprosthetic skill acquisition (Sadtler et al. 2014). It remains unresolved whether the intuitive space available for such neuroprosthetic control might be larger than that revealed by such highly constrained task. As a preliminary step toward mapping the volume of the manifold of possible neural activity

patterns, we measured structure in sensorimotor cortical population responses during unconstrained natural behavior in the Common marmoset using wireless multielectrode array recordings.

In what follows, we summarize the marmoset's behavioral repertoire and show that more than 90 percent of a waking hour is spent sitting, vertically clinging, and engaging in locomotion, leaping, foraging or food manipulation behaviors. These activity budget estimates accord with field studies of wild marmosets (Stevenson and Rylands 1988). Our initial characterizations of sensorimotor cortical population responses during natural behaviors suggest that more dimensions are required to account for the variance in neuronal activity than would be predicted from more constrained behaviors. However, state specific structure is still recoverable with many fewer dimensions than the number of neurons recorded.

4.2 Methods

Neurophysiology

One marmoset monkey was implanted with a 96-channel Utah array (Blackrock Microsystems) with electrodes of length 1 mm using the surgical procedure described in the previous chapter. The array targeted the forelimb array of primary sensorimotor and premotor cortex, guided by published stereotaxic coordinates (Burman et al. 2008, [a] 2014; Burish, Stepniewska, and Kaas 2008; Paxinos et al. 2012). The University of Chicago Institutional Animal Care and Use Committee approved all procedures. Neural recordings were performed with a custom configuration of the W64 wireless headstage (Triangle Biosystems International) described in the previous chapter, and the Cerebus Neural Data Acquisition System (Blackrock Microsystems). The wireless headstage transmits 60 channels of neural data and three channels of accelerometer

data. Neural signals were bandpass filtered from 0.8 Hz to 7 kHz and sampled at 30 KHz. Regions of 1.6 ms around threshold crossings below -6.25 RMS were extracted from each channel as putative spike waveforms and were sorted offline using Plexon Offline Sorter. Twenty-five units with a signal to noise ratio (SNR) of 2.8 of greater were used in the following analysis.

Behavioral Recording and Annotation

A webcam (Logitech 910) was used to record RGB video of a freely moving marmoset engaging in unconstrained natural behaviors within its home cage. The behavioral recordings were synchronized to the start of neural data acquisition through TTL pulses and an Arduino MEGA connected to the Cerebus GPIO ports. Behavioral annotation of this videos was performed manually using ELAN (Brugman and Russel 2004). Briefly, ELAN is program for annotating videos of conversations for linguistic analysis, but provides a convenient set of tools to align and annotate video and time series, in this case accelerometer data.

