

Final Version published as: Baldwin MKL, Cooke DF, Krubitzer L (2017). Intracortical microstimulation maps of motor, somatosensory, and posterior parietal cortex in tree shrews (*Tupaia belangeri*) reveal complex movement representations. *Cerebral Cortex*, 27 (2), 1439-1456. DOI 10.1093/cercor/bhv329

Intracortical microstimulation maps of motor, somatosensory, and posterior parietal cortex in tree shrews (*Tupaia belangeri*) reveal complex movement representations

Mary K. L. Baldwin, Dylan F. Cooke, Leah Krubitzer
Center for Neuroscience, University of California Davis, Davis CA

Running Title: Complex motor movement representations in tree shrews

Key Words: motor cortex, primate, evolution, reaching, grasping

Pages: 51

Figures: 13

Tables: 4

Correspondence to:
Leah A Krubitzer
University of California, Davis
Center for Neuroscience
1544 Newton Court
Davis, CA 95618
Phone: 530 757 8560
Fax: 530 757 8827
E-mail: lakrubitzer@ucdavis.edu

This research was supported by a grant from NIH NINDS NS035103 to LK, NIH NEI T23 EY015387, and NIH NINDS F32 NS093721-01 to MLKB.

ABSTRACT

Long-train intracortical microstimulation (LT-ICMS) is a popular method for studying the organization of motor and posterior parietal cortex (PPC) in mammals. In primates, LT-ICMS evokes both multi-joint and multiple-body-part movements in primary motor, premotor, and posterior parietal cortex. In rodents LT-ICMS evokes complex movements of a single limb in motor cortex. Unfortunately, very little is known about motor/PPC organization in other mammals. Tree shrews are closely related to both primates and rodents and could provide insights into the evolution of complex movement domains in primates. The present study investigated the extent of cortex in which movements could be evoked with ICMS and the characteristics of movements elicited using both short-train (ST) and LT-ICMS in tree shrews. We demonstrate that LT-ICMS and ST-ICMS maps are similar, with the movements elicited with ST-ICMS being truncated versions of those elicited with LT-ICMS. In addition, LT-ICMS evoked complex movements within motor cortex similar to those in rodents. More complex movements involving multiple body parts such as the hand and mouth were also elicited in motor cortex and PPC, as in primates. Our results suggest that complex movement networks present in PPC and motor cortex were present in mammals prior to the emergence of primates.

INTRODUCTION

Tree shrews are the closest living relative to primates, but they are also closely related to rodents (Fig. 1: Lin et al. 2014). Their relationship to primates is reflected in the organization of their neocortex. Specifically, tree shrews have a well-developed visual cortex with a relatively large expansion of their temporal lobe (Wong et al. 2009 more refs). However, features of their body morphology are more similar to rodents such as rats and squirrels (Le Gros Clark 1959; Jenkins Jr 1974; Kirk et al. 2008). Because of their distinct phylogenetic position and a mixture of features indicative of mammals in general and primates in particular, tree shrews serve as an ideal animal model for comparing across primate and rodent orders and for providing insights into how complex networks for movements such as reaching and grasping have emerged in primates.

Recent intracortical microstimulation (ICMS) studies in both primates and rodents, have revealed regions of sensorimotor cortex where complex multi-joint, and multi-body-part movements can be elicited (Graziano et al. 2002a,b; Graziano et al. 2005; Graziano 2008; Stepniewska et al. 2005, 2009a; Gharbawie et al. 2011a,b; Overduin et al. 2012; Bonazzi et al. 2013, Brown and Teskey 2014). Unlike many earlier stimulation studies using short train (ST: (40- or 50-ms trains) ICMS, these movements were observed using long-train (LT; 500-ms trains) ICMS. When LT-ICMS is applied in primates, complex multi-joint (such as wrist + elbow) and multi-body-part (such as forelimb + mouth) movements can be elicited in motor, premotor, and posterior parietal cortex (PPC) (Graziano et al. 2002a, 2005; Stepniewska et al. 2005, 2009a,b; Gharbawie et al. 2011a,b; Kaas et al. 2012; 2013). Within each of these cortical areas in primates, there are specific, well-characterized, and consistent movements, which are organized

into domains. These domains have been termed hand-to-mouth, defensive, etc., which reflect the observed movements when LT-ICMS is presented. Further, each domain shares strong and specific connections with matching domains across the network of motor, premotor, and PPC (Stepniewska et al. 2009b; 2011; Gharbawie et al. 2011a,b). Taken together, it has been suggested that these domains provide a basic motor network for ethologically relevant movements such as those used for reaching, grasping, defensive, or eating behaviors (Graziano et al. 2002; 2005; Kaas et al. 2013; Kaas and Stepniewska 2015).

Similar, but less complex movements have been observed in rodents such as rats, but only when stimulating motor cortex (Ramanathan et al. 2006; Harrison et al. 2012; Bonazzi et al. 2013; Brown and Teskey 2014). Although, it should be noted that PPC has yet to be explored using similar methods in rodents or mammals within other orders. Further, most studies in rodents observe multi-joint movements of the forelimb almost exclusively, with only a few references to the presence of movements involving multiple body parts (Li and Waters 1991; Gioanni and Lamarche 1985; Tandon et al. 2008; Tennant et al. 2011).

To date, multi-joint, and multi-body-part movements have not been observed in tree shrews, and until recently it was not even known if tree shrews had a premotor, posterior parietal, or even simply a true motor cortex that was separate from somatosensory cortex, as both electrophysiological studies using surface stimulation (Lende 1970) and anatomical evidence (Jane et al. 1969; Nudo and Masterton 1990) provided inadequate confirmation. However, Remple and colleagues (2006, 2007), using ST-ICMS, anatomical connections, and architectonic characteristics, demonstrated that

tree shrews do indeed have distinct motor and somatosensory cortical areas. Further, Remple and colleagues (2006) described multiple areas (M1 and a field they termed M2), which may be similar to primary and premotor cortex in primates, and found that movements involving single joints could be elicited from both of these motor areas as well as area 3a, primary somatosensory cortex (3b), and the rostral half of the caudal somatosensory area (Sc). Finally, regions of cortex caudal to Sc, which includes the posterior parietal region (PP), share strong connections with motor cortex (Remple et al., 2007), similar to findings in primates (Stepniewska et al. 2009b, 2011; Gharbawie et al. 2011a, b). Therefore, it seemed possible that tree shrews might have some of the same basic cortical networks as those observed in primates. However, LT-ICMS was not thoroughly tested in this species, and therefore movements, similar to those observed in primates were not observed in the posterior parietal region, nor were complex movements observed elsewhere in sensorimotor cortex.

The main goal of the current study was to compare the organization of tree shrew motor and parietal cortex, as revealed through LT-ICMS, with that of primates and other mammals such as rodents. Specifically, we reassessed the organization of evoked movements in tree shrew motor cortex to determine whether complex movements can be elicited, and whether such movements are more similar to those observed in rodents or primates. Further, we wanted to determine if movements could be elicited in the posterior parietal cortical region of tree shrews using LT-ICMS to appreciate whether such domains or motor primitives are present only in primates or whether basic features of this primate network emerged earlier in mammalian evolution.

MATERIALS AND METHODS

We examined the organization of motor maps using long-train intracortical microstimulation (LT-ICMS) in ten adult tree shrews (Table 2). All tree shrews (*Tupaia belangeri*) were female between the ages of 4 months and 6 years and weighed 122-153 g. Animals were obtained from a breeding colony at the University of Louisville. All surgical procedures were approved by the UC Davis IACUC and followed NIH guidelines.

Surgical procedures

Animals were anesthetized with intramuscular injections of ketamine (Ket: 100 mg/kg) and xylazine (Xyl: 6.8 mg/kg). Anesthetic levels were maintained using supplemental doses of Ket/Xyl as needed for the remainder of the experiment. Respiration rate, body temperature, muscle tone, and reflexes were monitored throughout the experiment in order to assure steady levels of anesthesia. Once animals were sufficiently anesthetized, they were given subcutaneous injections of lidocaine (2%) behind the ears and placed into a stereotaxic apparatus. Ophthalmic ointment was placed in the eyes to prevent desiccation. Subcutaneous injections of lidocaine (2%) were placed at the scalp, a surgical incision was made along the midline to expose the skull, and the temporal muscles were retracted. A craniotomy was made to expose frontal, parietal, and occipital cortex. The dura was removed, silicone was placed over the cortex in order to prevent desiccation, and the cortical surface was digitally imaged so that ICMS sites could be related to cortical vasculature. Small screws were then placed along the contralateral intact skull and later secured to a head post. Animals were then placed in a

specially constructed sling that supported the torso but allowed all four legs to move naturally (Fig. 2). This also allowed for an unobstructed view of ICMS-evoked movements. The head post was then secured and stabilized to a stereotaxic frame, which also supported the body sling.

ICMS motor mapping

ICMS was delivered through low impedance (0.1-1 M Ω) microelectrodes. Trains of pulses were generated using a Grass S88 stimulator and two SIU6 stimulus isolation units and measured by the voltage drop across a 10-k Ω resistor in series with the return lead of the stimulation isolation units. The stimulator was also connected to an LED light source, which was illuminated during the stimulation train, and signaled stimulus onset and offset during our recording analysis. For LT-ICMS, stimuli consisted of long (500 ms) trains of biphasic pulses delivered at 200 Hz. Each pulse phase was 0.2 ms. In some cases, we also measured evoked movements using short-train (ST) ICMS parameters (50 ms), or 'extra-long' (xLT) ICMS parameters (800 ms) at the same sites where LT-ICMS stimulation was used (see Table 2 for case summaries). For trains of 50 and 800 ms, all other stimulus parameters were the same as those described for LT-ICMS (500 ms). We varied the order of presentation of different train lengths at different sites and often these stimulation parameters were repeated and alternated several times within a single penetration. For each site, at least three stimulation trains of any given train length were applied.

We began exploring evoked movements across a relatively large portion of cortex extending from V1 to the frontal pole with widely spaced stimulation sites to delineate the total extent of cortex where movements could be evoked. Then we explored this

defined region in more detail. An electrode was lowered into the cortex using a micromanipulator to a depth of 1500-1800 μm , which corresponds to the depth of cortical layers V and VI (Wong and Kaas 2009).

Initially, stimulation pulses with an amplitude of 50 μA were used, however, this was increased if this current was not strong enough to elicit a response from an individual site. If no movement was detected for amplitudes of up to 500 μA , the site was considered to be nonresponsive. Stimulation threshold values were defined as the current at which there was a 50% chance of evoking a movement. To confirm the stability of anesthesia, and therefore our ability to consistently evoke movements, we periodically returned to stimulation sites to re-test threshold values throughout the experiment.

All movements were recorded (Sanyo Xacti VPC-HD2000A, 1920x1080 resolution, 60 frames/s) and analyzed off-line (see movement analysis below). Penetration sites were marked on a high-resolution digital image of the exposed cortex and, in some cases, fiducial probes (fluorescent dyes) were placed at strategic locations within cortex to aid the alignment of functional and histological data.

Histological procedures

Once ICMS mapping was complete, animals were given a lethal dose of sodium pentobarbital intraperitoneally, and perfused transcardially with saline, followed by 2% paraformaldehyde, then 2% paraformaldehyde with 10% sucrose added (pH 7.3). The brains were then removed. The cortex was separated from underlying brain structures and artificially flattened under a glass slide. Once flattened, the brains were post-fixed with

4% paraformaldehyde for 0.5 - 3 hours, and then placed in a 30% sucrose solution for 24 to 48 hours.

Flattened sections were cut into 3 or 4 series of sections, at a thickness of 40 μm . (However, the first four sections were cut at a thickness of 60-80 μm and processed for cytochrome oxidase (CO) in order to visualize the blood vessel pattern at the brain's surface (Fig. 3a). One series was processed for myelin (Gallyas 1979), and the remaining series were either processed for CO (Wong-Riley 1979), directly mounted onto glass slides for fluorescent probe analysis, or saved for another study.

Physiological and anatomical alignment/reconstruction

The images of the cortical surface taken during the microstimulation experiment were aligned to the most superficial CO sections by matching the brain surface blood vessel pattern using Adobe Illustrator (Adobe Systems Inc.). Deeper sections processed for myelin or CO were used to determine the cortical boundaries and were aligned to one another using common blood vessels (Fig. 3). When fiduciary probes were placed, we also used these markers to help in our reconstruction (Fig. 4). This process allowed us to accurately align cortical boundaries to our microstimulation maps.

Illustrated motor maps were created using Voronoi tessellation procedures within Illustrator (Adobe Illustrator script, <http://fabiantheblind.github.io/Illustrator-Javascript-Voronoi/>) based on the location of stimulation sites. All images of anatomical tissue sections were adjusted for brightness and contrast using Adobe Photoshop (Adobe Systems Inc.) but were otherwise unaltered.

Movement analysis

All movements were characterized by two independent observers, and recorded during the experiment. These characteristics were later confirmed and movements were further analyzed offline. Movements were characterized as either being “simple”, “multi-joint”, or “multi-body-part” depending on whether the observed movement involved muscle contractions around a single joint (simple), multiple joints within the same limb or body part (multi-joint), or muscles and joints across multiple body parts (multi-body-part) (Table 3).

ICMS-evoked movements were quantified by importing the experimental recordings into Tracker analysis and modeling software (<http://physlets.org/tracker/>), which was used to measure movement displacement and latencies. Different positions of a given body part were analyzed during each frame (1/60th of a second) within the recording. The change in position across recording frames was calibrated using a scale bar present within the frame close to the location of the studied body part movement. In this way plots of movement displacement were generated and compared within stimulation sites for different stimulus train lengths.

RESULTS

In the current study we stimulated 471 sites across 10 animals with 323 of these sites resulting in movements (Table 2). First we will describe how we determined where in the cortex our tested sites were located. Then we will describe the types of movements and differences in movements elicited using ST-, LT-, and xLT-ICMS parameters. Finally, we will describe how the motor maps are organized within various cortical fields.

