Distinct Cortical Circuit Mechanisms for Complex Forelimb Movement and Motor Map Topography

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SUMMARY

Cortical motor maps are the basis of voluntary movement, but they have proven difficult to understand in the context of their underlying neuronal circuits. We applied light-based motor mapping of Channelrhodopsin-2 mice to reveal a functional subdivision of the forelimb motor cortex based on the direction of movement evoked by brief (10 ms) pulses. Prolonged trains of electrical or optogenetic stimulation (100–500 ms) targeted to anterior or posterior subregions of motor cortex evoked reproducible complex movements of the forelimb to distinct positions in space. Blocking excitatory cortical synaptic transmission did not abolish basic motor map topography, but the site-specific expression of complex movements was lost. Our data suggest that the topography of movement maps arises from their segregated output projections, whereas complex movements evoked by prolonged stimulation require intracortical synaptic transmission.

INTRODUCTION

The motor cortex has long been known to play a central role in the generation of movement (Fritsch and Hitzig, 1870), but fundamental questions remain to be answered about the functional organization of its subregions and their neuronal circuits. Results from electrical brain stimulation have traditionally been interpreted with an emphasis on somatotopy (Penfield and Boldrey, 1937; Asanuma and Rosén, 1972), but the utility of this principle has diminished with the discovery of multiple representations of the body (Neafsey and Sievert, 1982; Luppino et al., 1991; Schieber, 2001). A more nuanced view has since developed, with recordings made during voluntary movements in monkeys demonstrating that neurons in motor cortex encode information related to the force (Evarts, 1968), direction (Georgopoulos et al., 1986), and speed of movements (Moran and Schwartz, 1999; Churchland et al., 2006). The activity of cortical neurons also reflects both preparation for movement (Sanes and Donoghue, 1993; Paz et al., 2003) and the interpretation of actions performed by others (Gallese et al., 1996; Hari et al., 1998). Recently, experimentation with prolonged trains of stimulation has suggested that the brain’s multiple motor representations may be organized according to classes of behavior (Graziano et al., 2002; Stepniewska et al., 2005; Ramanathan et al., 2006).

Despite the detailed knowledge gleaned from these efforts, our understanding of the macroscopic organization of motor cortex remains incomplete. Much of our understanding about the motor cortex comes from experiments in which stimulation or recording is performed at a few cortical points. Technical limitations have traditionally made it difficult to probe the cortical circuitry underlying motor representations in a uniform, quantitative manner. Recently, we and others have developed a novel method for rapid automated motor mapping based on light activation of Channelrhodopsin-2 (ChR2) that has facilitated experiments which were previously impossible (Ayling et al., 2009; Hira et al., 2009; Komiyama et al., 2010). This technique has the advantage of objectively and reproducibly sampling the movements evoked by stimulation at hundreds of cortical locations in mere minutes. Here, we apply light-based motor mapping to investigate the functional subdivisions of the motor cortex and their dependence on intracortical activity.

The ability to repeatedly map the motor cortex over timescales ranging from minutes to months has allowed us to appreciate the dynamic nature of movement representations and facilitated the comparison of motor maps generated before and after pharmacological perturbations of the intracortical circuitry. We have exploited the predominant expression of Channelrhodopsin-2 in layer 5B pyramidal neurons of Thy-1 transgenic mice (Arenkiel et al., 2007; Wang et al., 2007; Yu et al., 2008; Ayling et al., 2009) to target this class of corticofugal cells directly, exposing their contribution to motor cortex topography and identifying a functional subdivision of the mouse forelimb representation based on movement direction. Prolonged trains of light or electrical stimulation revealed that activation of these subregions drives movements to distinct positions in space. To identify mechanisms that could account for the different movement types evoked by stimulation of these cortical subregions, we performed pharmacological manipulations of the intracortical circuitry and targeted anatomical tracing experiments.
RESULTS