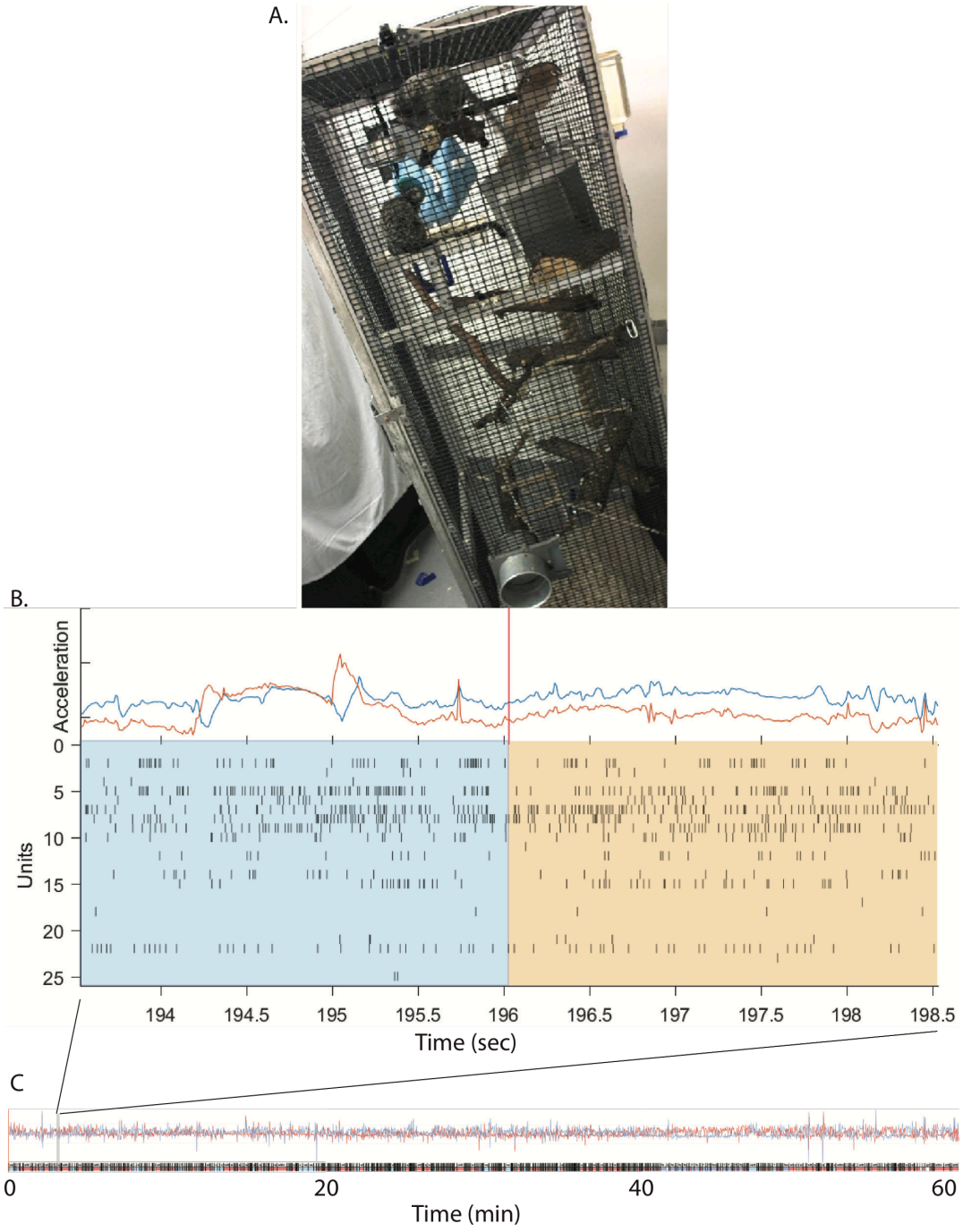

Figure 23 Neural recording and behavioral annotation across the marmoset's natural behavioral repertoire

A) Single frame video recording of unconstrained marmoset behavior. B) Five seconds of accelerometer and neural spiking data centered on this video frame. Colors indicate behavioral annotation (sitting in blue and foraging in yellow). C) One hour of behavioral annotation and accelerometer data. Grey highlight indicated data segment in (B).

4.3 Results

Marmoset Behavioral Repertoire

To sample sensorimotor cortical population responses across the marmoset behavioral repertoire we recorded population responses during one hour of unconstrained behavior (Figure 23). Over the course of the recording, we found that the marmoset spent almost half of that time sitting. Over 90 percent of that time was spent engaging in one of the following six behaviors: sitting, vertical clinging, food manipulation, foraging, leaping and locomotion (Figure 24, Table 1). Of the 1194 behavioral epochs observed, there are more than 150 epochs of each of the following behaviors: sitting, vertical clinging, leaping and locomotion. While there are fewer examples of foraging and food manipulation (Figure 24A,D), each example is long enough, on average 6 and 9 seconds respectively, to permit inclusion in the subsequent analysis (Figure 24C).

Behavior	Description
Sitting	Stationary on horizontal surface
Vertical clinging	Stationary on vertical surface
Food manipulation	Holding food item with hands while eating
Foraging	Actively searching for food items in substrate
Leaping	Bounding vertical distance
Locomotion	Moving body primarily along horizontal surface

Table 1 Description of criteria used for annotating six behaviors included within analysis