Determining the location of stimulation sites (architecture)

Flattened cortical sections processed for myelin (Fig. 5) were used to determine the boundaries of various cortical areas. This technique has been described in detail previously (Wong and Kaas 2009) and myeloarchitectonic boundaries have been directly related to the functional boundaries of M, 3b, V1 and auditory cortex in tree shrews (Remple et al. 2006, 2007). Motor cortex is moderately myelinated in deeper cortical layers. However, it was not possible to make distinctions with motor cortex so that M1 and M2 described previously for tree shrews could not be distinguished based on myeloarchitecture in the current study. Areas 3b, V1, auditory cortex (Aud), and orbital frontal cortex (OFC) can easily be identified because of their dark myelin staining. On either side of 3b are bands of tissue that are less myelinated. These correspond to area 3a rostrally and Sc caudally. Along the rostrolateral border of V1 is a lightly staining band of cortex, area 18/V2. V2 directly borders an area that has been termed the temporal anterior area (Ta) in previous studies (Jain et al. 1994; Lyon et al. 1998; Remple et al. 2006, Wong and Kaas 2009) although in the current study it corresponds in location, appearance and functional organization to posterior parietal cortex and is thus termed PP

(Figs. 6, 7, 11, 12). We use the term PP rather than Ta because of its location in the parietal lobe (rather than the temporal lobe) and because it appears to correspond to posterior parietal cortex described in other animals. The myelination of PP is much lighter laterally than it is medially. Determining all of the borders of PP based on myelin alone is difficult as there are only slight transitional differences in the myelination of this region. However, we can identify the rostral border of V2, which serves as the caudal boundary of PP, and the caudal boundary of Sc, which serves as the rostral boundary of PP.

The extent and organization of cortex in which movements could be elicited

We were able to elicit motor movements using both LT-ICMS and ST-ICMS from the frontal pole (in some cases 2 mm rostral to the 3b border) to the rostral border of area 18/V2 (Figs. 6, 7). This range of stimulation sites included motor (M), somatosensory (areas 3a, 3b, and Sc), and posterior parietal cortex (PP). However, in most cases we were only able to consistently elicit movements caudal to Sc when using LT-ICMS parameters (Fig. 7). In general, the extent of cortex where motor movements can be elicited was much larger for LT-ICMS than it was for ST-ICMS.

The topography of movement maps was similar for both ST- and LT-ICMS (Figs. 6, 7). Movements involving the hindlimb were evoked at the most medial locations within dorsal cortex, while facial and tongue movements were evoked at the most lateral stimulation sites. For the most part, movements involving the hindlimb were sparse and were located mainly within 3a, primary somatosensory, Sc, and in one case PP: we observed no sites within motor cortex associated with hindlimb movements (except one

site in one case, not shown). It is likely that the hindlimb representation of motor cortex is located along the medial wall (Remple et al. 2006), which was not tested in the current study. In between hindlimb and jaw movement sites was a large region in which forelimb movements of the shoulder, elbow, wrist, and digits could be elicited (Figs. 6, 7, 11, 12).

Short-train vs. long-train ICMS-evoked movements

In three animals we stimulated with three different stimulation train durations (Table 2). For all three durations, all other parameters were kept the same (amplitude, frequency, and shape of waveform). We either stimulated for 50 ms (ST), 500 ms (LT), or 800 ms (xLT). In general, movements elicited from ST-ICMS were less complex than those observed using LT-ICMS (Figs. 6, 7). Not surprisingly, movements elicited from ST-ICMS were truncated versions of movements observed during longer duration trains. That is, these movements were merely the beginning 50 ms of what was observed using LT-ICMS (Fig. 8). At no sites were ST and LT movements different from one another with the caveat that ST movements lacked certain joints/body parts that may have only been visible after 50 ms into the LT-ICMS. This is readily observed when directly comparing ST with LT maps (Figs. 6C, D; 7B, C). Movements elicited with 800 ms duration trains either extended the movements observed for 500 ms, or the movements stayed at their endpoint position until the stimulation duration was complete (Fig. 8). In some instances where repeated movements were observed, such as those involving the jaw opening and tongue licking, or forepaw movements much like those the animal would make when running or digging, the resultant movement from the extra-long stimulation resulted in additional cycles of the repetitive movement (Fig. 9).

At all sites from which movements could be evoked by 500 ms stimulation, 800 ms stimulation also evoked movements. However, this was not the case for stimulation durations of 50 ms. Specifically, stimulation at sites in the most caudal (in PP) or rostral (rostral motor cortex) portions of the motor map failed to elicit movements under ST-ICMS parameters when LT-ICMS did produce movements (e.g. Figs. 6E, 7D).

Threshold values were much lower for LT-ICMS than they were for ST-ICMS (Figs. 6E, 7D). For some sites, a six-fold current increase was required to produce movements using ST-ICMS compared to LT-ICMS. However, the majority of sites only required a two-fold increase or less. There were smaller differences between the required threshold currents eliciting movements for LT- and xLT-ICMS stimulation parameters; for many of the sites both stimulus durations shared the same threshold values (Fig. 7E).

Simple and Complex movements

In seven cases we used only LT-ICMS to explore motor, parietal, and posterior parietal cortex. We chose this stimulation duration because we were most interested in determining the extent of cortex in which movements could be evoked and exploring how complex movements are represented in the neocortex. As noted above, LT-ICMS produced complex movements in all areas explored (Figs. 6, 7, 11, 12; Table 3). Most of these movements involved a combination of forelimb joint and facial movements, such as multi-joint movements mimicking reaching and grasping behaviors, multi-body-part movements involving both ipsilateral and contralateral forelimbs, movements that involved forelimbs and hindlimbs, or a combination of facial movements such as the eye and ear (Figs. 8, 10B, C, D). Complex movements involving multiple body parts (e.g.

Fig. 10B, C, D) were located at junctions between movement domains that elicited single movements, such as face and the forelimb, yet rather than being an artificial superimposition of unrelated movements, the movements involved body parts that are naturally used together in behaviors such as feeding (Fig. 10C), defensive postures (Fig. 10D), or locomotion (Fig. 10B).

The motor map

Movements evoked by ICMS in motor cortex

It was difficult in the present study to assign a functional border between the presumptive M1 and M2 described by Rempel and colleagues (2006, 2007) based on a difference in architecture or threshold values, and therefore we combined all sites rostral to area 3a and termed the region as motor cortex (M). Hindlimb movements were not observed using either ST- or LT-ICMS parameters at any tested sites within motor cortex (Figs. 6, 7, 11, 12), except for one site in one case (not shown). The most common movements involved either forelimb or jaw and tongue movements, with movements involving the jaw and tongue located lateral to forelimb movements (Figs. 6, 7, 11, 12). In one case vibrissae movements were also observed after stimulation of sites within the most rostral aspect of motor cortex.

Many of the movements evoked from motor cortex were complex, involving both multi-joint and multi-body-part movements. Examples included movements that involved both the jaw opening and closing with the proximal aspect of the tongue moving up and down (similar to movements evoked from area 3a; Fig. 9), combined digit and elbow flexion (resembling grasping movements; Fig. 8), as well as elbow, digit, and jaw

movements that mimicked eating behaviors similar to “hand-to-mouth” movements observed in primates (Fig. 10C). In some instances the combined hand and mouth movements were bilateral, such that both ipsi- and contralateral hands moved towards the mouth (Figs. 10C), while in other cases it was only the contralateral hand that moved in conjunction with the mouth opening. Additionally, movements involving the jaw were often repetitive such that the jaw opened and closed multiple times during LT-ICMS but only once during ST-ICMS (similar to movements evoked from area 3a; Fig. 9).

Of all the sites tested in motor cortex (84 total across 10 cases), 31 sites involved movements of the face, 25 involved movements of the forelimb, 2 involved movements of the vibrissae, 1 involved a movement the trunk, 1 the hindlimb, and 1 involved a movement of the ear. Thirty-one sites did not evoke a movement at current levels up to 500 μ A, though it is important to note that some of these sites were located well rostral to the border of M1 and M2 as depicted by Remple and colleagues (2006). Using LT-ICMS parameters, just over half (27/53) of the movements elicited from motor cortex in all cases were complex. Of these, 26% (7/27) involved simultaneous hand and mouth movements (Fig. 10C), 37% (10/27) were multi-joint forelimb movements, and 35% (10/27) involved movements of the jaw and tongue together.

Motor cortex thresholds had a large range (25-475 μ A; average of 190 μ A). However, it is again important to note that all sites rostral to area 3a were included in our motor cortex analysis. The threshold values that were more caudal within this region (within 1mm of the rostral border of 3a) were more similar to thresholds observed in areas 3a and 3b, while threshold values at more rostral locations in M (beyond 1 mm of the rostral border of area 3a, i.e. perhaps in Remple’s M2) tended to be much higher (See

Figs. 11, 12). There was also a tendency for thresholds of more lateral sites involved in jaw and tongue to be slightly lower than those of more medial sites within motor cortex (e.g. Figs. 11C, D, 12C, D).

Movements evoked by ICMS in area 3a

Movements evoked from area 3a included those described for motor cortex, but also included movements of the hindlimb evoked from 7 sites (e.g. Figs. 6D, 7C, 11B). Of all of the sites tested within area 3a, 29% (15/51) resulted in simple movements, 59% (30/51) resulted in complex movements, and 12% (6/51) resulted in no movements when stimulated up to 500 μ A. Similar to motor cortex, many of the complex movements elicited from responsive sites involved simultaneous hand and mouth movements (22%: 10/45 sites), and multi-joint forelimb movements (16%: 7/45 sites), 13% (6/45 sites) included simultaneous jaw and tongue movements, which were often repetitive in nature (Fig. 9), and 16% (7/45 sites) included both hindlimb and forelimb movements that were similar to locomotor behaviors (Fig. 10B). The lowest threshold value was 15 μ A and the average threshold was 105 μ A.

Movements evoked by ICMS in area 3b

Area 3b takes up the largest surface area of all of the cortical areas studied here and contained the most sites from which we elicited motor movements (113 of 131 sites tested: Table 3). Movements elicited from 3b were more diverse than movements elicited from other fields and included the entire body: digits, wrist, elbow, shoulder, trunk, hindlimb, jaw, tongue, vibrissae, and eye squints (e.g. Figs. 7C, 11B, 12B). Eye squints, which were not observed in motor cortex or in area 3a were elicited from sites located

along the most caudolateral aspect of 3b, often bordering area S2/Pv. Additionally, all eye squint movements were contralateral, with no sites eliciting bilateral eye squints.

At 14% (18/131) of the tested sites within area 3b movements could not be elicited. Stimulation at the majority of sites where movements could be elicited involved the forelimb 66% (75/113), 33% (37/113) involved movements of the face (jaw, tongue, vibrissae, and/or eye squint), and 18% (20/113) involved movements of the hindlimb. Therefore forelimb movements had a large representation within the region of area 3b that was mapped in the current study. Movements of the hindlimb were represented along the most dorsomedial aspect of area 3b. This representation could possibly also extend onto the medial wall, but this was not tested.

Movements at many of these sites were complex 52% (59/113), involving multiple joints of the forelimb (Fig. 11B), or a combination of the jaw and tongue, hindlimb and forepaw, hand and mouth, or hand and eye squint (Figs. 6, 7, 11, 12). There were a number of bilateral movements involving the forelimbs, as well as repeated movements involving the jaw and tongue at the most rostral aspect of area 3b.

Threshold values for sites within 3b were generally low, similar to those observed in area 3a. The lowest threshold was 17 μ A and the average threshold was 148 μ A. Generally, threshold values were lowest for movements involving the forelimb, and highest for movements involving the hindlimbs (Figs 11C, D, 12C, D).

Movements evoked by ICMS in area Sc

Sc is a thin strip of cortex just caudal to area 3b. Thirty-four sites were tested in area Sc across 10 cases. Of these, stimulation at 18% (6/34) of the sites did not elicit movements up to 500 μ A of current. Stimulation at sites that did elicit movements

involved movements of the hindlimb (18%: 5/28 sites), forelimb (46%: 13/28 sites), or face (39%: 11/28 sites). Therefore facial movements, but more specifically eye squint movements, which composed 25% (7/28) of all elicited movements, are much more prevalent in area Sc compared to motor cortex, area 3a, and area 3b. Among the 13 sites involving forelimb movements, only one involved digit movements with the majority of forelimb movements involving the shoulder, elbow, or a combination of the two. Therefore the forelimb movements were more proximal involving mostly shoulder and elbow movements, than those observed in more rostral areas. Additionally, few facial movements involving the jaw (2/11 sites) and no movements of the tongue were elicited from area Sc.

Stimulation at 29% (8/28) of sites involved complex movements with two being multi-joint movements of the shoulder and elbow (Fig. 11A), and the others involving a combination of a forelimb and facial movement such as an eye squint and elbow flexion (Fig. 10D, 11A), hindlimb and shoulder movements (Fig. 7) or jaw and eye squint movements (Fig. 12).

The average threshold value for Sc was 220 μ A, which is greater than the average threshold value of more rostral cortical areas, but lower than the average threshold value of the posterior parietal region.

Movements evoked by ICMS in PP

We defined PP as the region of cortex caudal to Sc and rostral to V2. For the purposes of this paper we also included the region of cortex from the medial wall to the region of cortex just medial to S2/Pv (Figs. 6, 7, 11, 12). This region likely included several subdivisions such as PPc, PPd, and PPv as described by Rempel and colleagues

(2007), but we were unable to distinguish differences from our myeloarchitecture. A total of 48% (67/139) of the total sites tested across all cases resulted in motor movements of any kind (Table 2) when stimulated. Stimulation at 40% (27/67) of these sites resulted in complex movements but the majority of sites resulted in simple movements (60%: 40/67 sites). In general, most sites within PP evoked contralateral eye squints (42%: 28/67 sites), ear/pina movements (24%: 16/67 sites), or a combination of the two (21%: 14/67 sites). There were no observed movements of the jaw or tongue, or of the digits. Medially within PP, elicited movements involved the shoulder, trunk, and/or hindlimb, while laterally elicited movements involved the face (ear, vibrissae, or eye squints).

The threshold values for sites within PP were much higher than sites within more rostral areas of cortex. The average threshold value was 261 μ A with most sites requiring more than 200 μ A of current to elicit a movement.

Movements evoked by ICMS in S2/Pv

In S2/Pv, movements were elicited at 56% of the sites tested (18/32), 6 (33%: 6/18 sites) of which resulted in complex movements. Most movements were associated with vibrissae (6/18), eye squint (6/18) ear (3/18), shoulder (4/18), or digits (3/18). Just over half of the sites within S2/Pv were found to be complex (10/18), while 44% (8/18) were simple. Threshold values varied considerably, between 55 and 300 μ A with an average threshold value of 159 μ A. Determining a topography within S2/Pv was difficult given the small number of sites tested across cases.