Movement-Based Mapping of Mouse Motor Cortex

We used optogenetic motor mapping to rapidly stimulate hundreds of cortical points in ChR2 transgenic mice (Arenkiel et al., 2007) and assemble maps based on evoked movements of the contralateral forelimb and hindlimb (Figures 1A–1C, see Ayling et al., 2009 for methodological details). In these experiments, anesthetized mice were head-fixed in the prone position with their contralateral limbs suspended. In this posture, the limbs were able to move freely along the axis of measurement of a laser range finder. The resultant movement maps were centered at positions consistent with those obtained by EMG recording or visual observation (forelimb: 2.2 ± 0.1 mm lateral, 0.05 ± 0.09 mm anterior of bregma; hindlimb: 2.0 ± 0.11 mm lateral, 0.21 ± 0.1 mm posterior of bregma, n = 14 mice, all values ± SEM) (Pronichev and Lenkov, 1998; Ayling et al., 2009; Hira et al., 2009; Tennant et al., 2011). Composite maps based on the average of three repetitions were highly reproducible, with a shift in center position of 0.19 ± 0.02 mm (n = 12 mice) between mapping trials (∼30 min per composite map). In a separate group of animals implanted with cranial windows, maps remained stable for months (Figure S1 available online). Movement maps could also be generated in animals where ChR2 was expressed in pyramidal neurons of both superficial and deep cortical layers by transduction with adeno-associated virus (Figure S2).

Forelimb Motor Cortex Is Subdivided into Functional Subregions

Consistent with previous results, forelimb movements could be elicited by stimulation (10 ms pulses, 0.5–10 mW or 63–1270 mW/mm²) of a broad cortical area, up to 2 mm anterior and posterior of bregma (Ayling et al., 2009; Tennant et al., 2011). However, when forelimb movements were examined at stimulation sites across the motor cortex, a diversity of response types became apparent (Figures 1C–1F). Evoked movements were divided into two classes depending on the direction of forelimb movement (abduction or adduction, Figures 1D–1F). Stimulation sites that produced movements containing both abduction and adduction components were considered as regions of overlap between abduction and adduction maps. This analysis revealed a functional subdivision of the motor cortex that was not apparent from EMG-based maps, even when antagonistic muscle pairs were compared (Ayling et al., 2009).

The motor cortex abduction representation (here termed Mab) was not different from the adduction representation in area (Mad) (4.7 ± 0.6 versus 4.9 ± 0.7 mm², n = 14 mice), but movements evoked from the center of Mab tended to be smaller than those
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The centers of gravity of Mab and Mad were separated from each other by an average of 0.6 ± 0.06 mm (p < 0.0001, single sample t test versus hypothetical mean 0, n = 14 mice). When a threshold was applied at 50% of each map’s peak amplitude, separation between Mab and Mad increased to 1.2 ± 0.07 mm (n = 14 mice), which is comparable to the distance between the centers of forelimb and hindlimb somatosensory maps (1.2 ± 0.2 mm, n = 7 mice). These observations demonstrate that the mouse forelimb motor cortex can be reproducibly subdivided according to a simple assay of evoked movement direction.

Prolonged Stimulation of Abduction and Adduction Representations Drives Movements to Distinct Positions in Space

It has been proposed that long stimulus trains may be more effective than shorter bursts at producing ethologically relevant movements and identifying cortical movement representations (Graziano et al., 2005). Despite the ability of light-based mapping to rapidly, quantitatively, and uniformly sample the motor output of a large cortical area, the restricted sampling of forelimb displacement in our method limits the information that can be gathered about the movements generated by stimulation of any particular cortical location. To better describe the properties of the Mab and Mad motor subregions, we used a high-speed CCD camera to record forelimb movements evoked by stimulation of sites near the center of each map. In these experiments, the centers of the Mab and Mad maps were defined with the mouse lying prone and the contralateral forelimb suspended parallel to the ground (Figure 3A, left). The anesthetized mice were then moved to a sitting posture, with their heads fixed and their forelimbs hanging free (Figure 3A, center).