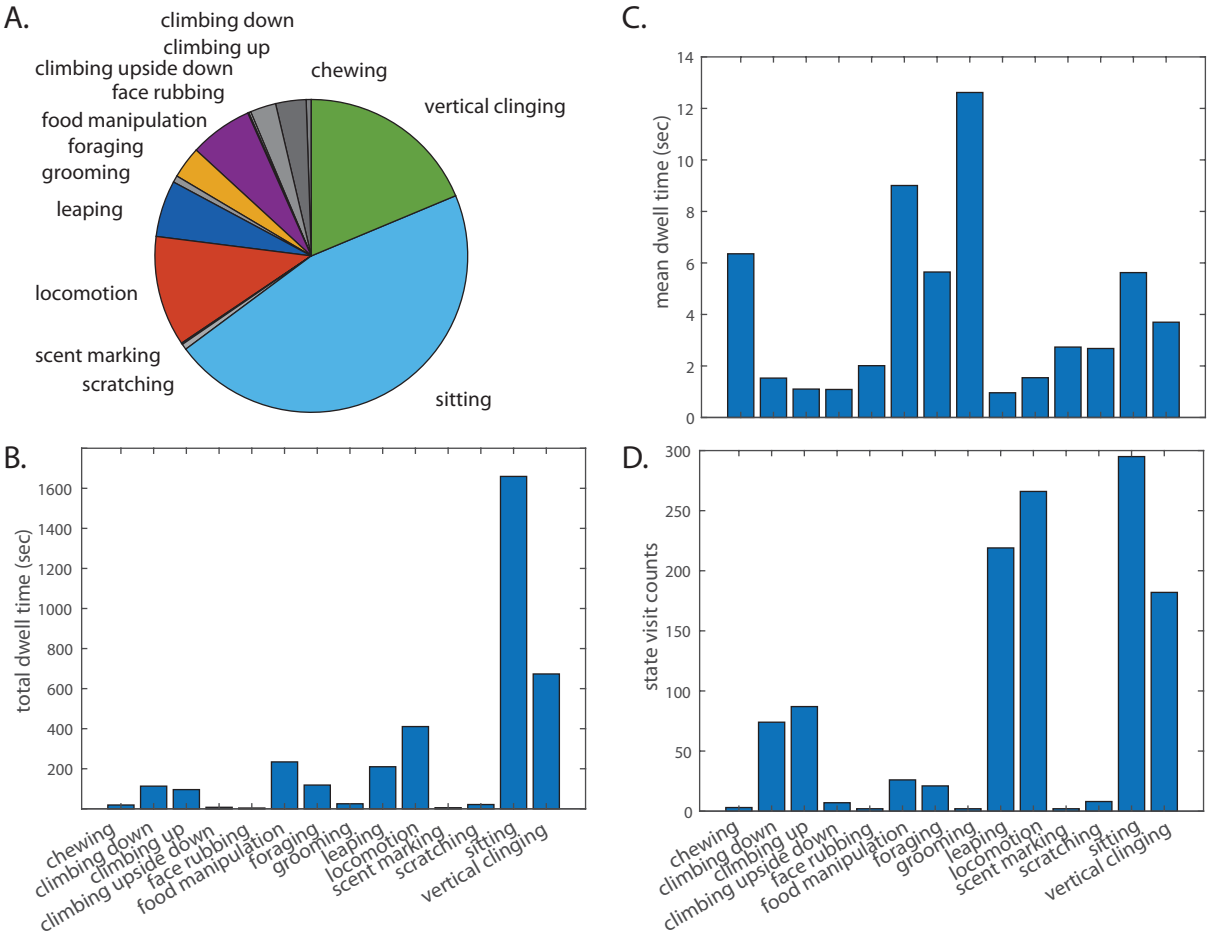

Figure 24 Summary of marmoset's unconstrained behavioral repertoire

A) Proportion of time spent in each behavioral state observed over the course of recording. B) Total time spend in each behavioral state during the recording. C) Mean dwell time per state. D) Counts of the number of times each state was visited during recording.