Summary of movements

In summary, our motor maps generated using LT-ICMS consisted of movement representations that were both simple and complex in nature. Complex movements could be evoked in motor cortex (51%: 27/53 of elicited movements), areas 3a (59%: 30/51), 3b (52%: 59/113), PP (40%: 27/67), Sc (29%: 8/28), and S2/Pv (33%: 6/18). Additionally, the variety of movements across all sites within area 3a and across all sites within 3b included a greater number of body parts than those in other cortical fields. Evoked movements across all sites within motor cortex involved the jaw, tongue, and forelimb, with an emphasis on movements of the digits and elbow. Only one site within motor cortex in one case involved movements of the hindlimb. ICMS stimulation to the most caudal area, PP, elicited few to no movements involving the jaw, tongue, or digits. Instead, most elicited movements from stimulation at lateral sites in PP involved eye squint and ear movements, while at medial sites shoulder movements were most common. Most bilateral and repetitive movements were evoked from areas 3a, 3b, and motor cortex, with few bilateral movements, and no repetitive movements evoked from stimulation in more caudal cortical fields (Figs. 6, 7, 11, 12). The lowest thresholds were observed for sites in areas 3a, 3b, and the caudal portion of motor cortex, and the highest thresholds were observed for sites in Sc, PP, and the most rostral aspect of motor cortex (Table 4). In general, thresholds were also much higher for sites associated with movements involving the hindlimbs than those involving the forelimbs and face (e.g. Fig. 12).

DISCUSSION

The current study focused on comparing the organization and types of movements represented within different cortical areas using LT-ICMS in tree shrews. Our results indicate that complex movements, similar to those described in primates can be evoked from a number of cortical areas in tree shrews, not just in motor cortex as in rodents (Fig. 13). These movements include those involving multiple joints of the forelimb that under natural conditions are associated with reaching and grasping as well as more complex movements involving multiple body parts such as simultaneous hand and mouth, ear and forelimb, and forelimb and hindlimb movements. However, it is important to note that the greatest diversity of complex movements was observed during stimulation of different sites within primary motor and somatosensory cortex, and not within the posterior parietal region.

We also compared the effects of different stimulation durations including short-train (ST; 50 ms), long-train (LT; 500 ms), and extra-long-train (xLT; 800 ms). Overall, each stimulation duration produced cortical motor maps with the same general topography (Figs. 6, 7, 11, 12). However, the movements elicited using ST-ICMS were truncated, simple versions of those movements elicited using LT- and xLT-ICMS (Figs. 8, 9). Extra-long-train ICMS resulted in either a repeated movement or a movement that maintained a similar endpoint to movements observed using LT-ICMS (Figs. 8, 9). For the most part, ST-ICMS current thresholds were much greater than those for LT-, and xLT-ICMS possibly because latencies are longer at lower current stimulation. Additionally, the overall motor maps observed using LT-, and xLT-ICMS encompassed a larger region of cortex, extending more rostrally into the frontal pole, and more caudally

into posterior parietal cortex, than the map generated using ST-ICMS parameters (Figs. 6, 7). This extent of ‘excitable’ cortex is significantly larger than that reported in any other small brained mammal. Finally, complex, multi-joint movements were most common when stimulating sites in M, 3a, and 3b, and less prevalent in Sc and PP. The diverse repertoire of elicited movements using LT-ICMS in the current study has not been described in any other mammal except primates.

Comparisons with previous studies of motor cortex in tree shrews

Only a few studies using cortical stimulation methods have been used to identify and study the organization of motor cortex in tree shrews. Lende (1970) used surface macroelectrodes to stimulate cortex in tree shrews and found that movements could be evoked from a large region, including somatosensory cortex. Thus, Lende concluded that somatosensory and motor cortex were indistinguishable and completely overlapped. Recently, through a series of anatomical and short-train intracortical microstimulation studies, Remple and colleagues (2006, 2007) were able to differentiate two motor areas (M1 and M2) using differences in threshold values in the different areas. However, they found that anatomical analysis was essential for determining the borders of all other areas, as ICMS threshold values were indistinguishable.

Our results support but greatly extend those of Remple and colleagues. Although we did not distinguish two motor fields, there was a tendency for threshold values in the most rostral aspects of our motor maps to be higher than those close to the area 3a border (Figs. 11C, D, 12C, D), suggesting the presence of the two motor fields described by Remple and colleagues (2006). As with previous reports, we too were able to elicit ST-

ICMS movements across a large region of cortex, including M (M1 and M2/premotor cortex), 3a, 3b, and the rostral portion of Sc (Lende 1970; Remple et al. 2006). However, our LT- and ST-ICMS motor maps extended more rostrally and far more caudally than those reported previously, and included the posterior parietal region up to the V2 rostral border (Fig. 6C). The reason for this difference in the overall size of our maps compared to previous studies in tree shrews is not clear, but it may be due to differences in the stimulation parameters. For instance, we used a biphasic pulse allowing for higher current amplitudes versus a cathodal pulse at lower current amplitudes used by Remple and colleagues (2006). Another possibility is that the level of anesthesia may have differed across studies. Finally, we positioned our animals in a hammock allowing the limbs to move more easily, and the detection of such movements to be more distinct.

Overall, the general topographic organization observed across fields in the present study is similar to previous reports in that movements associated with the face are represented laterally, while movements associated with the trunk and hindlimb are represented medially in the cortex (Lende 1970; Remple et al. 2006). However, there are some differences in the proportion of cortex devoted to specific representations of various body parts across the three studies, such as the size of the forelimb representation. For instance, though Remple and colleagues (2006) only found a few sites across all cases that elicited movements of the distal forelimb, and these movements consisted of simple flexions of the wrist or all digits together. In the current study, we often observed a large region of cortex from which digit movements could be evoked including flexion, extension, and at some sites, single digit movements (Figs. 6, 7, 11, 12). We did not find any sites at which eye movements could be elicited, like Remple and colleagues (2006)

but unlike Lende (1970) who was able to elicit eye movements at stimulation sites both rostral and caudal to somatosensory cortex.

Comparison with motor cortex in other mammals

Tree shrews have a unique phylogenetic relationship to primates as well as to well-studied rodents such as mice and rats. In fact, part of our motivation for studying tree shrews was to facilitate comparisons between rats/mice and primates. Motor cortex or cortex in which ICMS elicits movements has been primarily examined in rodents such as rats (Hall and Lindholm 1974; Gioanni and Lamarche 1985; Sanderson et al. 1983; Neafsey et al. 1986; Brecht et al. 2004; Ramanathan et al. 2006; Tandon et al. 2008), mice (Li and Waters 1991; Pronichev and Lenkov 1998; Tennant et al. 2011), and squirrels (Cooke et al. 2012), as well as prosimian primates (Fogassi et al. 1994; Wu et al. 2000; Stepniewska et al. 2005; 2009; 2011), New World (Strick and Preston 1982; Gould et al. 1986; Donoghue et al. 1992; Stepniewska et al. 1993; Burish et al. 2008; Stepniewska et al. 2014; Gharbawie et al. 2011a) and Old World monkeys (Kwan et al. 1978; Sessle and Wiesendanger 1982; Graziano et al. 2002a, b, 2005, Cooke et al. 2003; Overduin et al. 2012; Gharbawie et al. 2011b). For all Euarchontoglires species, including rodents and primates, there is a gross somatotopic organization of body movements with a medial-to-lateral progression of hindlimb, trunk, forelimb, and facial movements within motor cortex. However, in rodents this progression seems to be slightly rotated relative to that of primates (Fig. 13). This medial-to-lateral progression of movements is also observed within premotor and posterior parietal cortex of primates (see Geyer et al. 2000; Kaas and Stepniewska 2015 for review). Tree shrews are no

different from their close relatives, (primates and rodents), sharing the same general topographic organization within motor cortex, and a similar gross organization within posterior parietal cortex to that described in primates. The details of that organization, however, vary considerably across species. Non-human primates, for example, have a greater repertoire of evoked movements, which are similar to natural behaviors involving multiple joints and body parts. In primates, at least eight complex movements have been characterized (Fig. 13), and are represented in organized domains in motor, premotor, and posterior parietal cortex (see Kaas and Stepniewska 2015 for review).

In contrast to primates, in rodents, multi-joint and multi-body part movements have only been described within motor cortex (Li and Waters 1991; Ramanathan et al. 2006; Tandon et al. 2008; Harrison et al. 2012; Tennant et al. 2011; Bonazzi et al. 2013; Brown and Teskey 2014; Hira et al. 2015); however, it is important to note that the presence of evoked movements have not been well explored in posterior parietal cortex of rodents using LT-ICMS. The most studied movements within the rodent literature pertain to two multi-joint movement domains of the forelimb, which coincide with the rostral forelimb area (RFA) and the caudal forelimb area (CFA), which have been associated with grasping- and reaching-like behaviors respectively (Neafsey and Sievert 1982; Brown and Teskey, 2014). Other complex movements have been observed throughout motor cortex after stimulation of sites along the borders of their component movement domains. For instance, Li and Waters (1991), observed simultaneous hindlimb and forelimb movements when they stimulated cortex near the borders of the individual hindlimb and forelimb movement domains of motor cortex in mice, and Gianni and Lamarche (1985) first observed an overlap of evoked movements when stimulating near

the borders of vibrissae and forelimb domains in rats. Further, in primates, the “hand-to-mouth” domain, which includes movements of both the forelimb and mouth, lies between the region of motor cortex representing the forelimb and mouth (Graziano et al. 2002a; Stepniewska et al. 2009), similar to our current results in tree shrews (Figs. 6, 7, 11, 12). In humans, this type of clustering of complex movements has not been reported (Desmurget et al. 2014), but the data are too limited to come to any firm conclusions since only six stimulation sites per subject were evaluated.

The fact that such complex multi-joint and multi-body-part movements are represented along borders of the representations of their component parts has led to the suggestion that such ‘complex’ movements are merely the result of current spread, and are not useful for understanding how motor cortex generates movements that ultimately coalesce into context appropriate behaviors (Strick 2002). However, a recent deactivation study in rats, to assess the influence of RFA and CFA in reaching and grasping behaviors, suggests that these specific domains are integral, and have specific roles in reach shaping (elevate/advance/retract) (CFA) and grasping (RFA) behaviors (Brown and Teskey 2014).

The level of complexity of motor map organization likely comes from the complexity of the repertoire of motor behavior of the species studied. Notably, the natural forelimb movements of tree shrews are not as intricate, nor do they have the same degrees of freedom as forelimb movements generated by primates, especially with respect to digit control (Sargis 2001). This increased flexibility in the movement repertoire of primate forelimbs presumably requires a greater amount of cortex devoted to the expanded representations of such movements and a greater number of domains

associated with forelimb control. As stated earlier, primates have at least eight main movement domains that are associated with specific ethologically relevant movements (i.e., hand-to-mouth/body, defensive face, defensive forelimb, reaching, grasping, object manipulation, aggressive, combined hindlimb and forelimb, as well as looking/eye movements) represented within each of the cortical areas within the frontoparietal network: motor, premotor, and posterior parietal cortex. In rodents, multiple movement domains are not observed (see above), but similar LT-ICMS techniques have not been used to explore cortex beyond motor cortex. Complex movements, when observed, are mostly elicited when stimulating motor cortex; although it has been suggested that RFA may be within the homologue of the primate supplementary or premotor cortex, while CFA may be within the homologue of primary motor cortex (Neafsey and Sievert, 1982; Rouiller et al. 1993).

Our current results indicate that the frontoparietal network in tree shrews is a hybrid of the networks reported in primates and rodents (Fig. 13). For example, combined hand and mouth movements were represented lateral to possible reaching and grasping movements in motor cortex, similar to descriptions in primates (Graziano et al. 2002a, 2005; Stepniewska et al. 2011; Gharbawie et al. 2011a, b; Kaas and Stepniewska 2015), and combined forelimb and hindlimb movements were located medial to all other movement domains in PPC (Fig. 13). On the other hand, not all movement domains are similar across tree shrews and primates. For instance, within tree shrew motor cortex, facial defensive- and grimace-like movements found in primates, were not observed; only an eye squint representation was observed. Similarly, we failed to elicit either mouth or digit movements in posterior parietal cortex suggesting that “hand-to-mouth or

“grasping,” domains in this region are a specialization of primates. Further, we did not find many complex movements within the presumptive premotor cortex of tree shrews (Figs. 6, 7, 11, 12), but we did find possible movement domains associated with grasping within primary somatosensory cortex (Fig. 8, 13), which has not been reported in primates. However, it is important to note that our motor maps may not have been sufficiently dense, especially within posterior parietal cortex, to fully reveal the presence, absence, or full organization of complex movement domains.

In the current study we were able to evoke movements as far caudal as the V2 border. However, in prosimian primates, whose brain organization is thought to resemble that of the ancestral primate, microstimulation fails to elicit movements caudal to the rostral half of PPC. Thus there is a region of cortical tissue just rostral to the V2 border where movements cannot be elicited in prosimians (Stepniewska et al. 2009). This difference reflects the expansion of the caudal portion of posterior parietal cortex and visual areas along the dorsorostral border of V2 in primates relative to tree shrews.

Short- versus long-train stimulation

The use of electrical stimulation to determine the organization of motor cortex dates back to the studies of Frisch and Hitzig (1870) and Ferrier (1875), who used large cortical surface electrodes. It is interesting to note that even at this time, there was much debate about the type of stimulation parameters that should be used to determine the borders of sensorimotor cortex and its function (see Sherrington and Grünbaum 1901; Penfield 1937; Graziano 2009 for reviews). In the late 1960s, investigators began using small intracortical microelectrodes through which low electrical currents could be passed

to elicit movements (Asanuma and Sakata 1967; Stoney et al. 1968). With this advancement, many researchers began using short stimulation trains lasting only 40 ms, which evoked small muscle twitches around single joints. The goal was to stimulate with the least amount of current that could elicit a movement in order to produce a motor map that represented individual muscles and joints.

In 2002, Graziano and colleagues increased the stimulation duration, which was approximately the same duration of a natural reach in a monkey (500 ms). This stimulus duration resulted in movements that started with the same observed muscle twitches evoked by short duration stimulation, but then progressed into more complex movements, often involving multiple joints and body parts. More importantly, the use of these longer stimulation parameters has been found to reveal complex movement domains within motor, premotor, and posterior parietal cortex overlaid upon a very general topographic map in a number of primate species (for review see Kaas and Stepniewska 2015).

