Towards Cell and Subtype Resolved Functional Organization: Mouse as a Model for the Cortical Control of Movement

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Abstract—Despite a long history of interrogation, the functional organization of motor cortex remains obscure. A major barrier has been the inability to measure and perturb activity with sufficient resolution to reveal clear functional elements within motor cortex and its associated circuits. Increasingly, the mouse has been employed as a model to facilitate application of contemporary approaches with the potential to surmount this barrier. In this brief essay, we consider these approaches and their use in the context of studies aimed at resolving the logic of motor cortical operation.

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INTRODUCTION

That movement may be governed by specialized cortical regions has long been appreciated (Ferrier and Yeo, 1884; Horsley and Schafer, 1888), but how these motor cortical regions are organized to perform this role remains unresolved. Early stimulation studies that culminated in Penfield’s mapping in neurosurgical patients revealed a rough somatotopy (Penfield and Boldrey, 1937), suggesting the existence of an array of descending channels that could be engaged to move particular body parts. Later attempts using more controlled stimulation and spike-triggered averaging of muscle recordings revealed a fundamental complexity, whereby the activity of individual cells is linked to direct responses in multiple muscles across multiple limb joints (Cheney and Fetz, 1985; Kalaska, 2009). The relevance of classical motor cortical somatotopy has been challenged by more recent results with longer stimulation trains that drive ethologically relevant movement components (Graziano et al., 2002; Harrison et al., 2012; Brown and Teskey, 2014). Moreover, the predominance of oligosynaptic control of muscle activity by motor cortical output has been countered by the observation of a relatively long lag between activity in cortical neurons and muscles, even for corticospinal neurons synapsing directly onto spinal motor neurons (Schieber and Rívis, 2007).

These results highlight a fundamental ambiguity: we lack a clear view of the basic functional elements that underlie the cortical control of movement. Identifying such elements can rapidly catalyze mechanistic insight, as has been demonstrated by the discovery of subatomic particles in physics or of DNA structure in genetics. An emerging view posits that neuronal subtypes defined by features like axonal target region, target cell identity, and gene expression may reflect functional elements in neural systems (Zeng and Sanes, 2017; Arber and Costa, 2018). However, a primary barrier to testing this view and resolving functional elements within motor cortical circuits has been our inability to measure and perturb activity specifically in neuronal subtypes defined by features of cell identity that may differentiate their function.

Recognition of this barrier has led to increasing use of the mouse as a model organism for probing cortical movement control. The general power of the mouse lies in relating the organization of neural systems to their function at a fine scale where relevant mechanisms may operate and thus where relevant functional elements may lie. The comparative ease in mice of optically recording neural activity with calcium indicators, during both head-fixed (Dombeck et al., 2007, 2009) and free-behaving (Ghosh et al., 2011; Cai et al., 2016) tasks, enables experimentalists to measure neural activity in genetically-defined neuronal subpopulations at cellular resolution. The comparative ease of subcortical recording permits activity measurements from important cortical targets that historically have been harder to access. The comparative ease of optogenetic manipulations enables activity perturbation of brain areas (Arenkiel et al., 2007), neuronal subpopulations (Adamantidis et al.,...
range projection neurons like corticospinals (Tervo et al., 2016) or in subsets thereof that synapse onto specific neuron types (Wickersham et al., 2007b; Kim et al., 2016; Reardon et al., 2016). In addition, single cell sequencing has now identified many new genetically distinct classes of cortical neurons (Economou et al., 2018; Tasic et al., 2018), greatly facilitating the search for distinct functional elements within neuronal populations. The precision of molecular manipulation and advances in physiological methods together create a broad range of opportunities for identifying functionally distinct cell types at a new level of granularity.