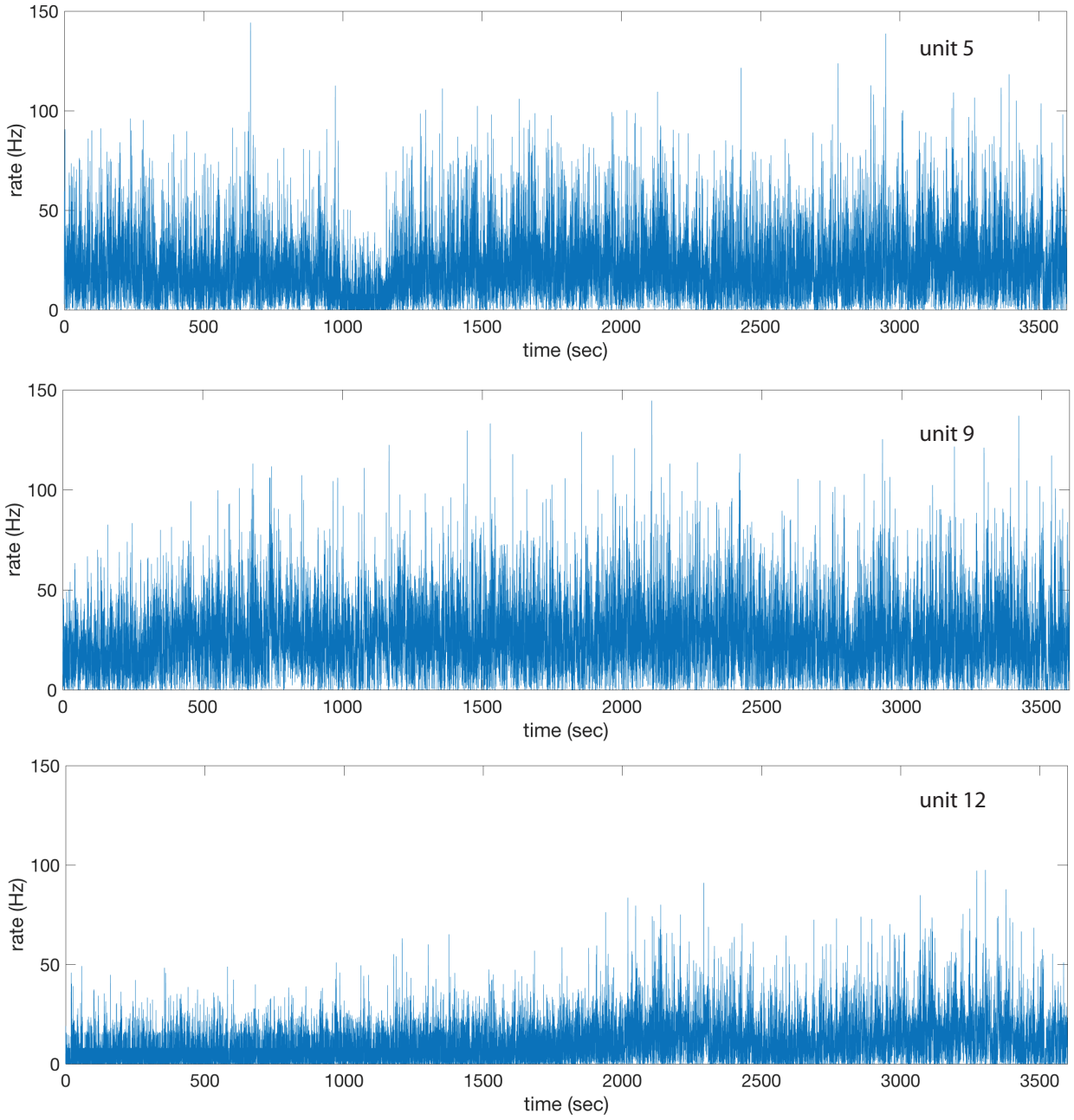

Figure 25 Example firing rates of three neurons across the entire recording

Firing rates were estimated by binning spikes in 1 millisecond bins and convolving with a Gaussian kernel with a 50 millisecond standard deviation.

Neural responses across the behavioral repertoire

Across the entirety of the recording, units had a range of firing rates (Figure 25). Firing rates were estimated by convolving spikes with a Gaussian kernel with a standard deviation of 50 milliseconds. About half of recorded units maintained high baseline firing rates, whereas the activity of the other half was more sparse (Figure 26). We separated responses according to behavior and examined state specific structure in firing rate distributions by randomly drawing 500 millisecond data segments from each state (Figure 27). For some behaviors and some units (5/21) we did find state specific separation of firing rate distributions (Figure 27A-D). Though for many units (16/21), we did not observe any differences in firing rate distributions across states (Figure 27E-F).

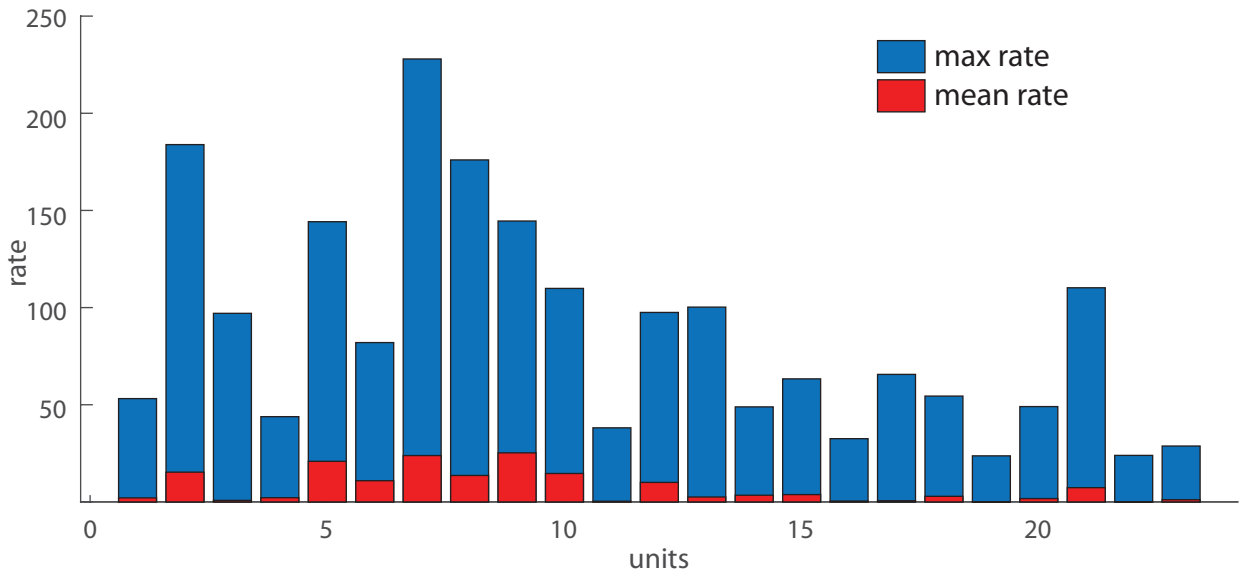

Figure 26 Summary of firing rates across the entirety of recording
Mean and maximum rates of each neuron within the recorded population.