The use of long duration stimulation has been controversial since the 1800s, and many of the past arguments posed against its use when stimulating the cortex through surface electrodes are being echoed more than a hundred years later for intracortical microstimulation. Critiques include: the notion that “high currents and long pulse trains allow current to spread far beyond the site of stimulation” and may also result in the activation of “indirect routes that could mediate the effects of stimulation” (Strick 2002) and that the resultant movements are an artifact of a stimulus driving muscle ‘length-tension’ equilibrium (Van Acker et al. 2013; 2014; Griffin 2014), rather than ethologically relevant and purposeful movements (Graziano et al. 2009). Regardless of the arguments for or against the use of LT-ICMS, the direct comparisons of different

stimulus train durations made in the current study revealed several important findings. 1. The gross topographic organization of movement maps obtained using LT- and ST-ICMS were highly similar (Figs. 6, 7, 11, 12). 2. ST-ICMS resulted in truncated versions of movements elicited by LT-ICMS, and movements from ST-, and LT-ICMS were identical for the first 50 ms after stimulation (Fig. 8). 3. Extensions of the LT-ICMS (i.e. xLT-ICMS, 800 ms duration) resulted in either a maintained endpoint fixation, a progression of the movement beyond the previous endpoint, or a repeated cycle of a given movement (Fig. 9) The presence of repeated movements suggests that long duration stimulation is not merely evoking a posture via static equilibrium of muscle tension. 4. The latencies of both ST- and LT-ICMS were indistinguishable (Figs. 8, 9). 5. LT-ICMS reveals an organization within posterior parietal cortex of tree shrews that is not apparent using ST-ICMS or anatomy alone. Therefore, the resultant repertoire and organization of movements revealed through LT-ICMS across cortical areas, especially within posterior parietal cortex, can provide insights in to the possible changes and specializations that have emerged across different cortical fields and mammalian orders. Indeed, with a lack of robust anatomical markers, and with the difficulty of obtaining and comparing physiological recordings of sensory responses within PPC of various species, LT-ICMS may be the best tool, at this point in time, for revealing homological characteristics of this cortical region.

What is ‘motor’ cortex?

Notably, we found that ICMS could elicit movements not only from motor cortex but also from much of cortex extending caudally, to the rostral border of V2, including

area 3b. Evoked movements in area 3b have also been reported in early motor stimulation experiments of humans (Penfield 1937), non-humans primates (Welker et al. 1957), and a number of other eutherian mammals (Saraiva et al. 1975; Sanderson et al. 1983, Lende and Woolsey 1956). Further, though not always emphasized, evoked movements from ST-ICMS stimulation of area 3b have consistently appeared in the figures of more recent studies in galagos (Wu et al. 2000), New World monkeys (Stepniewska et al. 1993; Burish et al. 2008), rodents (Donoghue and Wise 1982; Matyas et al. 2010; Cooke et al. 2012), and humans (Nii et al. 1996). Movements elicited by stimulation of area 3b appear to be independent of motor cortex (Matyas et al. 2010) and may propagate through direct projections to subcortical motor structures including the spinal cord (Nudo and Masterton 1990; Remple et al. 2006). With these results in mind, we might ask: What and exactly where is “motor” cortex? The most straightforward answer to that question is that motor cortex is the region that contains neurons projecting to the spinal cord. However, this definition is limited to representations of the body below the neck and ignores large regions of the brain representing facial movements, as well as regions of the brain associated with motor control that have indirect pathways to the spinal cord. Further, this definition would then include much of the classical “sensory” cortex, including area 3b caudal to M1.

The current theoretical framework of functionally distinct motor and somatosensory cortical areas is rooted in the physiological work of Sherrington and his colleagues (Dusser de Barenne 1935; Penfield 1937; Uematsu et al. 1992). Sherrington used surface recording and short-duration, low-current stimulation procedures to explore cortical functions along the central sulcus in a number of primate species. The results of

these studies were that evoked movements could only be elicited when stimulating the precentral gyrus and evoked potentials for peripheral sensory stimulation were only recorded from the postcentral gyrus (Sherrington and Grünbaum 1901; Leyton and Sherrington 1917). It is likely that both Sherrington and many other prominent researchers at the time were significantly influenced by the anatomical work of Campbell (1905), who conducted histological analysis on Sherrington's experimental brains and found distinct anatomical differences between the pre- and postcentral gyrus (see Uematsu et al. 1992 for review). Even though many studies, including the current study, demonstrate considerable motor and somatosensory overlap in this region of cortex, the idea that there are functionally distinct and separate motor and somatosensory areas is most prevalent today, and might even be considered dogma.

While all eutherian mammals studied have an architectonically distinct agranular region of cortex from which movements can be consistently evoked rostral to a granular sensory region where neurons respond to somatosensory stimulation, movements can also be evoked from granular cortex, as well as posterior parietal cortex. Therefore, based on our own observations and data from other laboratories, it appears that our definition of motor cortex should be reconsidered as we consistently discover that a huge swath of cortex in addition to the traditionally defined motor and sensory cortices appears to have dual roles.

SUMMARY

Our results suggest that the organized/modular representation of complex movements observed in posterior parietal cortex likely evolved with the emergence of

primates, yet the representation of some complex movements, in motor cortex, such as those associate with reaching and grasping, as well as simultaneous hand and mouth movements, were likely present in the ancestor of tree shrews, and primates.

Acknowledgements

We thank Dr. Heywood Petry and for supplying the tree shrews used in this study. We also thank Cindy Clayton and her staff within the UC Davis Psychology Department for animal care, Michaela Donaldson for histological and experimental assistance, and Adam Gordon and James Dooley for experimental assistance.

This research was supported by a grant from NIH NINDS NS035103 to LK, NIH NEI T23 EY015387, and NIH NINDS F32 NS093721-01 to MLKB.

REFERENCES

- Asanuma H, Sakata H. 1967. Functional organization of the cortical efferent system examined with focal depth stimulation in cats. *J Neurophysiol.* 30:35-54.
- Bonazzi L, Viaro R, Lodi E, Canto R, Bonifazzi C, Franchi G. 2013. Complex movement topography and extrinsic space representation in the rat forelimb motor cortex as defined by long-duration intracortical microstimulation. *J Neurosci.* 33:2097-2107.
- Brecht M, Krauss A, Muhammad S, Sinai-Esfahani L, Bellanca S, Margie TW. 2004. Organization of rat vibrissa motor cortex and adjacent areas according to cytoarchitectonics, microstimulation, and intracellular stimulation of identified cells. *J Comp Neurol.* 479:360-373.
- Brown AR, Teskey GC. 2014. Motor cortex is functionally organized as a set of spatially distinct representations for complex movements. *J Neurosci.* 34:13574-13585.
- Burish MJ, Stepniewska I, Kaas JH. 2008. Microstimulation and architectonics of frontoparietal cortex in common marmosets (*Callithrix jacchus*). *J Comp Neurol.* 507:1151-1168.
- Campbell AW. 1905. *Histological studies of the localization of cerebral function.* Cambridge: Cambridge University Press.
- Cooke DF, Taylor CS, Moore T, Graziano MS. 2003. Complex movements evoked by microstimulation of the ventral intraparietal area. *Proc Natl Acad Sci.* 100:6163-6168.
- Cooke DF, Padberg J, Zahner T, Krubitzer L. 2012. The functional organization and cortical connections of motor cortex in squirrels. *Cereb Cortex.* 22:1959-1978.
- Desmurget M, Richard N, Harquel S, Baraduc P, Szathmari A, Mottalese C, Sirigu A. 2014. Neural representations of ethologically relevant hand/mouth synergies in the human precentral gyrus. *Proc Natl Acad Sci USA.* 111:5718-5722.
- Donoghue JP, Wise SP. 1982. The motor cortex of rat: cytoarchitecture and microstimulation mapping. *J Comp Neurol.* 212:76-88.
- Donoghue JP, Leibovic SJ, Sanes JN. 1992. Organization of the forelimb area in squirrel monkey motor cortex representation of digit, wrist, and elbow muscles. *Exp Brain Res.* 89:1-19.
- Dusser de Barenne JG. 1935. Central levels of sensory integration. *Arch Neurol Psych.* 34:768-776.

- Ferrier D. 1975. Experiments on the brain of monkey. No. 1 Proc R. Soc. Lond. 23:409-430.
- Fritsch G, Hitzig E. 1870. Ueber die elektrische Erregbarkeit des Froschhirns. Arch Anat Physiol Wiss Med. 37:300-332.
- Fogassi L, Gallese V, Gentilucci M, Luppino G, Matelli M, Rizzolatti G. 1994. The fronto-parietal cortex of the prosimian galago: patterns of cytochrome oxidase activity and motor maps. Behav Brain Res. 60:91-113.
- Gallyas F, 1979. Silver staining of myelin by means of physical development. Neurol Res. 1:203-209.
- Geyer S, Matelli M, Luppino G, Zilles K. 2000. Functional neuroanatomy of the primate isocortical motor system. Anat Embryol. 202:443-474.
- Gharbawie OA, Stepniewska I, Kaas JH. 2011a. Cortical connections of functional zones in posterior parietal cortex and frontal cortex motor regions in new world monkeys. Cereb Cortex. 21:1981-2002.
- Gharbawie OA, Stepniewska I, Qi H, Kaas JH. 2011b. Multiple parietal-frontal pathways mediate grasping in macaque monkeys. J Neurosci. 31:11660-11670.
- Gioanni Y, Lamarche M. 1985. A reappraisal of rat motor cortex organization by intracortical microstimulation. Brain Res. 344:49-61.
- Gould HJ, Cusick CC, Pons TP, Kaas JH. 1986. The relationship of corpus collosum connections to electrical stimulation maps of motor, supplementary motor, and the frontal eye fields of owl monkeys. J Comp Neurol. 247:297-325.
- Graziano MS, Taylor CS, Moore T. 2002a. Complex movements evoked by microstimulation of precentral cortex. Neuron. 34:841-851.
- Graziano MS, Taylor CS, Moore T, Cooke DF. 2002b. The cortical control of movement revisited. Neuron. 36:349-362.
- Graziano MS, Aflalo TN, Cooke DR. 2005. Arm movements evoked by electrical stimulation in the motor cortex of monkeys. J Neurophysiol. 94:4209-4223.
- Graziano MS. 2008. The Intelligent Movement Machine: An Ethological Perspective on the Primate Motor System. Medicine & Health Science Books
- Graziano MS, Aflalo TN, Cooke DF. 2005. Arm movements evoked by electrical stimulation in the motor cortex of monkeys. J Neurophysiol. 94:4209-4223.

- Griffin DM, Hudson HM, Belhaj-Saïf A, Cheney PD. 2014. EMG activation patterns associated with high frequency, long-duration intracortical microstimulation of primary motor cortex.
- Hall RD, Lindholm EP. 1974. Organization of motor and somatosensory neocortex in the albino rat. *Brain Res.* 66:23-38.
- Harrison TC, Ayling OGS, Murphy TH. 2012. Distinct cortical circuit mechanisms for complex forelimb movement and motor map topography. *Neuron.* 74:397-409.
- Hira R, Terada SI, Kondo M, Matsuzaki M. 2015. Distinct functional modules for discrete and rhythmic forelimb movements in the mouse motor cortex. *J Neurosci* 35:13311-13322.
- Jain N, Preuss TM, Kaas JH. 1994. Subdivisions of the visual system labeled with the Cat-301 antibody in tree shrews. *Vis Neurosci.* 11:731-41.
- Jane JA, Campbell CBG, Yashon D. 1969. The origin of the corticospinal tract of the tree shrew (*Tupaia glis*) with observations on its brain stem and spinal terminations. *Brain Behav Evol.* 1:160-182.
- Jenkins Jr F. 1974. Tree shrew locomotion and the origins of primate arborealism. *Primate Locomotion.* Academic Press Inc.: 85-113.
- Kaas JH, Stepniewska I, Gharbawie OA. 2012. Cortical networks subserving upper limb movements in primates. *Eur J Rehabil Med.* 48:299-306.
- Kaas JH, Gharbawie OA, Stepniewska I. 2013. Cortical networks for ethologically relevant behaviors in primates. *Am J Primatol.* 75:407-414.
- Kaas JH, Stepniewska I. 2015. Evolution of posterior parietal cortex and parietal-frontal networks for specific actions in primates. *J Comp Neurol.* (Epub ahead of print).
- Kirk EC, Lemelin P, Hamrick MW, Boyer DM, Bloch JI. 2008. Intrinsic hand proportions of euarchontans and other mammals: implications for the locomotor behavior of plesiadapiforms. *J Hum Evol.* 55:278-99.
- Kwan HC, MacKay WA, Murphy JT, Wong VC. 1978. Spatial organization of precentral cortex in awake primates. II. Motor outputs. *J Neurophysiol.* 44:1120-1131.
- Le Gros Clark WE. 1959. *The antecedents of man.* Edinburgh: Edinburgh University Press.
- Lende RA, Woolsey CN. 1956. Sensory and motor localization in cerebral cortex of porcupine (*Erethizon dorsatum*).