Historically, motor system studies have been carried out in mammals much larger than the humble *Mus musculus* that are anatomically and behaviorally divergent. The benefits of working with primates are clear: their anatomy and physiology are closer to that of humans, they are able to perform highly dexterous movements, and they have the cognitive capacity to master complicated tasks. However, their size, long gestational cycles, and human-like traits reduce experimental throughput and render interventional approaches costly and ethically challenging. As the mouse gains prominence as a motor system model, potential value emerges in coordinating approaches across model organisms, in comparative inquiry, and in identifying opportunities for generalizable findings from mice. In this review, we explore the study of motor cortical functional organization in the mouse. We consider a range of technical approaches that can identify manifestations of functional organization in neural circuit architecture and activity. These approaches include circuit tracing, activity measurement and perturbation, and studies across motor learning. Our goal is to help clarify the specific contribution the mouse can make to questions of general relevance.

## COMPARATIVE STUDIES OF CORTICOSPINAL ORGANIZATION

Despite appreciable homology in motor system organization between rodents and primates, certain distinctions may prove functionally significant. All placental mammals have a cortical motor area rostral to somatosensory cortex, but in primates a larger portion of this region is dedicated to hand and digit control (Preuss et al., 1997; Kaas, 2012). Evidence of functional distinction and anatomical hierarchy between premotor and primary motor cortices in rodents remains limited, though recent observations (Kimura et al., 2017; Saiki et al., 2017; Yoshida et al., 2018; Fulcher et al., 2019) suggest rodent motor cortices may have something of a primate-like hierarchy. Relative to that of primates, mouse motor cortex appears more heavily interconnected with primary and secondary somatosensory cortices, and less connected with parietal and prefrontal association areas (Oh et al., 2014; Mohammed and Jain, 2016). Recent studies in rodents have shown that prolonged (hundreds of milliseconds) stimulation in different motor cortical regions evokes different classes of movements, reminiscent of previous findings in monkeys, although the diversity of movement types evoked in rodents is more limited (Harrison et al., 2012; Brown and Teskey, 2014). The relatively small scale of the mouse motor cortex (10^6 neurons, 10^6 corticospinal neurons) enables a comprehensiveness of assessment not readily achievable in larger mammals, but this scale may have constrained functional organization and motor behavioral strategies in divergent ways across evolution (Krubitzer and Seelke, 2012).

This diversity across species presents an opportunity to explore how evolutionary divergence translates into functional differences. The value of comparative approaches in the study of motor control is thus far best exemplified by considering the anatomy of the corticospinal pathway. The seminal work of Lawrence and Kuypers over 50 years ago first clearly implicated this pathway as crucial for both the agility of mammalian limb movements, and the fractionation of limb joint control (Lawrence and Kuypers, 1968). Unlike spinal motor neurons whose organization is stereotyped and highly conserved across mammalian species (Jessel et al., 2011), it has long been appreciated that corticospinal neurons exhibit striking heterogeneity across mammals in the organization of their projections to the spinal cord. Kuypers highlighted the importance of these spinal terminations, writing that:

> ‘When considering the connections of the descending pathways…, it should be realized that their motor capacities are not so much determined by the location of their cells of origin…as by the motor capacities of the interneurons and motoneurons on which these pathways terminate’ (excerpt from pg 83 Porter and Lemon, 1995, originally from Kuypers, 1973).

Kuypers observed a substantial variation in the rostrocaudal organization of corticospinal projections across species. For example, in marsupials (e.g. kangaroos), ungulates (e.g. goats), and rabbits the corticospinal tract terminates at cervical/thoracic levels and therefore does not play a direct role in lower limb control (Kuypers, 1981). This raises questions about whether descending projections from cortex play distinct roles at different segmental levels of the spinal cord. Here the mouse presents a useful model for identifying and selectively accessing caudally projecting neurons in order to address their impact on motor output.

In cats, dogs, rodents, and a subset of New World monkeys (e.g. marmosets), corticospinal projections extend into the lumbar cord but are excluded from the ventral-most region of the spinal gray matter. This is the region where motor neuron cell bodies lie, suggesting that motor output is indirectly influenced via spinal interneurons in these species. This is in contrast to the organization of projections in most primates where axons extend into the ventral gray matter of the spinal cord (Kuypers, 1981) thereby permitting corticospinal neurons to exert a direct excitatory influence on spinal motor neurons (Lemon and Griffiths, 2005). These con-
nections are strongest onto motor neurons innervating distal hand muscles (Fritz et al., 1985) and emanate principally from primary motor cortex (Rathelot and Strick, 2006), suggesting the emergence of an evolutionarily newer mode of control within the motor cortex of most primates (Levine et al., 2012).