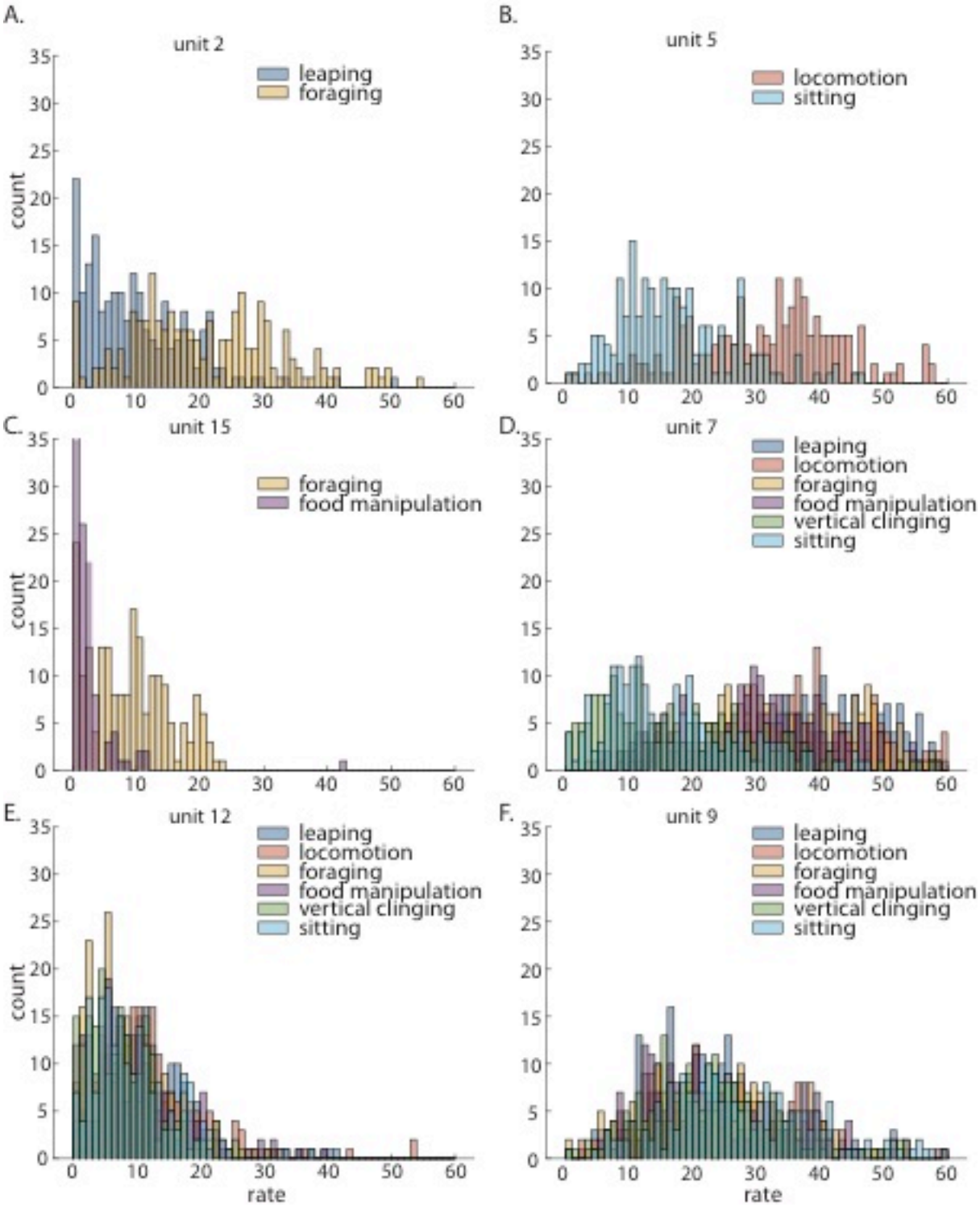

Figure 27 State specific firing rate distributions

A-D) Examples of state specific firing rate distributions.
E-F) Examples of units with firing rate distributions that do not vary with behavioral state.