- Lende RA. 1970. Cortical localization in the tree shrew (*Tupaia*). *Brain Res.* 18:61-75.
- Leyton ASF, Sherrington CS. 1917. Observations on the excitable cortex of the chimpanzee orang-utan, and gorilla. *J Expt Physiol.* 11:135-222.
- Li CX, Waters RS. 1991. Organization of the mouse motor cortex studied by retrograde tracing and intracortical microstimulation (ICMS) mapping. *Can J Neurol Sci.* 18:28-38.
- Lin J, Chen G, Gu L, Shen Y, Zheng M, Zheng W, Hu X, Zhang X, Qui Y, Liu X, Jiang C. 2014. Phylogenetic affinity of tree shrews to Glires is attributed to fast evolution rate. *Mol Phylogenet Evol.* 71:193-200.
- Lyon DC, Jain N, Kaas JH. 1998. Cortical connections of striate and extrastriate visual areas in tree shrews. *J Comp Neurol.* 401:109-128.
- Matyas F, Sreenivasan V, Marbach F, Wacongne C, Barsy B, Mateo C, Aronoff R, Petersen CC. 2010. Motor control by sensory cortex. *Science.* 330(6008):1240-3.
- Neafsey EJ, Sievert C. 1982. A second forelimb motor area exists in rat frontal cortex. *Brain Res.* 232:151-156.
- Neafsey EJ, Bold EL, Haas G, Hurley-Gius KM, Quirk G, Sievert CF, Terreberry RR. 1986. The organization of the rat motor cortex: a microstimulation mapping study. *Brain Res.* 396:77-96.
- Nii Y, Uematsu S, Lesser RP, Gordon B. Does the central sulcus divide motor and sensory functions? Cortical mapping of human hand areas as revealed by electrical stimulation through subdural grid electrodes. *Neurology.* 46:360-367.
- Nudo RJ, Masterton RB. 1990. Descending pathways to the spinal cord, III: sites of origin of the corticospinal tract. *J Comp Neurol.* 296:559-582.
- Overduin S, d'Avella A, Carmena JM, Bizzi E. 2012. Microstimulation activates a handful of muscle synergies. *Neuron.* 76:1071-1077.
- Penfield W, Boldrey E. 1937. Somatic motor and sensory representation in the cerebral cortex of man studied by electrical stimulation. *Brain.* 37:389-443.
- Pronichev IV, Lenkov DN. 1998. Functional mapping of the motor cortex of the white mouse by a microstimulation method. *Neurosci behave Physiol.* 28:80-85.
- Ramanathan D, Conner JM, Tuszynski MH. 2006. A form of motor cortical plasticity that correlates with recovery of function after brain injury. *Proc Natl Acad Sci.* 103:11370-11375.

- Remple MS, Reed JL, Stepniewska I, Kaas JH. 2006. Organization of frontoparietal cortex in the tree shrew (*Tupaia belangeri*). I. Architecture, microelectrode maps, and corticospinal connections. *J Comp Neurol.* 497:133-154
- Remple MS, Reed JL, Stepniewska I, Lyon DC, Kaas JH. 2007. The organization of frontoparietal cortex in tree shrew (*Tupaia belangeri*): II Connectional evidence for a frontal-posterior parietal network. *J Comp Neurol.* 501:121-149.
- Rouiller EM, Moret V, Liang F. 1993. Comparison of the connectional properties of the two forelimb areas of the rat sensorimotor cortex: support for the presence of a premotor or supplementary motor cortical area. *Somatosens Mot Res.* 10:269-89.
- Sanderson KJ, Welker W, Shambes GA. Motor cortex and sensorimotor overlap in cerebral cortex of albino rats. *Brain res.* 292:251-260.
- Saraiva PES, Magalhaes Castro B. 1975. Sensory motor representation in the cerebral cortex of the three-toed sloth (*Bradypus tridactylus*). *Brain Res.* 90:181-193.
- Sargis EJ. 2001. The grasping behavior, locomotion and substrate use of the tree shrews *Tupaia minor* and *T. tana* (Mammalia, Scandentia). *J Zoology.* 253:485-490.
- Sessle BJ, Wiesendanger M. 1982. Structural and functional definition of the motor cortex in the monkey (*Macaca fascicularis*). *J Physiol.* 323:245-265.
- Sherrington CS, Grünbaum ASF. 1901. An address on localization in the “motor” cerebral cortex. *Br Med J.* 2:1857-1859.
- Stepniewska I, Preuss TM, Kaas JH. 1993. Architectonics, somatotopic organization, and ipsilateral cortical connections of the primary motor area of owl monkeys. *J Comp Neurol.* 330:238-271.
- Stepniewska I, Fang PC, Kaas JH. 2005. Microstimulation reveals specialized subregions for different complex movements in posterior parietal cortex of prosimian galagos. *Proc Natl Acad Sci.* 102:4878-4883.
- Stepniewska I, Fang PC, Kaas JH. 2009a. Organization of the posterior parietal cortex in galagos: I. Functional zones identified by microstimulation. *J Comp Neurol.* 517:765-782.
- Stepniewska I, 2009b. Organization of the posterior parietal cortex in galagos: II. Ipsilateral cortical connections of physiologically identified zones within anterior sensorimotor region. *J Comp Neurol.* 517:783-807.
- Stepniewska I, Friedman RM, Gharbawie OA, Cerkevich CM, Roe AW, Kaas JH. 2011. Optical imaging in galagos reveals parietal-frontal circuits underlying motor behavior. *Proc Natl Acad Sci USA.* 108:E725-32.

- Stepniewska I, Gharbawie OA, Burish MJ, Kaas JH. 2014. Effects of muscimol inactivations of functional domains in motor, premotor, and posterior parietal cortex on complex movements evoked by electrical stimulation. *J Neurophysiol.* 111:1100-1119.
- Strick, PL. 2002. Stimulating research on motor cortex. *Nat. Neurosci.* 5:714-715.
- Stick PL, Preston JB. 1982. Two representations of the hand in area 4 of primate. I. Motor output organization. *J Neurophysiol.* 48:139-149.
- Stoney SD, Thompson WD, Asanuma H. 1968. Excitation of pyramidal tract cells by intracortical microstimulation: effective extent of stimulating current. *J Neurophysiol.* 31:659-669.
- Tandon S, Kambi N, Jain N. 2008. Overlapping representations of the neck and whiskers in the rat motor cortex revealed by mapping at different anesthetic depths. *Eur J Neurosci.* 27:228-237.
- Tennant KA, Adkinds DL, Donian NA, Asay AL, Thomas N, Kleim JA, Jones TA. 2011. The organization of the forelimb representation of the C57NL/6 mouse motor cortex as defined by intracortical microstimulation and cytoarchitecture. *Cereb Cortex.* 21:865-878.
- Teskey GC, Kolb B. 2011. Functional organization of rat and mouse motor cortex. *Animal models of movement disorders. Vol II, EL Lane and SB Dunnett (Eds). Springer Science+Business Media, New York, USA. pp117-137.*
- Uematsu S, Lesser RP, Gordon B. Localization of sensorimotor cortex: the influence of Sherrington and Cushing on the modern concept. 1992. *30:904-912.*
- Van Acker GM 3rd, Amundsen SL, Messamore WG, Zhang HY, Luchies CW, Kovac A, Cheney PD. 2013. Effective intracortical microstimulation parameters applied to primary motor cortex for evoking forelimb movements to stable spatial end points. *J Neurophysiol.* 110:1180-1189.
- Van Acker GM 3rd, Amundsen SL, Messamore WG, Zhang HY, Luches CW, Cheney PD. 2014. Equilibrium-based movement endpoints elicited from primary motor cortex using repetitive microstimulation. *J Neurosci.* 34:15722-34.
- Welker WI, Benjamine RM, Miles RC, Woolsey CN. 1957. Motor effects of stimulation on cerebral cortex of squirrel monkey (*Saimiri sciureus*). *J Neurophysiol.* 20:347-364.
- Wong P, Kaas JH. 2009. Architectonic subdivisions of neocortex in the tree shrew (*Tupaia belangeri*). *Anat Rec.* 292:994-1027.

Wong-Riley M. 1979. Changes in the visual system of monocularly sutured or enucleated cats demonstrable with cytochrome-oxidase histochemistry. *Brain Res.* 171:11-28.

Welker WI, Benjamin RM, Miles RC, Woolsey CN. 1957. Motor effects of stimulation of cerebral cortex of squirrel monkey (*Saimiri Sciureus*). *J Neurophysiol.* 30:347-364.

Wu CW, Kaas JH. 2000. Converging evidence from microstimulation, architecture, and connections for multiple motor areas in the frontal and cingulate cortex of prosimian primates. *J Comp Neurol.* 423:140-177.

TABLES

Table 1. List of Abbreviations

3a	Area 3a
3b	Area 3b
CO	Cytochrome oxidase
M	Motor cortex
M1	Primary motor cortex
PP	Posterior parietal region
PPC	Posterior parietal cortex
Sc	Caudal somatosensory area
V1	Primary visual cortex
V2	Secondary visual area

Table 2. Case Overview

Case #	Stimulus Duration Tested (ms)	Number of Sites Tested in Each Brain Area (sites with movements/ <i>total sites tested</i>)						Total
		M	3a	3b	Sc	PP	S2/Pv	
14-14	500	0/3	4/4	12/12	2/2	5/13	0/0	23/34
14-20	500	2/4	6/7	10/12	2/3	7/16	1/1	28/43
14-34	500	2/5	2/4	7/9	1/2	2/8	2/5	15/33
14-36	500	11/13	5/6	10/11	3/3	7/10	2/5	38/48
14-39	500	5/7	7/7	14/16	4/4	5/12	3/4	38/50
14-49	500	3/6	3/3	9/13	2/3	12/25	2/3	31/53
	50	1/2	2/2	5/6	0/0	0/0	0/0	8/10
	800	1/2	2/2	6/6	0/0	0/0	0/0	9/10
14-88	500	5/7	4/4	7/8	2/2	11/17	3/4	32/42
	50	3/7	3/4	7/8	1/2	8/17	2/4	24/52
	800	5/7	4/4	7/8	2/2	11/17	3/4	32/52
14-89	500	9/16	8/8	22/24	6/8	10/20	3/8	58/84
	50	8/16	5/8	20/24	2/8	2/20	3/8	40/84
	800	9/16	8/8	22/24	6/8	10/20	3/8	58/84
14-93	500	8/11	5/6	12/14	2/3	5/12	2/2	34/48
14-124	500	8/12	1/2	10/12	4/4	3/6	0/0	25/36
TOTAL	500	53/84	45/51	113/131	28/34	67/139	18/32	323/471
	50	12/25	10/14	32/38	3/10	10/37	5/12	72/136
	800	15/25	14/14	35/38	8/10	22/37	6/12	100/136

Sites that were tested but found to be within V1 or V2 (See Figs. 6 and 7) are not included in the above table.

Table 3. Complex and Simple Movements with LT ICMS

Case #	Total Sites Tested	Sites with Movements Using LT-ICMS	Complex Movements			Total Complex Movements
			Multi-Joint	Multi-body-part	Multi body-part + multi-joint	
14-14	34	23	1	10*	Unknown	11*
			unknown			
14-20	43	28	2	11	1	11
			unknown		Unknown	
14-34	33	15	1	3	1	3
14-36	48	38	4	17	1	20
14-39	50	38	15	14	6	22
14-49	53	31	10	17	5	22
14-88	42	32	8	14	2	20
14-89	84	58	12	18	4	26
14-93	48	34	7	17	5	19
14-124	36	26	7	4	1	10

* denotes cases where we were unable to determine specific movements (because we did not thoroughly record all movements)

Table 4. Average Thresholds Across Areas and Across Cases (μA) with LT-ICMS

Case #	Average Threshold (μA) with LT-ICMS/Brain Area					
	M	3a	3b	Sc	PP	S2/Pv
14-14	n/a	89 n=4	107 n=11	78 n=2	155 n=5	n/a
14-20	nm	nm	nm	nm	nm	nm
14-34	205 n=2	75 n=2	157 n=7	n/a	232 n=2	n/a
14-36	212 n=10	202 n=5	192 n=9	308 n=3	293 n=6	250 n=2
14-39	136 n=5	68 n=5	83 n=12	193 n=4	221 n=5	108 n=3
14-49	138 n=3	53 n=3	83 n=9	85 n=2	253 n=12	163 n=2
14-88	165 n=4	16 n=4	85 n=5	129 n=2	277 n=9	125 n=2
14-89	219 n=8	111 n=8	154 n=22	279 n=6	364 n=10	215 n=2
14-93	222 n=7	180 n=4	212 n=12	290 n=2	168 n=5	120 n=2
14-124	134 n=6	65 n=1	237 n=8	241 n=4	225 n=2	n/a
Average	190 N=45	106 N=36	148 N=95	220 N=25	261 N=56	159 N=13

nm indicates cases where the thresholds were not measured

FIGURE LEGENDS

Figure 1. Phylogenetic tree representing the relationship of mammals within the Euarchontoglires clade, which includes rodents, rabbits and pikas, tree shrews, and primates.

Figure 2. Experimental set up. Tree shrews were placed in individually designed hammocks such that all four limbs could move freely. Their heads were secured with a head post, and the electrode was lowered using a micromanipulator. The hammock, head post, and micromanipulator were all attached to the same stereotax.

Figure 3. Alignment of motor maps with cortical architecture. Photographs taken during cortical mapping (**A**) were aligned to architectonic sections by matching the surface blood vasculature patterns in the photos with the most superficial tissue sections stained for CO (**B**) to reveal surface blood vessel patterns. A prominent star-shaped vascular pattern in **B** is highlighted in **A**. (Additionally, during mapping, fluorescent fiducial probes were placed in various locations to aid in our alignment of ICMS maps to histologically processed tissue). Common blood vessel patterns (black and white arrowheads in **B** and **C**) were used to align deeper tissue sections that revealed areal borders.

Figure 4. Example of fluorescent fiduciary probes used to help align motor maps with cortical architecture. In this example, fiduciary probes were placed at the end of motor mapping using the anatomical fluorescent tracer, fluoro ruby (FR: 15% in phosphate

buffered saline: Thermo Fisher Scientific). **A.** Is a photograph of a myelin section under fluorescent light showing the location of the FR probes (two white sites within photograph), with the architectonic borders superimposed over the photograph (gray lines). **B.** Is a photograph of the same myelin section under brightfield highlighting the myeloarchitecture used to determine cortical borders (white and black lines).

Figure 5. Myeloarchitecture of the tree shrew cortex from a single section of flattened cortical tissue. Areal borders for areas 17/V1, 3b, and auditory cortex are easily identified as darkly staining regions of cortex. Scale bar is 2mm.

Figure 6. Short-train (ST, 50 ms) and long-train (LT, 500 ms) intracortical microstimulation (ICMS) maps of Case 14-88. **A.** Entire flattened cortical hemisphere with anatomical borders indicated with solid black lines. The gray region represents tissue that was located along the medial wall prior to flattening. The boxed region is enlarged in panels **C**, **D**, and **E**. **B.** Color-coded legend of joints and body parts moving during ICMS and the corresponding location on the lateral view of the tree shrew body. This legend is used for Figures 6, 7, 11, 12. Different colors are used to indicate the types of movements elicited at different locations within panels **C** and **D**. **C** ST-ICMS motor map. White dots represent the location of penetration sites where ICMS evoked movements. Surrounding color tiles represent the location of the body moving during ST-ICMS. Striped tiles indicate multiple simultaneous movements. Solid black lines represent borders of cortical areas based on myelin. **D.** LT-ICMS motor map. **E.** Map of ratios in stimulation current thresholds required to elicit motor movements. Darker

orange and red colored tiles indicate greater ratios of ST:LT thresholds. Burgundy tiles indicate no ST-evoked movements up to 500 μ A, and white tiles indicate sites where threshold values were not measured. For the most part, greater currents were required to elicit movements using ST- vs. LT-ICMS (at one site, shown in green, LT threshold > ST threshold).