This raises the question: what is the functional impact of this direct access to spinal motor neurons? Comparative studies have demonstrated that the more dexterous a species is, the more anatomically developed its cortico-motoneuronal connections are (Bortoff and Strick, 1993). Although cortico-motoneuronal connections are best developed in primates, mice have proven to be an effective model for assessing their role in behavior and examining the molecular mechanisms by which they form. A recent study by Gu et al., implicated Sema6D-PlexA1 signaling in mediating the selective elimination of direct contacts onto motor neurons by corticospinal axons (Gu et al., 2017). In keeping with this, they showed that motor cortical expression of PlexA1 is strong in wild type mice, where such connections are largely eliminated, but weak in humans where direct synapses are preserved. PlexA1 mutant mice retain cortico-motoneuronal connectivity into adulthood and perform significantly better on tasks requiring dexterous manipulation. Collectively, these findings support the hypothesis that cortico-motoneuronal connections are particularly involved in the selective and independent movement of the digits, which underlies hand dexterity (Lemon and Griffiths, 2005).

**CELL AND SUBTYPE RESOLVED CONNECTIVITY MAPPING**

In the late 1800s, Ramon y Cajal meticulously reconstructed the morphology of neuronal cells in Golgi impregnated brain tissue. Despite the vagaries of this technique, he made several prescient observations about the nature of connectivity between neuronal populations (Zergeroğlu and Nañççi, 2015). Since that era, neural tracing techniques have advanced significantly, often leveraging advances in genetics, virology, and imaging to visualize neural circuits and probe their functional significance. These techniques have enabled finer aspects of neural system organization to be charted, such as those dependent on the distinct identities of neuronal subtypes and the synaptic connectivity between them. In addition, large-scale RNA sequencing efforts are now uncovering an unprecedented diversity in cortical cell types; in two cortical regions alone over 100 transcriptionally distinct neuronal populations have been identified (Tasic et al., 2018). Arguably in no mammalian species have these emerging anatomical tools been more utilized than in the mouse.

The introduction of conventional tracers taken up by intact neurons represented a significant advance in neuroanatomical mapping that enabled observation of the input and output of a region of interest. Retrograde tracers such as Cholera toxin subunit B and Fluorogold enter neurons via receptor mediated uptake or vesicular endocytosis and are then transported to the cell body. Conversely, anterograde tracers such as biotinylated dextran amine are taken up by somata and dendrites and subsequently transported along the axon (Lanciego and Wouterlood, 2011). In motor circuits these tools have been widely adopted to map anatomical organization (VanderHorst and Ulfhake, 2006; Betley et al., 2009; Liang et al., 2011).

However, a significant drawback of conventional tracers is that bulk uptake from the injection site cannot allow for selective tracer entry into specific cell types that are spatially intermingled with other types. In the mouse, this was made possible by the emergence of genetic access to discrete cell types marked by the expression of a specific gene. Over the last 15 years, elucidation of cell types in rodent cortex has progressed rapidly (Arlotta et al., 2005; Kepecs and Fishell, 2014; Tasic et al., 2016) and alongside this there have been several adaptations in neurotropic viruses that allow for delivery of genes into neuronal cells. One of the most widely used viruses for in vivo gene delivery are adeno-associated viruses (AAVs), largely because they mediate perduring gene expression, rarely integrate into the host genome, and exhibit no detectable pathogenicity following infection (Penaud-Budloo et al., 2008; Hammond et al., 2017).

The mapping of inputs and outputs has also been significantly facilitated by the development of a toolkit of trans-synaptic neuronal viruses. As the name suggests, these viruses have the ability to spread between synaptically coupled neurons, revealing connectivity with cell and subtype resolution. One of the most efficacious of these has been rabies, a retrograde trans-synaptic virus. Two modifications have adapted rabies for the study of neural circuitry. First, the glycoprotein (G) gene, which is critical for viral trans-synaptic spread, was deleted and is expressed only in those cells whose inputs are to be traced (Wickersham et al., 2007a, b). Second, the initial rabies infection is targeted to the cell type of interest by pseudotyping the virus with the envelope protein EnvA, which renders it unable to infect mammalian cells unless they express the cognate avian receptor TVA (Young et al., 1993; Bates et al., 1998). As with the expression of the glycoprotein, TVA expression can also be restricted to the cell type of interest.