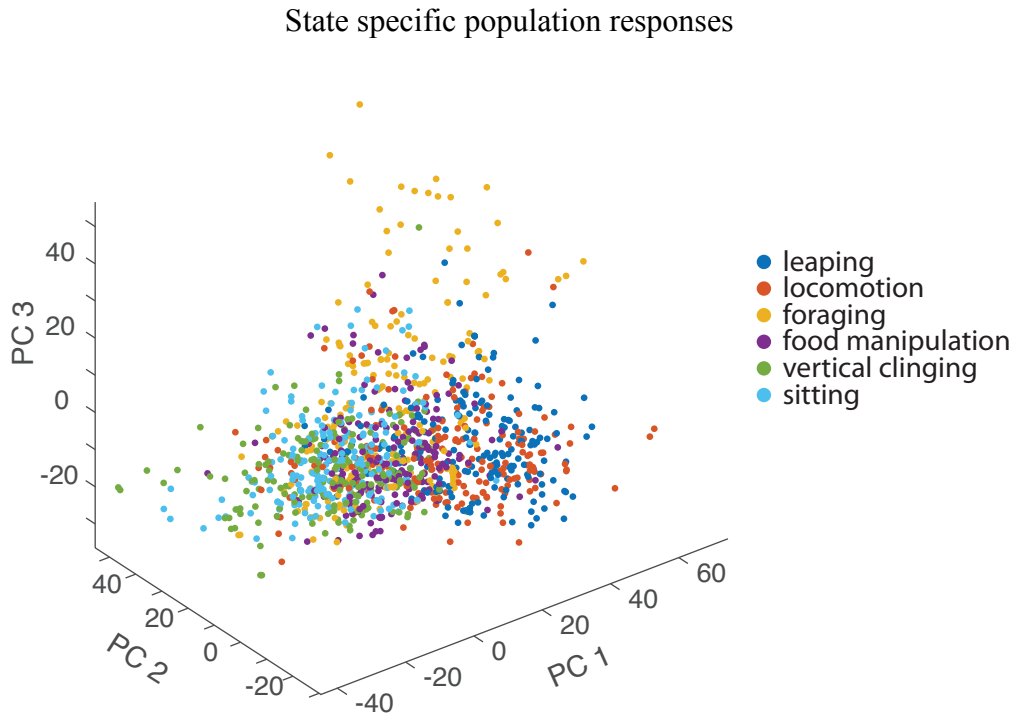

Figure 28 State specific separation of population responses in low dimensional principal component space

Average rates of randomly chosen data segments from epochs of leaping, locomotion, foraging, food manipulation, vertical clinging and sitting plotted in space defined by the first three principal components computed with pooled population responses.

To examine how this structure in individual units' firing rate distributions manifests at the population level, we pooled population responses across the six behavioral states of interest and performed principal component analysis (Figure 28). We found a consistent state specific separation of the population responses within principal component space. Data from the more dynamic behaviors of leaping and locomotion separated from the more static behaviors of vertical clinging and sitting. We also consistently found that the responses corresponding to food manipulation sat between the activity from the static and dynamic behaviors just mentioned.

Interestingly, the population responses corresponding to foraging were reliably positioned in a portion of principal component space separated from all of the other behaviors, but partially aligned with the responses corresponding to food manipulation. To get a sense of the dimensionality of the neural spaces occupied by the population responses corresponding to the six behaviors, we performed principal component analysis on the population responses for each behavior separately (Figure 29). We found that, for each behavior, it took at most six principal components to account for 80 percent of the variance in neural responses.

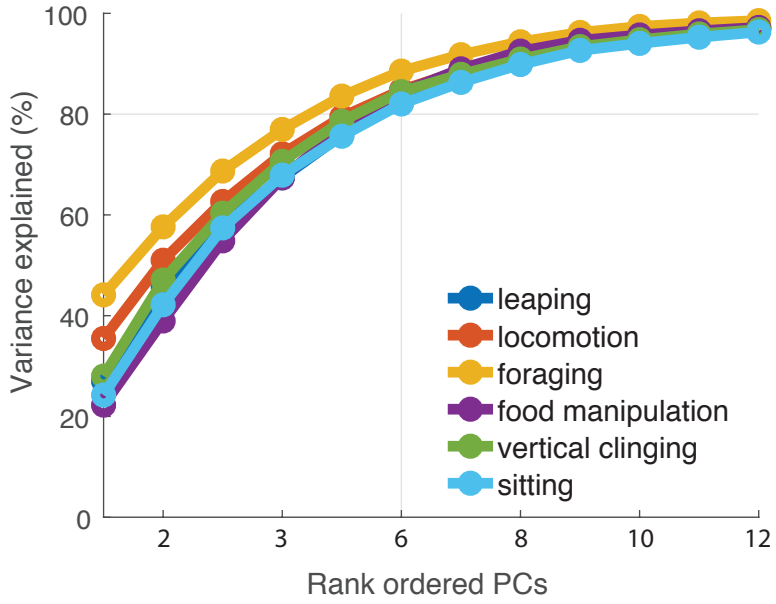

Figure 29 Dimensionality of population responses for each state estimated with principal component analysis