Figure 7. Short-train (ST, 50 ms) and long-train (LT, 500 ms) intracortical microstimulation (ICMS) maps of Case 14-89. **A.** Flattened cortical hemisphere with anatomical borders indicated. **B.** ST-ICMS motor map. **C.** LT ICMS motor map. **D** and **E.** Map of ratios in stimulation current thresholds required to elicit motor movements. Greater currents were required to elicit movements using ST- vs. LT-ICMS (**D**); however, most thresholds are similar for 500 and 800ms trains, i.e., $\text{threshold}_{500\text{ ms}} : \text{threshold}_{800\text{ ms}}$ ratio was near 1 (**E**). See Fig. 6 for figure conventions.

Figure 8. Example of ICMS-elicited movements using short-train (ST: 50 ms), long-train (LT: 500 ms), and extra-long-train (xLT: 800 ms) stimulation parameters. The black traces represent the baseline position of the forelimb. The trace with the darkest shade of gray represents the position of the hand 48 ms after stimulus onset, while progressively lighter shades of gray traces indicate progressively later positions of the arm and hand subsequent time points up to 800 ms. The black star represents the point on the hand used to measure the forelimb displacement, which is plotted below each movement example. The grey shading within the plot represents the period of time stimulation was presented.

Figure 9. Example of an ICMS-elicited repeated movement using 3 different stimulation train durations at a single penetration site within area 3a of case 14-88 (Fig. 6). The top panel shows the lateral view of the tree shrew face at different points in time during stimulation. Stimulation caused the jaw to move downward and then upward. This motion was repeated multiple times with increased stimulation duration (50 ms left, 500 ms center, and 800 ms right). Black tracings represent the resting state position of the jaw prior to stimulation, dark gray, the position of the jaw at the maximum extent of the first downward movement in the movement series. The black star represents the point on the jaw used to measure the displacement plotted at bottom. The grey shading within the plot represents the period of time stimulation was presented.

Figure 10. Examples of a simple and complex movements evoked from various cortical fields using long-train intracortical microstimulation. **A.** An example of a simple movement involving the forelimb evoked during stimulation to a site within the posterior parietal cortex. The black trace represents the location of the forelimb prior to stimulation, the gray trace represents the location of the arm at the apex of the movement. **B.** An example of complex movement involving both ipsilateral (ipsi—black and gray dashed line traces) and contralateral (contra—black and gray solid line traces) forelimbs and the contralateral hindlimb. **C.** Movement evoked by LT-ICMS in motor cortex. Both hands move upward towards the mouth with elbow flexion, while the jaw opens. **D.** Elbow flexion with eye squint evoked by LT-ICMS in Sc.

Figure 11. LT-ICMS maps of Case 14-39 (**A**) and Case 14-124 (**B**) with corresponding threshold maps in which larger black circles indicate sites where movements could be evoked with smaller currents (14-39: **C**), (14-124: **D**). Other conventions as in previous figures.

Figure 12. LT-ICMS maps for cases 14-36 (**A**) and 14-93 (**B**) with corresponding threshold maps (14-36:**C**), (14-93:**D**). Other figure conventions are the same as those described in previous figures.

Figure 13. Comparisons of motor maps across different mammals within the Euarchontoglires clade including rats (**A, B**), tree shrews (**C, D**), and prosimian galagos (**E,F**). General color-coded motor maps across all cortical areas are presented on the left column (**A, C, E**), while the location of possible movement domains within cortical areas are presented to the right (**B, D, F**). **C** and **D**, summarize the current results, with the location of possible movement domains within motor, posterior parietal cortex, as well as primary somatosensory cortex (3b). The rat map is based on results from Brecht et al., 2004; Tandon et al. 2008; and Brown and Teskey 2014. Movements involving the eyes are located along the medial wall (Brecht et al. 2004). Prosimian primate movement domains have been reported in motor, premotor, and posterior parietal cortex by Stepniewska et al. 2005, 2009, 2014.

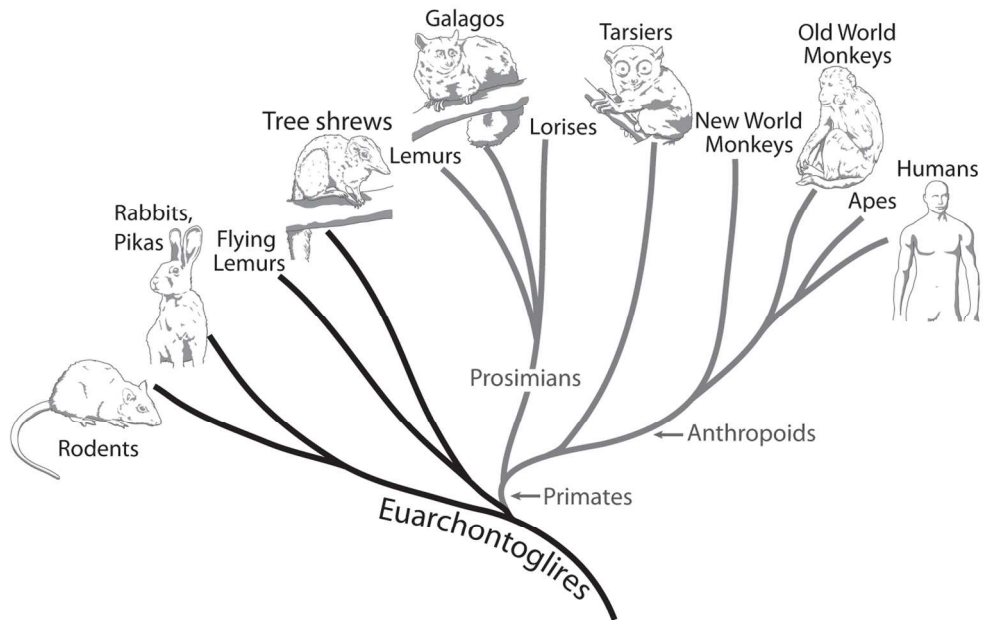


Figure 1. Phylogenetic tree representing the relationship of mammals within the Euarchontoglires clade, which includes rodents, rabbits and pikas, tree shrews, and primates.

180x113mm (300 x 300 DPI)

Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

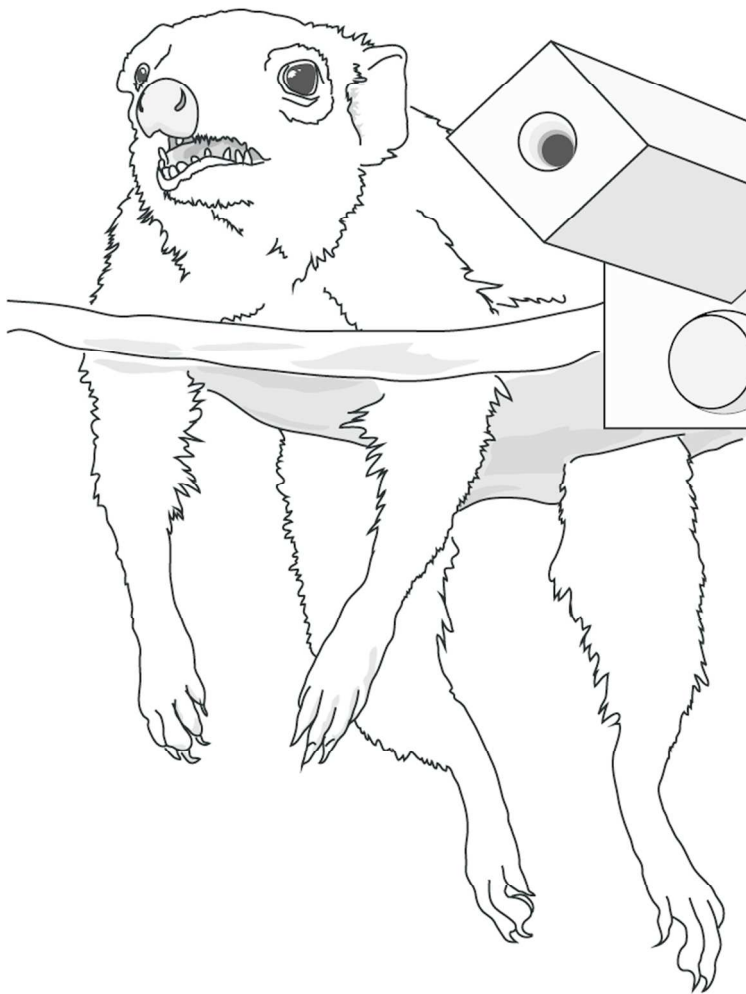


Figure 2. Experimental set up. Tree shrews were placed in individually designed hammocks such that all four limbs could move freely. Their heads were secured with a head post, and the electrode was lowered using a micromanipulator. The hammock, head post, and micromanipulator were all attached to the same stereotax. 85x137mm (300 x 300 DPI)

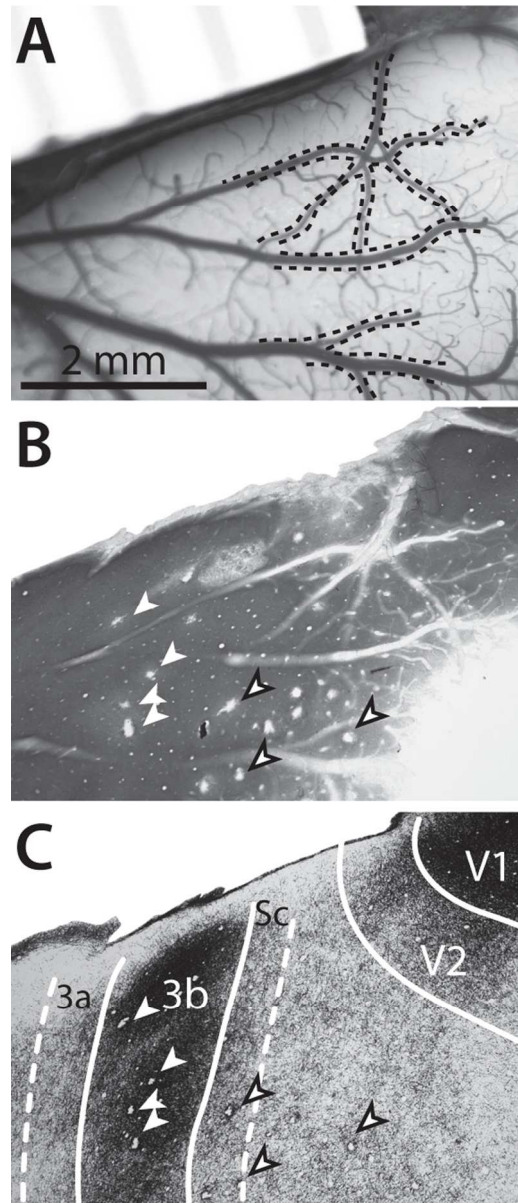


Figure 3. Alignment of motor maps with cortical architecture. Photographs taken during cortical mapping (A) were aligned to architectonic sections by matching the surface blood vasculature patterns in the photos with the most superficial tissue sections stained for CO (B) to reveal surface blood vessel patterns. A prominent star-shaped vascular pattern in B is highlighted in A. (Additionally, during mapping, fluorescent fiducial probes were placed in various locations to aid in our alignment of ICMS maps to histologically processed tissue). Common blood vessel patterns (black and white arrowheads in B and C) were used to align deeper tissue sections that revealed areal borders.

85x197mm (300 x 300 DPI)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

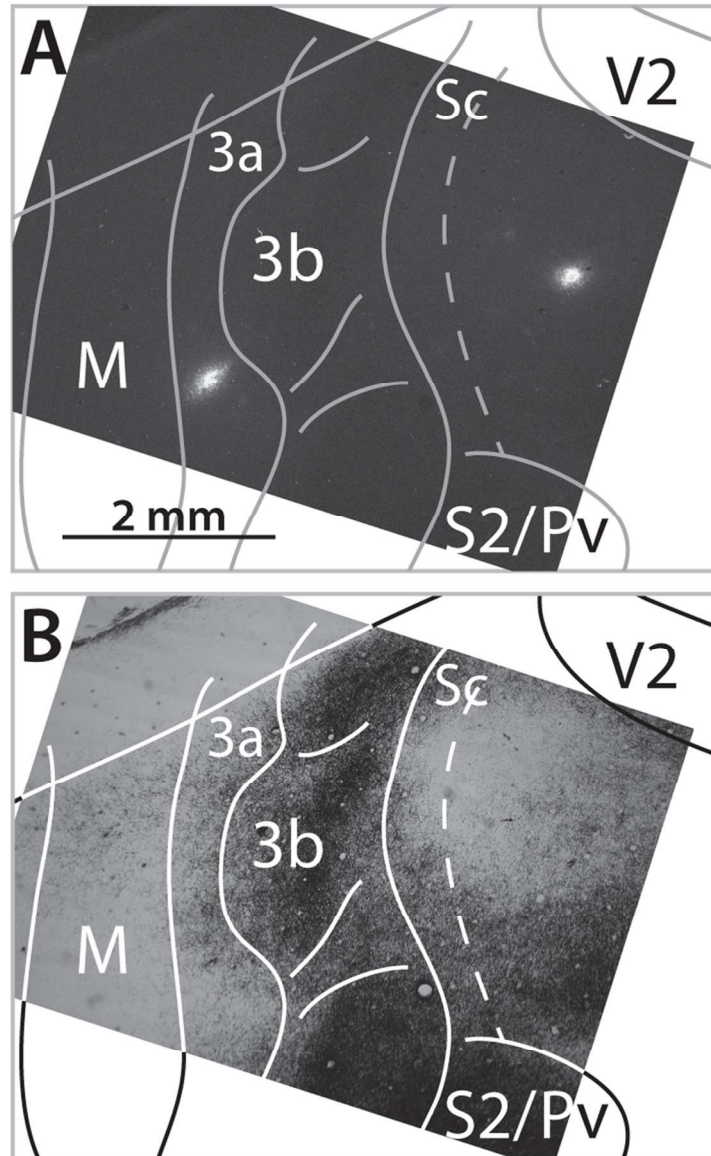


Figure 4. Example of fluorescent fiduciary probes used to help align motor maps with cortical architecture. In this example, fiduciary probes were placed at the end of motor mapping using the anatomical fluorescent tracer, fluoro ruby (FR: 15% in phosphate buffered saline: Thermo Fisher Scientific). A. Is a photograph of a myelin section under fluorescent light showing the location of the FR probes (two white sites within photograph), with the architectonic borders superimposed over the photograph (gray lines). B. Is a photograph of the same myelin section under brightfield highlighting the myeloarchitecture used to determine cortical borders (white and black lines).
85x134mm (300 x 300 DPI)