With prolonged stimulus trains (500 ms), the forelimb tended to reach a final position within ~300 ms and remain there for the duration of the stimulus. Stimulation of Mab caused the contralateral forelimb to be raised and then brought toward the midline, whereas stimulation of Mad typically produced rhythmic movements lower in space, often coupled with movement of the hindlimb (Figure 3B). These movements were reproduced in anesthetized mice where ChR2 was locally expressed using adeno-associated virus (Figure S2) and in awake, freely moving ChR2 transgenic mice stimulated within Mab and Mad via optical fibers (Figures 3A and 3B, right; Movie S2). In both anesthetized and awake mice, the displacement of the limb from its starting position was significantly greater when Mab was stimulated rather than Mad (Figures 3B and 3C). Although movement trajectories (Figure 3B) and displacements (Figure 3C) were clearly dependent on stimulus site for both awake and anesthetized mice, the speed profiles of Mab and Mad movements were nearly identical (Figure 3D). Movements evoked from each site were remarkably consistent from trial to trial, and the variability that they did exhibit had a temporal structure that depended on the site of stimulation (Figure S3). Increasing stimulus duration generally had little effect on movement map structure, despite changes observed in movement trajectories (Figure S4). Consistent with previous results from electrical...
stimulation (Ramanathan et al., 2006), modulating optogenetic stimulus intensity did not affect movement trajectories evoked by prolonged stimulation (Figure S5). These experiments complement the mapping study by exposing the distinct types of complex movement that can be evoked from Mab and Mad by prolonged stimulation in both anesthetized and awake mice.

Electrical and Optogenetic Stimulation Evoke Similar Movements

To determine whether these complex movements require selective stimulation of layer 5B neurons, we compared optogenetic stimulation (500 ms train of 5 ms, 5 mW pulses at 100 Hz) with trains of electrical intracortical microstimulation (ICMS) targeted to layer 5 of cortex (500 ms trains of 200 μs, 100 μA pulses at 200 Hz) (Ramanathan et al., 2006). Given the differences between ICMS and optogenetic stimulation, we were surprised to discover that ICMS was able to closely reproduce the complex movements characteristic of transgenic or viral optogenetic stimulation of Mab and Mad (Figure 4A, Figure S2). In addition to their overlapping trajectories, movements evoked by either method had comparable peak displacements, time to peak, and angle from origin at peak displacement (Figure 4B). Interestingly, although movements evoked by ICMS or optogenetic stimulation shared the same end point, ICMS-evoked movements were significantly slower (Figure 4C). These results suggest that the site of stimulation determines the trajectory of the resulting movement (Figure 3), whereas movement speed depends on the mechanism of stimulation (Figure 4).

Specificity of Complex Movements Evoked from Different Cortical Areas Requires Intracortical Synaptic Transmission

After characterizing the movement representations of the mouse motor cortex, we investigated their mechanistic basis. We hypothesized that the distinct movements produced by the Mab and Mad motor cortex subregions could be explained by differences either in their output projections (Rathelot and Strick, 2009; Matyas et al., 2010), or in the pattern of input they receive...
from recurrent intracortical circuits (Weiler et al., 2008; Anderson et al., 2010; Hooks et al., 2011) or subcortical loops (Hoover and Strick, 1993; Flaherty and Graybiel, 1991; Kelly and Strick, 2003).

To test the extent to which cortical synaptic input contributes to the differences between Mab and Mad motor subregions, we compared movement trajectories generated before and after the application of glutamate receptor antagonists (CNQX 4.5 mM and MK-801 0.3 mM) or saline to the cortical surface (Figure 5A). In the control condition Mab and Mad movements had nonoverlapping trajectories that could be distinguished by plotting the angle of the forelimb from the starting position (Figure 5B, left). Disrupting glutamatergic transmission increased the extent to which Mab and Mad trajectories overlapped, biasing both toward medial rotation (Figure 5B, right). Glutamate receptor antagonists also had a site-specific effect on speed profiles, causing a delayed increase in movement speed for Mad, but not Mab (Figure 5C). These results suggest that differences between movements evoked by prolonged stimulation of Mab and Mad may reflect variation in the patterns of glutamatergic synaptic input that these areas receive.

Movement Topography Is Preserved during Blockade of Intracortical Synaptic Transmission

We next examined the effects of pharmacological manipulations on the structure of motor maps evoked by brief (10 ms) pulses of light (Figures 6A and 6B). We had initially hypothesized that blocking cortical glutamatergic transmission would eliminate the contribution of facilitatory cortico-cortical projections from regions lacking direct motor output, causing a reduction in map area. Surprisingly, we found that Mab and Mad maps tended to increase in amplitude (Figure 6B) and expand in area (Figure 6C) after application of glutamate receptor antagonists, compared with no change after application of saline vehicle. This expansion in map area was also apparent in the hindlimb motor representation (134 ± 77%, p = 0.02, n = 9, paired t test), but the expansion was most pronounced in Mad (Figure 6C). The region of overlap between abduction and adduction representations increased in the presence of glutamate receptor antagonists, but was not significantly altered by application of saline (Figure 6D). Because of its influence on map area (Figures SSA and SSB), stimulus intensity was held constant within animals for all pharmacology experiments.