4.4 Discussion

Marmoset natural behavior repertoire

The summary of the marmoset's behavioral repertoire described above is an exceedingly brief snapshot and provides only a course description of marmoset behavior. Even so, it accords well with previous descriptions of marmoset behavior in captivity and in the wild. Multiple investigators note that marmosets begin to be active within a half hour of sunrise and return to their sleeping site shortly before sunset (Stevenson and Rylands 1988). Within that time, Maier estimated that C. jacchus spends 29% of waking time feeding on exudates (Maier et al. 1984 in Sussman and Kinzey 1984). Stevenson and Rylands (1988) estimate that C. jacchus spent about 53% of their time stationary, 35% of their time moving, including foraging, 10% engaging in social activities and 12% of that time feeding. While these are only rough estimates, they are consistent with the activity budgets we quantified for this study. Further, the behaviors observed also match well with more extensive characterizations of behavior reported by Stevenson and Poole (1976) and more recently by Schiel and Souto (2017) . However, the initial summary reported here is courser than the more extensive ethogram reported by Stevenson and Poole (1976). For example, whereas I group all horizontal locomotor behavior into a single category 'locomotion,' Stevenson and Poole (1976) describe two basic modes of locomotion with subdivisions of behavioral segments for each. They additionally, describe a consistent sequence of behavioral elements involved in prey capture. I did not provide prey with dynamic escape responses, and thus did not elicit these sorts of prey capture sequences. I additionally did not observe gummivory, a key component of the marmoset's behavioral repertoire in the wild, as there were no opportunities for gum feeding within the marmosets' home cages. We are currently exploring methods that would allow us to obtain behavioral segmentation of unconstrained behaviors at higher resolution (Egnor and Branson 2016; Kabra et al. 2012;

Berman et al. 2014). In the future, we plan to both sample behavior over longer durations and characterize behavior in greater depth.

Population responses during natural behavior

A consistent finding of studies designed around repeated trials of highly constrained behaviors is the presence of low dimensional structure in the responses of populations of neurons (Gao and Ganguli 2015). Many fewer dimensions than the number of recorded neurons are needed to explain substantial portions of the variance in population activity. However, it remains unresolved whether this low dimensional structure is a product of highly constrained behavioral tasks or whether it is a manifestation of deeper principals of neural organization. In a recent meta-analysis of such studies, Gao and Ganguli (2015) showed that this low dimensional structure is present across mammalian sensorimotor (Churchland et al. 2012; Paz 2005; Chapin and Nicolelis 1999), prefrontal (Machens et al. 2010; Mante et al. 2013; Narayanan and Laubach 2009), hippocampal (Peyrache et al. 2009; Sasaki et al. 2007), visual (Hegdé and Van Essen 2004), parietal (Matsumoto et al. 2005) and brainstem (Bromberg-Martin et al. 2010) systems. It has also been shown repeatedly in insect olfactory systems (Haddad et al. 2010; Raman et al. 2010; Assisi et al. 2007; Mazor and Laurent 2005; Stopfer et al. 2003) and in the sensory ganglion of the leech (Briggman et al. 2005).

If we plot number of dimensions needed to explain 80 percent of the variance in population responses corresponding to the unconstrained marmoset behavioral repertoire, we find that those data sit apart from those results obtained in the context of simpler and more highly constrained behavior (Figure 30). This finding suggests that the dimensionality of population responses during natural behavior might be higher than what one might expect from studies of more constrained behavior. However, there is at least one caveat to consider before

drawing this conclusion. With the Utah array, we are sampling across multiple areas of marmoset sensorimotor and premotor cortex. It may be the case that this increase in dimensionality could be the result of sampling multiple cortical areas. We are in the process of mapping the placement of the electrode array to determine which electrodes lay in which areas of cortex. Once this mapping is complete we could repeat this analysis with units for single functionally defined cortical areas. This will result in fewer neurons for this analysis, which could present a problem.