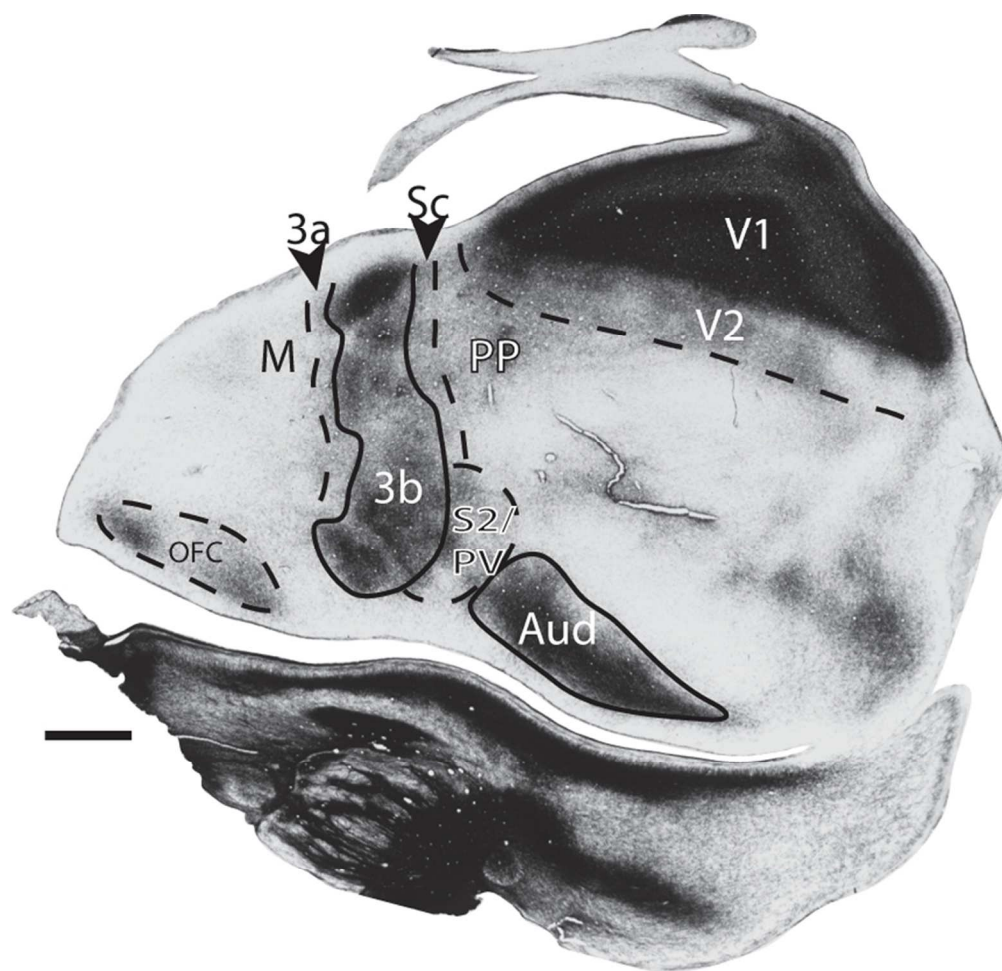


Figure 5. Myeloarchitecture of the tree shrew cortex from a single section of flattened cortical tissue. Areal borders for areas 17/V1, 3b, and auditory cortex are easily identified as darkly staining regions of cortex.

Scale bar is 2mm.

85x81mm (300 x 300 DPI)

Case 14-88

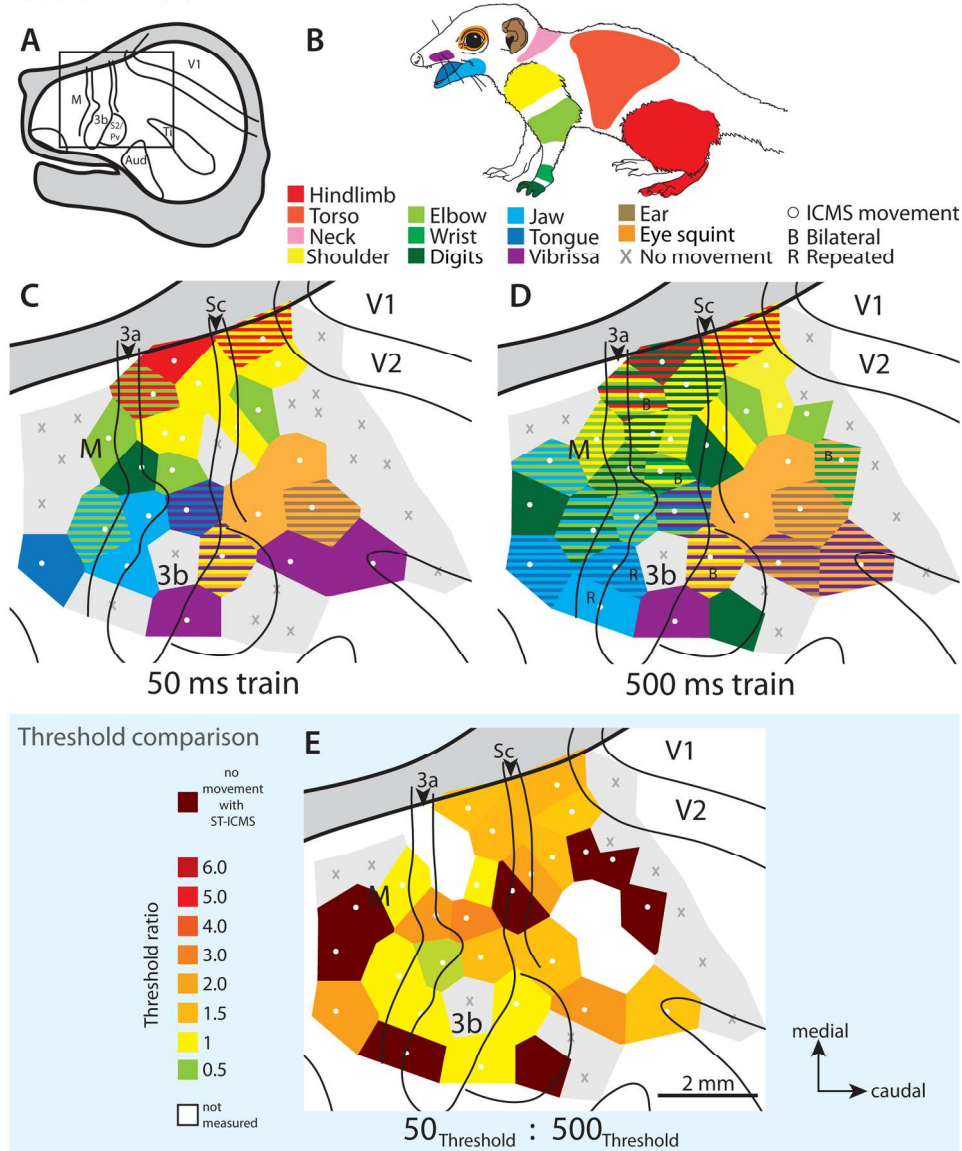


Figure 6. Short-train (ST, 50 ms) and long-train (LT, 500 ms) intracortical microstimulation (ICMS) maps of Case 14-88. A. Entire flattened cortical hemisphere with anatomical borders indicated with solid black lines.

The gray region represents tissue that was located along the medial wall prior to flattening. The boxed region is enlarged in panels C, D, and E. B. Color-coded legend of joints and body parts moving during ICMS and the corresponding location on the lateral view of the tree shrew body. This legend is used for Figures 6, 7, 11, 12. Different colors are used to indicate the types of movements elicited at different locations within panels C and D. C. ST-ICMS motor map. White dots represent the location of penetration sites where ICMS evoked movements. Surrounding color tiles represent the location of the body moving during ST-ICMS. Striped tiles indicate multiple simultaneous movements. Solid black lines represent borders of cortical areas based on myelin. D. LT-ICMS motor map. E. Map of ratios in stimulation current thresholds required to elicit motor movements. Darker orange and red colored tiles indicate greater ratios of ST:LT thresholds. Burgundy tiles indicate no ST-evoked movements up to 500 μ A, and white tiles indicate sites where threshold values were not measured. For the most part, greater currents were required to elicit movements using ST- vs. LT-

1
2
3 ICMS (at one site, shown in green, LT threshold > ST threshold).
4 180x219mm (300 x 300 DPI)
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review

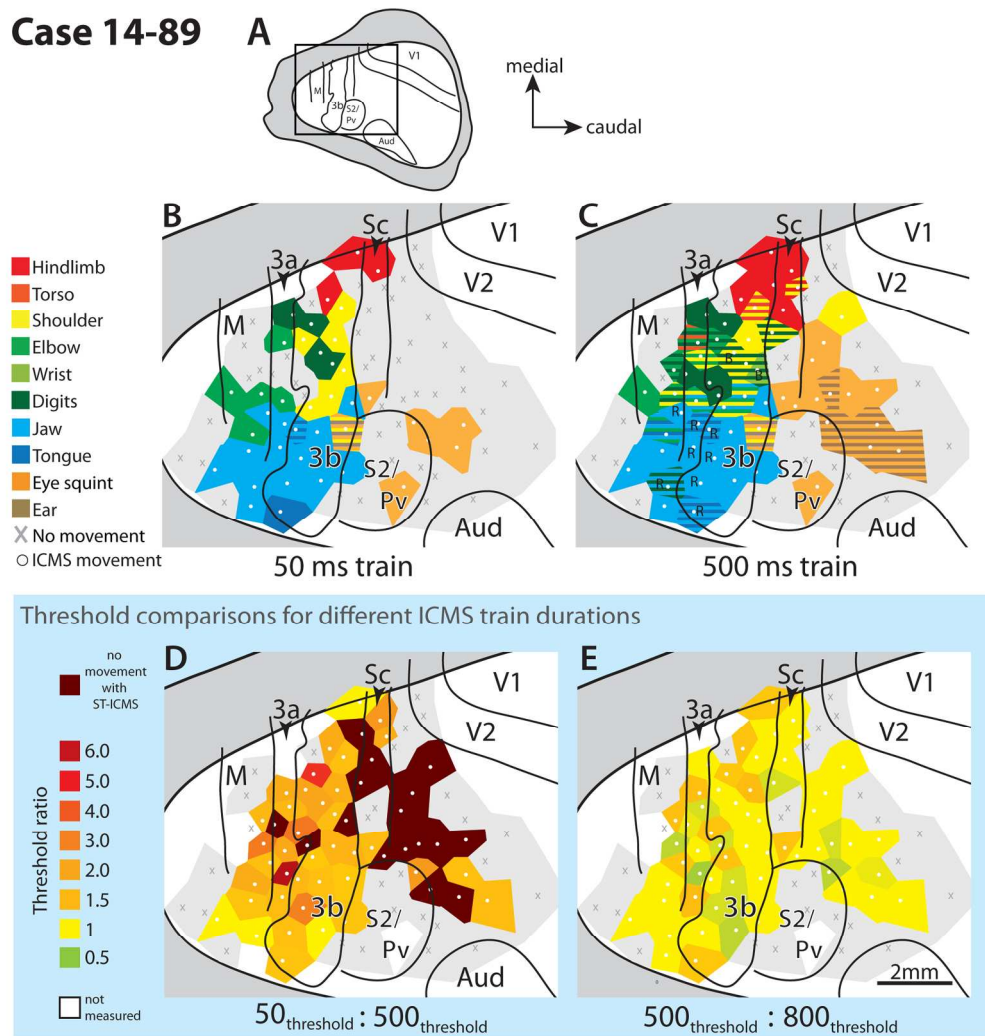


Figure 7. Short-train (ST, 50 ms) and long-train (LT, 500 ms) intracortical microstimulation (ICMS) maps of Case 14-89. A. Flattened cortical hemisphere with anatomical borders indicated. B. ST-ICMS motor map. C.

LT ICMS motor map. D and E. Map of ratios in stimulation current thresholds required to elicit motor movements. Greater currents were required to elicit movements using ST- vs. LT-ICMS (D); however, most thresholds are similar for 500 and 800ms trains, i.e., threshold_{500 ms}: threshold_{800 ms} ratio was near 1 (E). See Fig. 6 for figure conventions.