Despite the fact that glutamate receptor antagonists caused map expansion and increased overlap between Mab and Mad, movement topography was not abolished. The Mab and Mad maps could still be distinguished in the presence of glutamate receptor antagonists (Figure 6B), with no significant reduction in the separation between their centers of gravity (Figure 6D). Application of glutamate receptor antagonists did not cause a significantly greater shift in map centers from their baseline positions than application of saline for Mab (0.5 ± 0.09 versus 0.5 ± 0.09 mm, respectively, p = 0.96, n = 9 versus n = 5, t test) or Mad (0.5 ± 0.09 versus 0.2 ± 0.04 mm, respectively, p = 0.06).

Although the increased movement durations (Figure 5C) and expansion of motor maps (Figure 6C) caused by disruption of excitatory synaptic transmission were unexpected, this may be explained by a loss of disynaptic inhibition (Helmstaedter et al., 2009; Murayama et al., 2009; Adesnik and Scanziani, 2010; Silberberg and Markram, 2007; Kaper et al., 2007). To test this hypothesis, we repeated these experiments with...
GABA<sub>A</sub> receptor antagonists (gabazine 1 μM n = 4 or picrotoxin 100 μM n = 2, Figure S6). GABA receptor antagonists diminished differences between M<br/>and M<sub>ad</sub> movement trajectories, but had no significant effect on movement kinematics (Figure S6), and generally did not degrade functional subdivisions of the motor cortex. Disrupting GABAergic transmission did reproduce the increases in map amplitude (Figure S7C) and area (Figure S7D) seen during blockade of excitatory transmission. As with the delayed increase in movement speeds (Figure 5C), this effect was restricted to M<sub>ad</sub>. These effects are consistent with disinhibition causing the selective expansion of the M<sub>ad</sub> subregion. The separation between M<sub>ab</sub> and M<sub>ad</sub> and the region of overlap between them was unchanged (Figure S7E). Like glutamate receptor antagonists, GABA receptor antagonists did not cause greater displacement of map centers than saline treatment for M<sub>ab</sub> (0.6 ± 0.1 versus 0.5 ± 0.1 mm, p = 0.37, n = 6 versus n = 5, t test) or M<sub>ad</sub> (0.4 ± 0.1 versus 0.2 ± 0.04 mm, p = 0.24).

**Topical Application of Glutamate Receptor Antagonists Disrupts Cortical Input without Preventing Direct Activation of ChR2-Expressing Output Neurons**

The observation that disrupting intracortical synaptic transmission can impair the expression of diverse complex movements without abolishing the topography of movement maps was initially surprising, but may be explained by differences between
the roles of intracortical and corticofugal circuits. It is possible that cortical application of receptor antagonists interferes with local circuit function and the generation of complex movements by prolonged stimulation, but does not alter the movement maps generated by the output of corticofugal cells directly activated by brief pulses of optogenetic excitation. To measure the effect of glutamate receptor antagonists on cortical activity evoked by ChR2 stimulation, we recorded local field potentials (LFPs) in all cortical layers using a multichannel probe (Figure 7). These recordings confirmed that glutamate receptor antagonists blocked synaptic input to the cortex driven by electrical stimulation of the contralateral forelimb. Glutamate receptor antagonists did not block direct activation of ChR2, but they did cause a decrease in delayed, presumably synaptic, components (Figure 7A). This effect was evident at all depths recorded (Figure 7B), but may have been primarily due to inactivation of the upper cortical layers, where drug concentrations are expected to be highest after topical application. Because optogenetic stimulation of ChR2-expressing neurons does not require synaptic activation, corticofugal neurons could still propagate their action potentials beyond the influence of the cortically applied glutamate receptor antagonists to evoke movements.