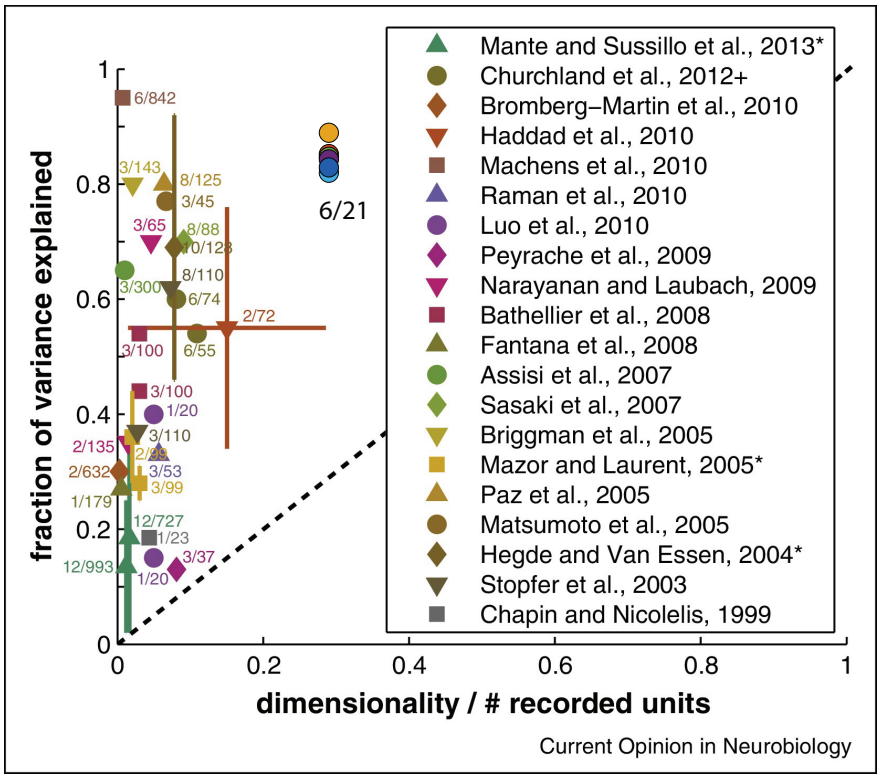

Figure 30 Dimensionality of sensorimotor population responses during unconstrained natural behavior is higher than expected from similar estimations based on more constrained behavior. Summary figure from meta-analysis of 20 studies estimating dimensionality of population responses during a variety of behaviors in a variety of systems. Circular points, to the right of the cluster of points hugging the ordinate, represent number of dimensions needed to explain 80 percent of the variance in population responses during marmoset sitting, vertical clinging, food manipulation, foraging, leaping and locomotion. Modified from Gao & Ganguly (2015).

Gao and Ganguli (2015) present this meta-analysis to motivate their theory of neural task complexity, which is meant, among other things, to address the question of how many neurons are needed to recover accurate descriptions of population dynamics. Their notion of neural task complexity is formulated in terms of task parameters, but it is not entirely clear how to parameterize natural unconstrained behaviors. Therefore further theoretical work will be needed to extend the theory of neural task complexity to natural behaviors and to estimate how many neurons might be needed to accurately capture a picture of population dynamics during unconstrained natural behaviors.

In the future, we plan to train marmosets to perform more traditional experimental tasks used to study motor control, such as the center out task, in concert with neural recordings during unconstrained naturalistic behaviors. We believe that such an approach will allow us to situate the results obtained from more highly constrained and trained behaviors within the context of the space spanned by the natural behavioral motor repertoire. It has been shown that an understanding of the structure in population responses during a given behavior can provide insight into why some tasks are more difficult to learn than others (Sadtler et al. 2014). Natural behaviors provide access to population responses that may span a larger neural repertoire and thus provide richer control space for decoding algorithms to take advantage of. The challenge for the designer of intuitive and embodied neuroprosthetic systems will be to understand the structure and dynamics of the neural repertoire spanning the space of natural behaviors and use that understanding to implement adaptive and effective systems that allow for intuitive control of neuroprosthetic devices that generalize beyond highly constrained contexts into daily life.

4.5 Conclusion

These very preliminary observations suggest that consistent findings of low dimensional structure from studies of highly constrained behaviors may be limited by the simplicity of behaviors studied. In the future we plan to examine the relationship between state specific subspaces by evaluating the variance explained when projecting population responses from one state into the subspaces computed using the population responses from other states (Elsayed et al. 2016). We also plan to incorporate more controlled experimental tasks that involve learning so that we might be able to place such tasks within the space circumscribed by population responses during natural behaviors. With this approach we hope to gain insight into the benefits of a balance between naturalistic and more constrained behavior when studying population codes.

Finally, while low dimensional structure does provide convenient summaries of population responses, and in some cases it does seem to have functional significance (e.g. Kaufman et al. 2014), without connecting these summaries to cell-type specific components of the neural circuits underlying behavior, they will remain superficial summaries. As the genetic tools to target cell-type specific components of neural circuits continue to mature in marmosets, I expect that one significant contribution of these tools would be to add depth to our understanding of these low dimensional summaries in terms of the circuit components underlying them.

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