One of the earliest applications of rabies retrograde tracing, absent these genetic modifications, was in the monkey, where it revealed surprising overlap in the motor cortical regions targeting muscles that act at different limb joints (Rathelot and Strick, 2009) and the presence of direct interactions between cerebellum and basal ganglia (Hoshi et al., 2005; Bostan et al., 2010). More recently, rabies modified as above has been used in the mouse spinal cord to map the organization of interneuron inputs to flexor and extensor motor neurons (Tripodi et al., 2011). This study revealed differences in the settling positions of premotor interneuron inputs to antagonistic muscles of the hindlimb, suggesting that they are differentially recruited by local spinal circuits. Indeed, these differences extended to the nature of the descending inputs they receive, with extensor motor neurons...
being preferentially targeted by vestibulospinal neurons (Basaldella et al., 2015).

These advances in trans-synaptic tracing have enabled experimenters to reconstruct synaptic inputs and draw conclusions that previously could only be made with electrophysiological recordings and electron microscopy, neither of which are amenable to brain-wide mapping. Combining rabies trans-synaptic technology with genetic tools in mice (e.g. the Cre/lox system for conditional gene expression) permits visualization of both the local and long-range inputs to defined neuronal cell types. In our recent work we have used this approach to demonstrate differences in the anatomical organization of corticospinal neurons that engage different target populations in the spinal cord (Fageiry et al., in preparation).

Looking to the future, there is scope to further extend the utility of these tools. For example, the lethality of rabies remains a significant limitation to its long-term use in physiological experiments. Continuing efforts to further diminish the pathogenicity of rabies (Reardon et al., 2016; Ciabatti et al., 2017) give hope for the use of this technique to deliver gene products that enable the observation and manipulation of neural activity in specific populations of presynaptic input neurons. This will enable exploration of how different neuronal subtypes inform the function of downstream populations.

THE DEVELOPMENT OF MOTOR BEHAVIORAL ASSAYS FOR CORTICAL INTERROGATION

As a new model for cortical control, appropriate behavioral paradigms in mice are still somewhat limited in number but growing quickly. Existing paradigms for use in mice differ from those typically used in primates in three primary ways. First, primate motor behavioral paradigms often involve complex behavioral sequences that separate different phases of motor behavior, like planning, initiation, execution, and sensory-guided modification, to distinct epochs so they can be separately examined. Second, primate paradigms often elicit performance of many limb movement variants, a task richness facilitated by the flexibility of primate limb movement and the ability of primates to generalize task rules (Churchland et al., 2012). This feature is particularly advantageous, as many imaginative characterizations of motor system function would benefit from observing recorded populations in a broad range of neural activity states. Yet many mouse behavioral paradigms do not emphasize the diversity of movement types needed to generate commensurate diversity in neural activity. Lastly, consistent with their lack of direct cortical projections to motor neurons, the behaviors that mice can perform exclude the most dexterous movements or too-use seen in primates, but do include some skilful manipulation using the digits.

Inspired by previous approaches in primates, there has been recent development of behavioral paradigms in which mice perform cortically-dependent single forelimb tasks while head-fixed, facilitating stable activity recording and stereotyped behavioral trials. In one such paradigm, mice reach toward and grasp food pellets (Fig. 1A; Guo et al., 2015). In another, a vibratactile stimulus applied to the forepaw prompts the mouse to reach toward and touch a sensor (Estebanez et al., 2017). In an elegant reprise of a classic human task, mice have also been trained to adapt to force-field perturbations while manipulating a joystick, which was found to involve sensorimotor cortex (Fig. 1B; Mathis et al., 2017). In another paradigm, a mouse learns to reach toward and grasp a water droplet, then bring the droplet to its mouth, a task which appears relatively easy to train and permits a large number of daily trials (Fig. 1C; Galiñanes et al., 2018).