Divergent Projections from Mab and Mad

The fact that cortical application of glutamate receptor antagonists does not abolish movement topography (Figure 6) or prevent direct activation of corticofugal ChR2-expressing neurons (Figure 7) suggests that cortical output circuits may differentiate the Mab and Mad subregions. To test this hypothesis, we injected the deep cortical layers of Mab and Mad with adeno-associated virus containing fluorescent marker constructs to label axonal projections throughout the brain (Figure 8A). In addition to reciprocal intracortical projections between these regions and trans-callosal projections to homotopic sensorimotor cortex, we observed adjacent, nonoverlapping projections in the striatum and internal capsule (Figures 8B and 8C), with fibers originating in Mab occupying positions medial to those from Mad (2.0 ± 0.1 versus 2.5 ± 0.07 mm from midline in the dorsolateral striatum, p = 0.03, n = 7, paired t test; Figure 8D). This observation further supports the hypothesis that movement map topology is a product of the pattern of corticofugal projections, whereas the generation of complex movements by prolonged stimulation requires input from recurrent intracortical circuits and/or loops with subcortical structures.

DISCUSSION

We have applied light-based motor mapping to reveal that the mouse forelimb motor cortex is subdivided into distinct movement representations. Prolonged stimulation of these regions drives movements with similar speed profiles, but which terminate at different positions in space. Although complex movements evoked by prolonged stimulation were sensitive to perturbations of intracortical synaptic transmission, the topography of movement direction was not abolished by blockade of either excitatory or inhibitory synaptic transmission. The persistence of movement topography in spite of disrupted intracortical synaptic transmission may be due to the presence of...
segregated corticofugal pathways from the two movement representations.

**Mechanistic Basis of Multiple Motor Representations**

Functional differences between movement representations are likely the product of both their intracortical circuits (Jacobs and Donoghue, 1991; Rouiller et al., 1993) and their corticofugal pathways (Brown and Hestrin, 2009; Rathelot and Strick, 2009). The recurrent circuitry of the neocortex (Douglas and Martin, 2004; Hooks et al., 2011) provides computational power and allows flexible control of the more stereotyped connections between the spinal cord and the periphery. We have shown that the ability of prolonged cortical stimulation to generate complex movement patterns depends upon these intracortical circuits, and can be blocked by pharmacological manipulations. The contribution of recurrent cortical circuitry to movement representations is evidenced by their rapid modification in response to pharmacological manipulations (Jacobs and Donoghue, 1991) or inhibition of protein synthesis (Kleim et al., 2003) and their rewiring after injury (Dancause et al., 2005). Expansion of representations after application of both glutamate and GABA receptor antagonists is presumably due to a loss of disynaptic inhibition, consistent with previous work (Jacobs and Donoghue, 1991; Aroniadou and Keller, 1993; Hess and Donoghue, 1994; Schneider et al., 2002; Foeller et al., 2005). The critical role of inhibitory circuits in cortical function and the profound change in brain state induced by application of GABA receptor antagonists complicates interpretation of our GABA experiments, but it is interesting to note that the effects of this manipulation were relatively specific to the **M**<sub> Mad </sub> representation (Figure S7).

Our observation that distinct cortical movement representations persisted after the pharmacological disruption of intracortical synaptic transmission suggests that the corticofugal projections made by these regions play a key role in shaping movement representations, as has been reported for the whisker motor pathway of mice (Matyas et al., 2010) and monkey motor cortex (Rathelot and Strick, 2009). Light-based motor mapping using line 18 Thy-1 transgenic mice (Ayling et al., 2009; Hira et al., 2009; Komiyama et al., 2010) is particularly well suited to defining the contribution of corticofugal projections to motor topography since layer 5b pyramidal neurons are preferentially labeled (Yu et al., 2008; Ayling et al., 2009).

The macroscopic parcellation of motor cortex into functionally distinct zones is particularly intriguing given that neuronal response types appear to be intermingled at the cellular level in rodents (Ohki et al., 2005; Dombeck et al., 2009; Komiyama et al., 2010; Wang et al., 2011). This apparent paradox may be resolved if movement representations are emergent phenomena that only materialize at the population level (Georgopoulos et al., 1986; Wessberg et al., 2000). Alternatively, this observation could reflect important differences between the layer 2/3 cortical neurons studied in many imaging experiments and the predominantly layer 5b neurons stimulated in light-based mapping.