Paradigms in which mice move freely allow for the expression of more ethologically relevant behavior, for which the motor system is more likely to be adapted. In one such paradigm, a mouse reaches through a narrow opening to retrieve a food pellet (Azim et al., 2014; Fink et al., 2014; Wang et al., 2017). The dexterous paw manipulations during feeding behavior are elicited in paradigms focused on pasta (Whishaw et al., 2017) and seed eating (Barrett et al., 2020). Free-moving paradigms also facilitate home cage training, which increases the number of behavioral trials. This was exploited recently for training a joystick-based center-out reach task (Bollu et al., 2019b). The continued development of methodology for neural recording in freely behaving rodents, including wireless electromyographic recording (Gutruf and Rogers, 2018) and head-mounted microscopes (Ziv et al., 2013; Cai et al., 2016) has expanded the range of behavioral contexts amenable to physiological study.

The advent of electromyographic (EMG) recording methods for mice has improved capacity for measuring motor output as mice behave. EMG electrodes for chronic recording have been adapted and miniaturized from those used previously in larger mammals (Pearson et al., 2005; Akay et al., 2006). The use of EMG recording is essential to studies involving isometric tasks or detection of direct effects of neural perturbations on muscle activity (Miri et al., 2017; Murray et al., 2018). EMG recordings can also be used in closed-loop fashion to determine reward and guide behavioral training, a feature exploited to train mice to simultaneously contract two forelimb muscles in our recent work on voluntary cocontraction (Warriner et al., in preparation).

Realizing many of the experimental opportunities in mice will require continued development of task paradigms. Though field expertise in mouse training remains in its infancy, ultimately it may prove challenging to achieve the movement diversity seen in primate paradigms. Moreover, the behavioral repertoire of mice reflects different evolutionary pressures and mice interact with the world via movement differently from primates. These are differences that must be negotiated when using mice to discover general principles of motor system function. Fortunately, new methods are emerging for wireless recording, markerless behavior tracking (Mathis et al., 2017), and achieving statistical power for hypothesis testing in behavioral paradigms lacking an explicit trial structure (Sarup et al., 2019). This is creating new opportunities...
for physiological interrogation during diverse and ethologically relevant behaviors.

**PERTURBATIVE INTERROGATION OF CORTICAL CIRCUITS**

Genetic access in mouse motor circuits facilitates optogenetic perturbation, which has substantial potential for probing the function of neuronal subpopulations and informing neural system models. The building and testing of models of motor system operation benefits from the ability to probe direct interactions between neuronal populations on the fast timescale that neurons communicate (1–10ms). Reagents for optogenetic perturbation continue to progress (Kim et al., 2017), and the spatial resolution of their application continues to improve. Fast timescale perturbations can be targeted to brain areas (Arenkiel et al., 2007), subpopulations within areas (Adamantidis et al., 2007), and now to individual neurons (Rickgauer et al., 2014; Chen et al., 2018). The ability to rapidly inhibit endogenous neural activity through optogenetic perturbation enables the testing of direct functional influence. The specific influence of detectable activity features can be addressed through so-called ‘closed-loop’ perturbation (Grosenick et al., 2015).

The ready applicability of optogenetic approaches in mice is beginning to produce categorically new observations of motor cortical operation. Rapid inhibition in transgenic mice expressing channelrhodopsin2 in all cortical inhibitory interneurons has helped distinguish the influence of motor cortical output on trained and untrained limb movements (Guo et al., 2015), and those involving the processing of unpredictable sensory input (Heindorf et al., 2018). Coupling the temporal precision of inhibition in these transgenic mice with EMG has helped reveal that motor cortical output directly drives limb muscle activity only during certain movement types (Miri et al., 2017). This temporal precision has also enabled localization of motor cortical influence to specific behavioral phases in decision-making paradigms (Guo et al., 2013; Goard et al., 2016), and to kinematically distinct movement features that likely have a distinct dependence on sensory feedback (Bollu et al., 2019a).