**Movement Representations in Rodents and Primates**

Multiple motor representations of the rodent forelimb have previously been described as the caudal and rostral forelimb areas (CFA and RFA) (Neafsey and Sievert, 1982). Although **M**<sub> Mab </sub> and **M**<sub> Mad </sub> occupy the same cortical territory as mouse CFA and RFA (Tennent et al., 2011), important differences exist between...
them. First, $M_{ab}$ and $M_{ad}$ are contiguous and equal in area, whereas CFA is larger than RFA and they are separated by a representation of the neck (Tennant et al., 2011). Second, RFA is not apparent in all experiments or animals (Tennant et al., 2011), whereas $M_{ab}$ and $M_{ad}$ almost always co-occur. It is interesting to note that in rats, mapping with short stimulus durations produces maps that include RFA and CFA, whereas long (500 ms) durations reveal maps containing movement representations similar to $M_{ab}$ and $M_{ad}$ (Ramanathan et al., 2006).

Primate motor cortex is commonly described as a hierarchical arrangement of primary motor cortex, premotor areas, and supplementary motor cortex where premotor areas can facilitate motor output from primary motor cortex (Cerri et al., 2003). It has been suggested based on their connectivity that rodent RFA and CFA are homologous to premotor and primary motor cortex, respectively (Rouiller et al., 1993). Our observation that $M_{ad}$ expands after application of GABA receptor antagonists but $M_{ab}$ does not suggests that these regions may be differentially regulated by feed-forward or lateral inhibition. Coupled with the relatively longer latencies for movements evoked from the more caudal $M_{ad}$ region, this could be viewed as evidence for a hierarchical arrangement of mouse motor cortex.

Although intracortical connections are obviously critical for motor function, it is also known that multiple motor cortical regions project in parallel to the spinal cord (Rouiller et al., 1993; Dum and Strick, 2002). This implies that multiple motor regions can contribute directly to movement, and may not be arranged hierarchically (Graziano and Aflalo, 2007). This view is corroborated by the results of our experiments with glutamate and GABA receptor antagonists, which demonstrated that the $M_{ab}$ and $M_{ad}$ representations could function independently after a diminution of intracortical synaptic transmission. If multiple motor regions do not form a hierarchical chain, they may instead encode various behaviors or postures (Graziano et al., 2002, 2005). This is consistent with our observation that stimulation of $M_{ab}$ and $M_{ad}$ drives limb movements to different
end positions in space. This result could be produced with opto-
genetic or electrical stimulation, suggesting that it is not an arti-
fact of passive electrical current spread from the stimulation site
(Strick, 2002). Although we sometimes observed movements that resembled locomotion (combined rhythmic movements of contralateral forelimb and hindlimb upon stimulation of M\text{ab}), or manipulation (stimulation of M\text{ab} generally caused elevation and medial rotation to bring the contralateral forelimb to a central position in front of the body, Movie S2), we chose to focus our analysis on basic measures of motor behavior, such as move-
ment direction.

Comparison of Optogenetic and Electrical Motor Mapping

Our light-based motor mapping technique has been optimized for speed and simplicity (Ayling et al., 2009); hence, measure-
ments of limb movement were made in a single dimension during mapping, or in two dimensions for video analysis. ICMS has been optimized to resolve select movements of single joints (Burish et al., 2008; Chakrabarty et al., 2009; Young et al., 2011), something that is not observed with our technique in its present form. As a consequence, we are overlooking some of the complexity of evoked movements during mapping, and it is likely that the mouse motor cortex could be subdivided more finely based on a more advanced quantitative assay. These disadvantages of light-based mapping are offset by its unique ability to rapidly, objectively, and noninvasively quantify motor output of a defined cell type across the entire sensorimotor cortex.

The spatial resolution of light-based mapping is determined by physical scattering of light and by active spread of excitation. The influence of these factors is apparent from the observation that motor map area is strongly related to both stimulus intensity (Figure S5) and anesthetic depth (Tandon et al., 2008). The influence of these factors is apparent from the observation that motor map area is strongly related to both stimulus intensity (Figure S5) and anesthetic depth (Tandon et al., 2008). A further limit on spatial resolution could be imposed by the width of ChR2-expressing pyramidal neurons’ overlapping dendritic arbor. Although the lateral resolution of light-based mapping may limit our ability to define exact boundaries of motor repre-
sentations, we are able to resolve functional subregions of the forelimb motor cortex and generate maps of the hindlimb motor cortex that are often less than a millimeter in diameter (Ayling et al., 2009). Furthermore, blocking the synaptic spread of acti-
vation does not decrease the size of motor maps, suggesting that active spread of excitation does not substantially degrade
d,eposition, since stimulation sites can be distributed uniformly,
spaced densely, and sampled repeatedly to accurately define the
center of a motor map. Despite the biophysical differences be-

neuronal populations are recruited by these methods. This finding supports the ability of ICMS to selectively
target restricted ensembles of cortical neurons.