These results collectively illustrate the newfound granularity of functional interrogation enabled by optogenetics, but two issues arise. First, the distinct timescale of these perturbations relative to previous approaches creates challenges for reconciling results with historical findings. This challenge arises in part because neural systems respond differently to perturbations over different timescales. Interestingly, in some instances results from fast timescale perturbation align well with predictions based on classical results with slower methods, but other recent results suggest that this similarity may diminish as task complexity increases (Pinto et al., 2019). Second, concerns have been raised about interpreting pharmacological and optogenetic perturbation results given the possibility of deficits from unintended effects downstream of the perturbation (Martin et al., 1993; Otchy et al., 2015). In general, these concerns warrant strong consideration when interpreting data. In certain cases, such concerns can be allayed though characterization of the temporal pattern (Sauerbrei et al., 2020) or latency (Miri et al., 2017) of perturbation effects on downstream targets.

**CELL AND SUBTYPE RESOLVED ACTIVITY MEASUREMENT**

The confluence of genetic and optical approaches has created new opportunities for resolving activity in neuronal subpopulations. Emerging methods enable the...
testing of hypotheses that impute function onto neuronal subtypes defined by features of cellular identity, as functional differences between subtypes should be reflected somehow in neural activity. One such method, referred to as ‘optical tagging,’ allows the identification of neurons within a recorded population according to their genetic identity through the expression of an opsin that yields light-dependent firing (Lima et al., 2009). This method has been used, for example, to show that parvalbumin-positive interneurons in motor cortex respond to sensory cues and the initiation of movement but do not seem to gate the activity of nearby pyramidal neurons (Estebanez et al., 2017). Concerns about the use of optical tagging for excitatory populations are allayed by the use of inhibitory optogenetic probes for identifying such neurons (Rowland et al., 2018). In projection neurons, such concerns can also be avoided through the use of an optical variant of the collision test, in which projecting axons of opsin-expressing neurons are stimulated with light. This has been used to distinguish the movement-related activity in intratelencephalic and pyramidal tract neurons (Li et al., 2015; Saiki et al., 2017). Optical collision was also used to parse the activity of two subtypes of pyramidal tract neurons (Economou et al., 2018) in a study that highlights the potential for functional differences among genetically distinct subtypes that share many morphological similarities.

The imaging of fluorescent calcium indicators has also spawned insights into functional organization at cellular resolution. Early in vivo two-photon imaging studies in forelimb motor cortex identified a clustering of neurons preferentially active during different movement types (Dombeck et al., 2009), reminiscent of functional organization on a larger spatial scale in monkeys (Graziano et al., 2002). Coupling imaging with access to genetic cell types enables a high-throughput approach for cellular resolution measurements across populations. This has been exploited recently to distinguish task-related activity in pyramidal tract neurons from that of other cortical pyramidal neurons (Heindorf et al., 2018). The relative ease of chronic activity recording with calcium indicator imaging has enabled recent studies examining changes across learning, including in movement encoding (Peters et al., 2017; Omlor et al., 2019), and activity correlations between motor cortical output and cerebellar granule cells (Wagner et al., 2019).

The ability to interrogate murine circuits with cellular and subtype resolution also enables testing long-held ideas of cell-based organization. Historical views of motor cortical organization all share a common feature: they implicate distinct functions onto distinct subsets of cells (Graziano, 2006; Darling et al., 2011). Attempts to map cortical organization with electrical stimulation (Fritsch and Hitzig, 1870; Penfield and Boldrey, 1937; Graziano et al., 2002) have led to views that neurons populating distinct subregions drive particular muscle groups or movement types. These ideas have been challenged by our recent work in mice supporting a nascent alternative view that it is instead the covariation of firing patterns across an entire population that dictates motor output (Miri et al., 2017; Warriner et al., in preparation). This has added to recent results suggesting that motor cortex leverages changes in population dynamics manifest in activity covariation to flexibly control a variety of motor outputs (Kaufman et al., 2014; Pandarinath et al., 2018; Perich et al., 2018). Adjudicating between cellular and newer covariation-based views of motor cortical organization will benefit from opportunities to both map activity at cellular resolution and perturb it in real time to assess functional roles. Here the smaller scale of the mouse motor cortex is advantageous as it permits a comprehensiveness of assessment not readily achievable in larger mammals.