A Rodent Model of Motor Circuitry for Complex Movements

The ability to reproducibly evoke distinct complex movements from multiple cortical sites presents an opportunity to perform further investigations of motor circuitry in a widely used model organism. More importantly, it will allow the advantages of genetic engineering in mice to be applied to the problem of motor cortex function and organization, either for optical circuit anal-
ysis (Zhang et al., 2007; Tian et al., 2009; Chow et al., 2010) or in the search for future treatments for movement disorders, cortical injuries, and paralysis (Hodgson et al., 1999; Dancause, 2006; Murphy and Corbett, 2009; Dawson et al., 2010; Vargas-Irwin et al., 2010).

EXPERIMENTAL PROCEDURES

Animals and Surgery

Animal protocols were approved by the University of British Columbia Animal Care Committee. Channelrhodopsin-2 transgenic mice (Arenkiel et al., 2007) from Jackson Labs (line 18, stock 007612, strain B6.Cg-Tg(Thy1-COP4/EYFP)18Gfng/J) established a breeding colony. Adult mice aged 2–6 months and weighing 20–30 g were used for these experiments. Isoflurane anesthesia was used during surgery and intrinsic optical signal imaging of somatosensory representations, but was replaced by ketamine/xylazine (1:100 mg/kg, supplemented at 1:10th initial dose as necessary) prior to motor mapping. Cranietomies were performed on transgenic mice used in acute experiments, but virally transduced mice (see section below for details on injections) were mapped through the intact skull due to concern that multiple cranial surgeries could damage the cortex. Chronic mapping was performed through a cranial window (Harrison et al., 2009).

Light-Based Motor Mapping

Light-based mapping methodology has been described in detail (Ayling et al., 2009). Briefly, we used a scanning stage (ASI MS-2000) controlled by custom Igor Pro software (Wavemetrics) to direct a fixed 473 nm laser beam (Crystalaser, focused to 100 μm diameter, 10 ms pulses, 0.5–10 mW total or 63–1,270 mW/mm²) to an array of cortical sites (typically 13 × 13, with 300 μm spacing between sites). This process was repeated three to five times to obtain a mean value for each pixel of the map. Stimulation was deliv-
ered in a semi-random order with identical stimulus intensity for all sites within a map. Movements were detected using laser range finders with mm sensi-
tivity targeted to the forelimb and hindlimb (Keyence LK-081). In order to exclude artifacts (e.g., from breathing or electrical noise), responses were considered to be genuine only if their amplitude exceeded three times the standard deviation of the 500 ms prestimulus period within 100 ms after stimulus onset.

Map Analysis

Motor maps were generated by plotting the peak amplitude of the mean move-
ment profile corresponding to each cortical site of stimulation. Amplitude was
quantified within a 300 ms time window after laser stimulation. If the amplitude of the movement evoked at that site was positive, the corresponding pixel was added to the abduction map. If the amplitude had a negative value with respect to the baseline, that site was added to the abstraction map. In the case of bidi-
rectional movement profiles where both the positive and negative components
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satisfied the amplitude criteria, the corresponding site was included in both the abduction and adduction maps and counted as overlap between maps. For each map, the center of gravity was calculated along with the mean amplitude and latency for the nine pixels closest to the center point. Maps with mean amplitude of <0.1 mm at the center were excluded from further analysis. Separation between Mab and Mad was defined as the distance between the centers of gravity for each map.