Thinking beyond cortex, the mouse also has particular value as a model for assessing interactions between motor cortex and other motor system regions. Functional units of cortical control may be distinguished not just by their collective firing patterns, but also by the way they respond to inputs and the changes they induce in the activity of downstream target neurons. Moreover, essentially all of our ideas about mechanism in motor control involve one neuronal population responding to input from another population. Yet historically it has been prohibitively difficult to directly observe these interactions at spike time resolution, especially when subcortical structures are involved. Fortunately, multielectrode electrode arrays newly enable the monitoring of activity in multiple connected neuronal populations (Jun et al., 2017). Methods from the rapidly evolving field of data science are radically improving our ability to find structure in the large datasets these arrays generate (Paninski and Cunningham, 2018). The relative ease of targeting subcortical structures in the mouse with such arrays makes it a particularly good system in which to characterize cortical organization based on interactions with subcortical regions.

**PROBING FUNCTIONAL ORGANIZATION THROUGH MOTOR LEARNING**

In addition to their role in probing cortical organization related to movement execution, rodent models have made substantial contributions to our understanding of how new movements are generated, improved, and changed over time. Early studies using corticospinal tract transection or cortical lesion implicated motor cortex in the learning of dexterous movements in rats (Castro, 1972; Whishaw et al., 1991, 1993). More recent work has disentangled this role from cortical involvement in movement execution, indicating a distinct cortical role in motor learning and stereotyping new movement sequences (Kawai et al., 2015; Hwang et al., 2019). The vital role motor cortex plays in learning suggests that its organizational logic should reflect this role. Thus, the reorganization of motor cortex that underlies learning could be useful in illuminating this logic.

The rodent has historically played a critical role in characterizing the many structural changes in cortex during motor learning. Extensive reorganization of dendritic arbors throughout motor cortex has been observed during the learning of forelimb tasks (Greenough et al., 1985; Withers and Greenough, 1989;
underlying cortical organization (Shmuelof and the theories of motor learning that themselves imply an activity chronically across large swaths of motor cortex to explore as mechanistic underpinning of motor learning may depend critically on task design. In this respect, the relative ease of engaging freely-behaving mice in motor learning could be advantageous for capturing aspects of learning in situ.

**LOOKING TO THE FUTURE**

Studies in mice are increasingly leveraging technological developments in molecular biology, virology, optogenetics, imaging, and neural recording to address questions of neural system function. This has occurred in parallel with an increase in the sophistication of behavioral tasks and methods to observe and quantify task performance. This positions mouse models to exploit the growth of these diverse tools to assess neuronal function at an increasingly fine level of granularity. Such efforts may ultimately resolve basic functional elements that underlie the cortical control of movement.

Moving forward, this control will be investigated via both population activity dynamics and cell type function. Recent studies have revealed a tremendous diversity of transcriptionally defined neuronal populations, adding to the range of cell identity features the functional relevance of which can now be assessed. At the same time, machine learning approaches have demonstrated a vast computational capacity in neural network models composed of homogeneous units connected in ways not obviously illustrative of function. The capacity for interfacing large-scale physiological assessment of motor circuit function with anatomical and genetic techniques in the mouse offers a unique opportunity to explore how computational capacity emerges from biological circuits. A key challenge here is the development of behavioral paradigms best suited to this endeavor.

As we improve our understanding of motor learning, planning, and execution, comparative studies can help illuminate functional organization and identify principles conserved across species. Thus, another ongoing challenge lies in identifying questions addressable in mice that are of general interest to the motor control field. This challenge is compounded by the novelty of experimental opportunities: historical limits on the resolution of activity measurement and perturbation have impeded the development of hypotheses regarding how neuronal populations directly interact on fast timescales as movement unfolds. The challenge of establishing general relevance is also compounded by the distinct features of rodent behavioral strategy and anatomical organization. Yet the power of comparative analysis may ultimately prove such differences integral to the impact of mouse studies.

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REFERENCES
Ferrier D, Yeo G (1884) A record of experiments on the effects of lesion of different regions of the cerebral hemispheres. Philos Trans Royal Soc Lond:479–564.