Video Capture of Evoked Movements in Anesthetized Mice
After completing two to five motor maps, mice were raised into a sitting posture with their forelimbs hanging freely. Stimulus sites were placed as close to the centers of the abduction and adduction representations as possible without targeting major blood vessels, since these absorb light strongly (Ayling et al., 2009). Fifty-one frames were captured at a rate of 100 Hz beginning 10 ms prior to laser stimulus onset, and paw trajectories were generated from the raw image sequences using the plugin “MTrack2” for ImageJ. Ten to 20 repetitions were then averaged for each trial, and speed and angle profiles were calculated based on this average trajectory.

Video Capture of Evoked Movements in Awake Mice
ChR2 transgenic mice were implanted with optical fibers (Thorlabs BFH48-200) extending to the cortical surface and terminating in a ferrule connector (Precision Fiber Products) fixed to the skull with dental acrylic and bone screws. Two fibers were implanted, targeted to the mean coordinates of the Mab and Mad map centers. These locations were stimulated alternately (5 mW 5 ms pulses at 100 Hz for 500 ms) using a 473 nm laser (IkeECOOL IKE-473-100-OP) connected via an optical coupler (Doric). Stimulus evoked behavior was recorded by a CCD camera (Dalsa 1M60) and frame grabber (EPix). Limb trajectories were analyzed in the same manner as the anesthetized data, except that paw position was tracked using the plugin “Manual Tracking” for ImageJ.

Intracortical Microstimulation
Glass pipets with a tip 10–20 μm containing a 0.25 mm bare silver wire were filled with 1% fast green in 3 M sodium chloride. A micromanipulator (Sutter) was used to advance the pipet to a depth of 700 μm. Stimulation sites were matched with those targeted by laser stimulation in the same animals. Trains of 200 μs 100 μA pulses at 200 Hz with 10–500 ms durations were generated by an AM systems stimulator and a WPI stimulus isolator.

Virus Injections and Anatomical Tracing
For motor mapping experiments involving virally transduced mice, 1–2 μl of adenovirus-based virus (serotype 2/1 CAG-ChR2-GFP) was injected through a burr hole into the sensorimotor cortex of ChR2-negative mice 2 mm lateral to bregma at a depth of 500 μm using a 5 μl Hamilton syringe with a 33 gauge needle and a syringe pump (WPI). Mice recovered for 2–4 weeks before being used in experiments. For anatomical tracing experiments, Mab and Mad were identified by light-based mapping through the intact skull of ChR2 transgenic mice (Hira et al., 2009). Injections were made using a custom pressure injection system (Cetin et al., 2006). At each site, 250 nl of virus (turboRFP, mCerulean, or eGFP, with matched serotypes 2/1 or 2/9) was injected over 10 min at a depth of 500 μm. Fluorophore placement in Mab versus Mad was alternated between animals. In three of seven animals, motor maps could not be produced by transcranial stimulation, and injections were targeted to the mean coordinates of Mab or Mad. Three weeks after injection, the mice were transecranially perfused and 100 μm coronal sections were sliced on a vibratome, with every third section mounted for epifluorescence imaging. Fluorescence plots from midline were smoothed and averaged, and the mean position of peak fluorescence was calculated for each animal.

Pharmacology
For experiments involving glutamate receptor antagonists, CNQX (4.5 mM) and MK801 (300 μM), gabazine (1 μM), or picrotoxin (100 μM) in physiological saline solution were applied to the craniectomy. The compounds were allowed to incubate for 30 min before mapping resumed, and were replenished (at the same concentration) every ~30 min throughout the experiment. Control experiments were identical except that saline solution was applied in place of the drugs.

Local Field Potential Recordings
A NeuroNexus multi-site electrode (A1-X16-3mm-50-413) was lowered 800 μm into sensorimotor cortex using a micromanipulator (Sutter), and a reference electrode was immersed in the saline bathing the cortical surface. In each experiment, at least 50 trials of 1 ms, 0.1 Hz electrical (1 mA), and ChR2 (10 mw 473 nm) stimulation were recorded, and then CNQX and MK801 were applied to the cortical surface as above and incubated for 30 min before recordings were repeated. The mean peak-to-peak amplitude was measured in a time window of 300 ms after stimulus onset for each electrode contact. The mean amplitude of the baseline noise was subtracted, and adjacent electrode contacts were binned by averaging.

SUPPLEMENTAL INFORMATION
Supplemental Information includes seven figures, Supplemental Experimental Procedures, and two movies and can be found with this article online at [doi:10.1016/j.neuron.2012.02.028].

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