180x187mm (300 x 300 DPI)

Complex: Multi-joint forelimb movement (evoked by ICMS in area 3b)

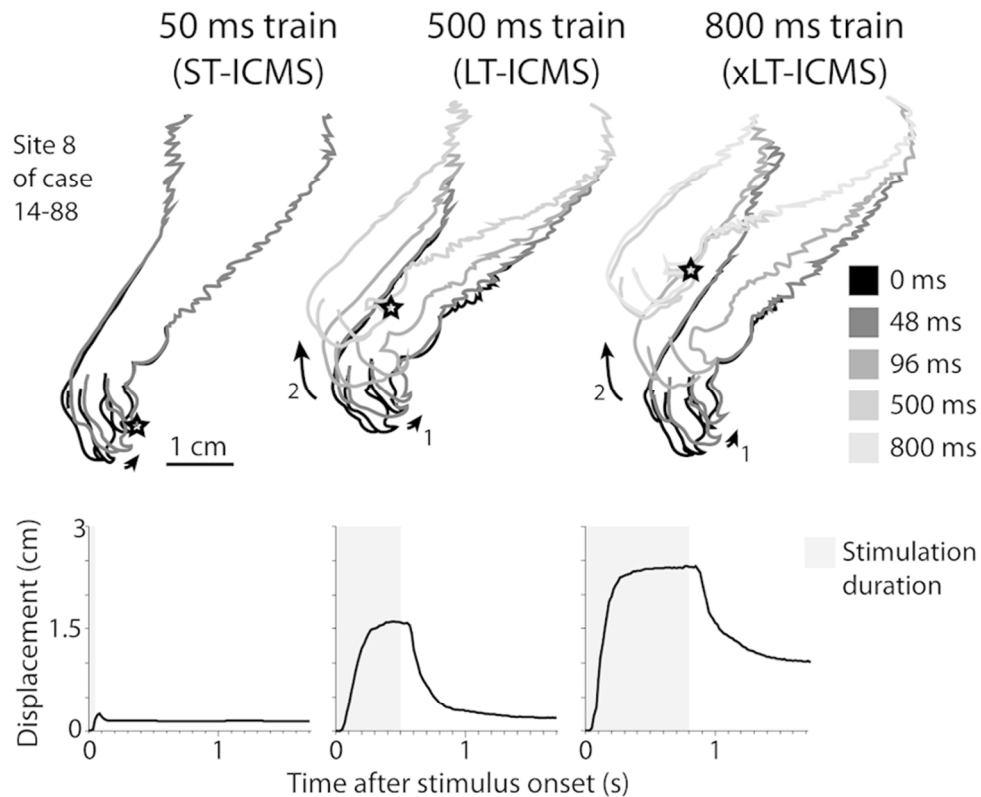


Figure 8. Example of ICMS-elicited movements using short-train (ST: 50 ms), long-train (LT: 500 ms), and extra-long-train (xLT: 800 ms) stimulation parameters. The black traces represent the baseline position of the forelimb. The trace with the darkest shade of gray represents the position of the hand 48 ms after stimulus onset, while progressively lighter shades of gray traces indicate progressively later positions of the arm and hand subsequent time points up to 800 ms. The black star represents the point on the hand used to measure the forelimb displacement, which is plotted below each movement example. The grey shading within the plot represents the period of time stimulation was presented.

85x79mm (300 x 300 DPI)

Repeated jaw movement (evoked by ICMS in area 3a)

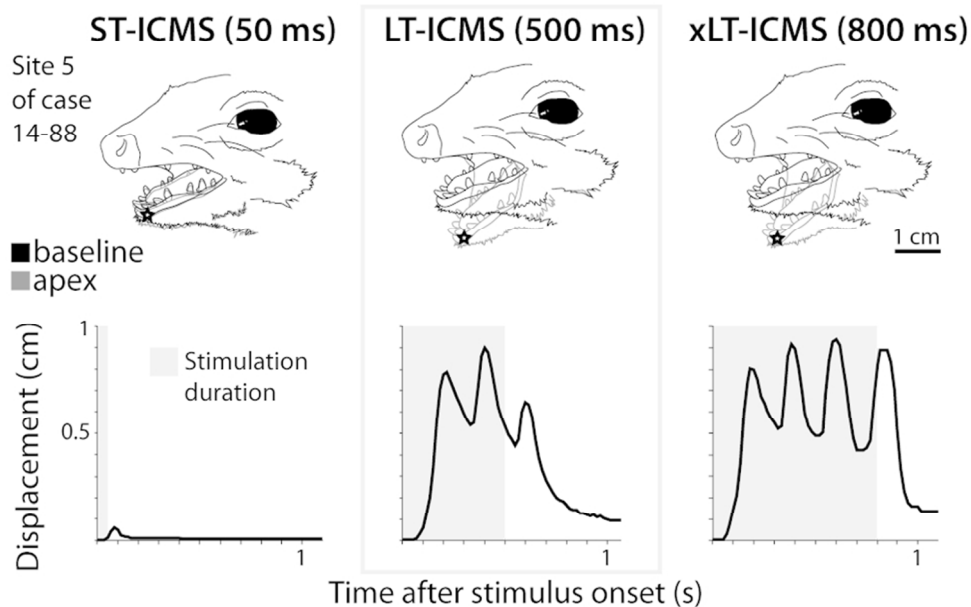


Figure 9. Example of an ICMS-elicited repeated movement using 3 different stimulation train durations at a single penetration site within area 3a of case 14-88 (Fig. 6). The top panel shows the lateral view of the tree shrew face traced from different video frames. Stimulation caused the jaw to move downward and then upward. This motion was repeated multiple times with increased stimulation duration (50 ms left, 500 ms center, and 800 ms right). Black tracings represent the resting state position of the jaw prior to stimulation, dark gray, the position of the jaw at the maximum extent of the first downward movement in the movement series. The black star represents the point on the jaw used to measure the displacement plotted at bottom.

The grey shading within the plot represents the period of time stimulation was presented.

85x56mm (300 x 300 DPI)

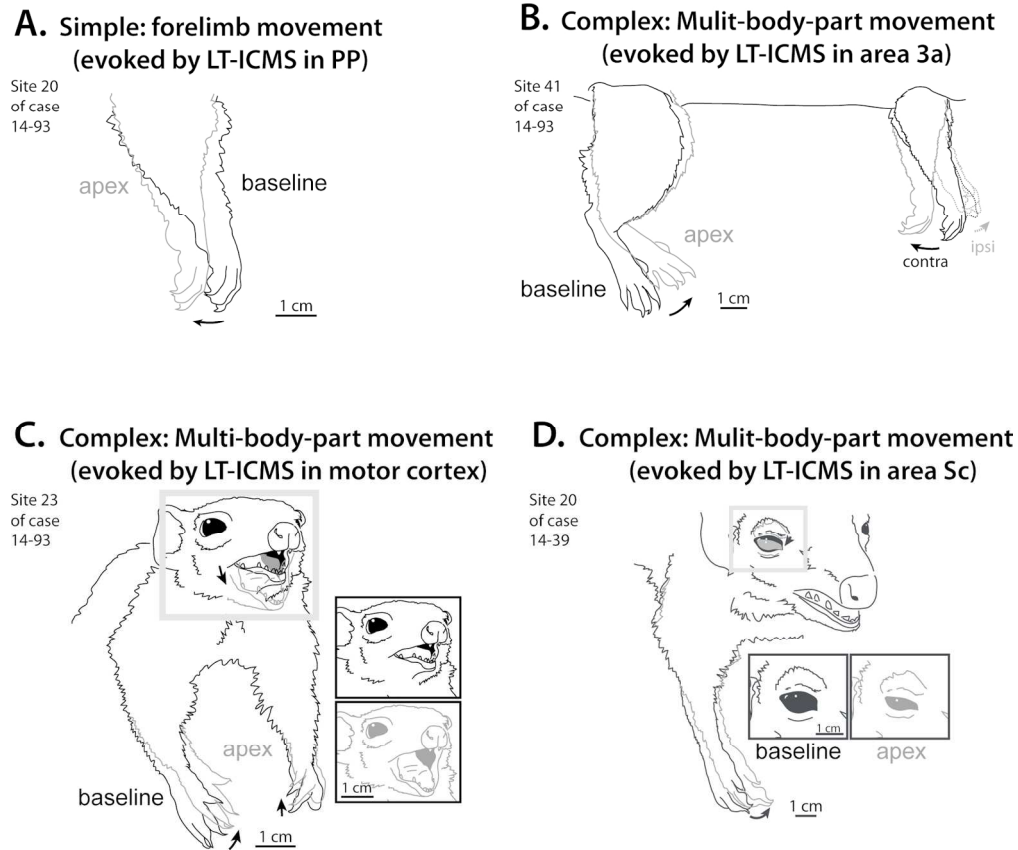


Figure 10. Examples of a simple and complex movements evoked from various cortical fields using long-train intracortical microstimulation. A. An example of a simple movement involving the forelimb evoked during stimulation to a site within the posterior parietal cortex. The black trace represents the location of the forelimb prior to stimulation, the gray trace represents the location of the arm at the apex of the movement. B. An example of complex movement involving both ipsilateral (ipsi—black and gray dashed line traces) and contralateral (contra—black and gray solid line traces) forelimbs and the contralateral hindlimb. C. Movement evoked by LT-ICMS in motor cortex. Both hands move upward towards the mouth with elbow flexion, while the jaw opens. D. Elbow flexion with eye blink evoked by LT-ICMS in Sc.

180x152mm (300 x 300 DPI)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

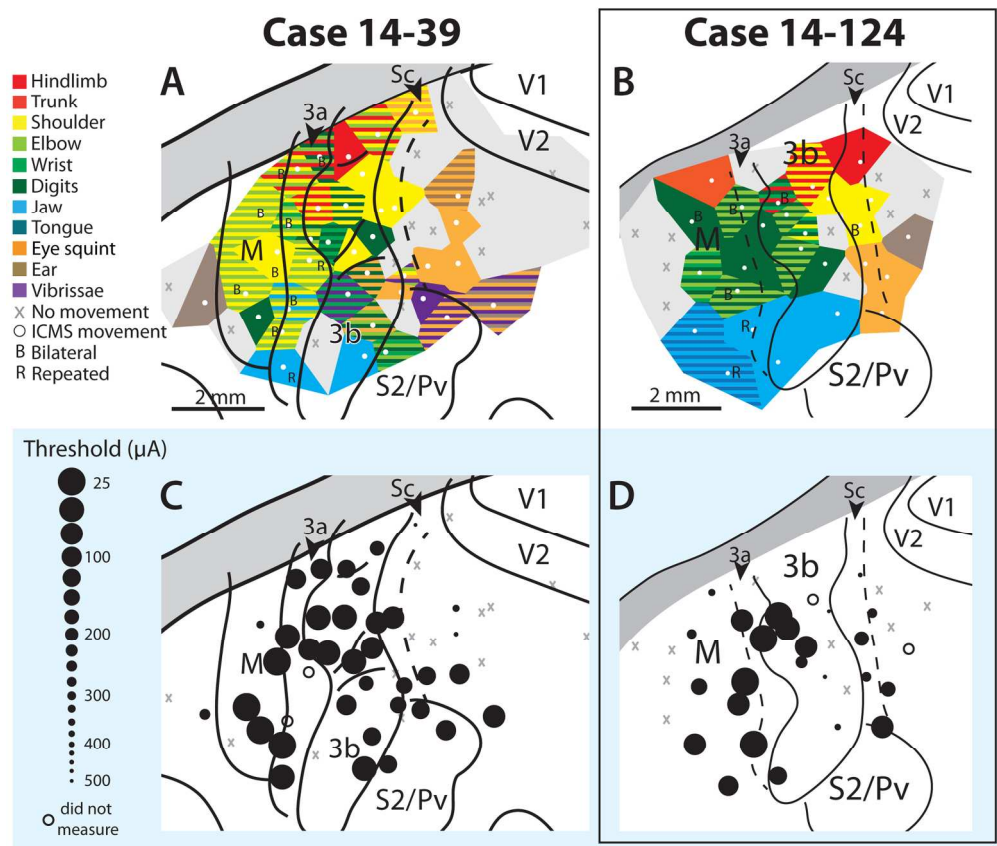


Figure 11. LT-ICMS maps of Case 14-39 (A) and Case 14-124 (B) with corresponding threshold maps in which larger black circles indicate sites where movements could be evoked with smaller currents (14-39: C), (14-124: D). Other conventions as in previous figures.
 180x154mm (300 x 300 DPI)

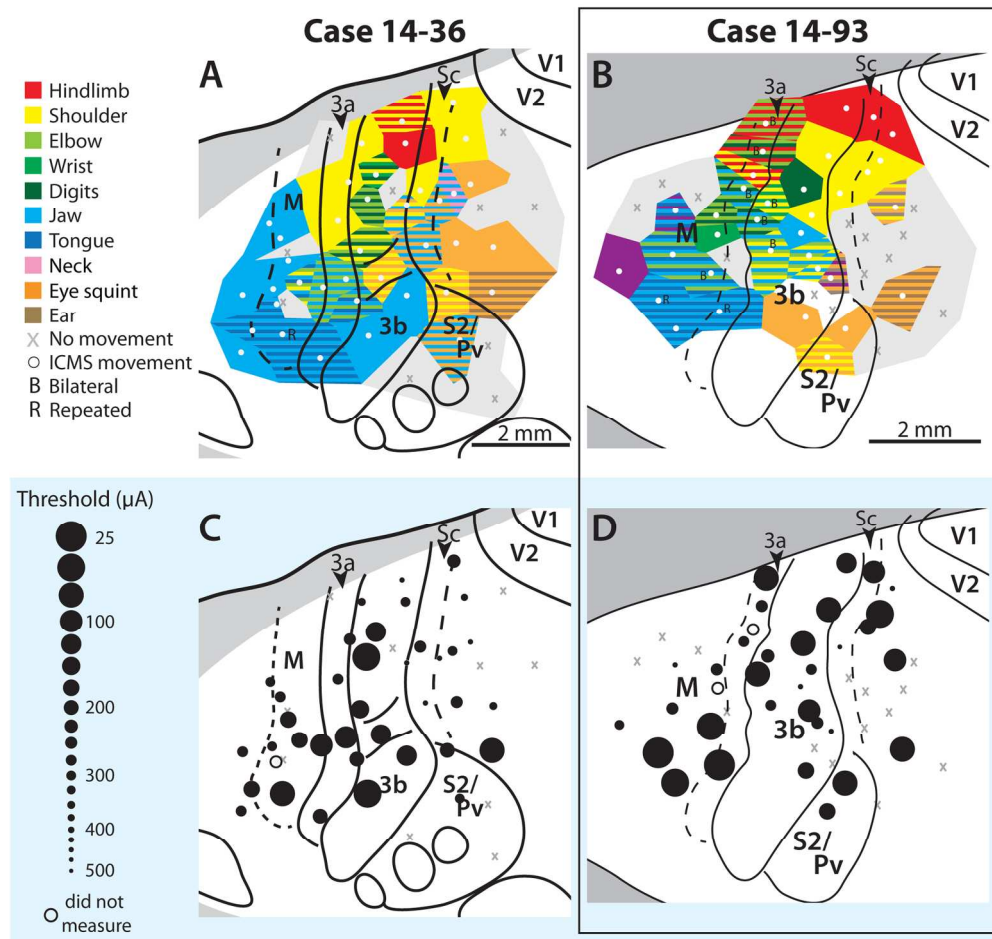


Figure 12. LT-ICMS maps for cases 14-36 (A) and 14-93 (B) with corresponding threshold maps (14-36:C), (14-93:D). Other figure conventions are the same as those described in previous figures.
180x170mm (300 x 300 DPI)



1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

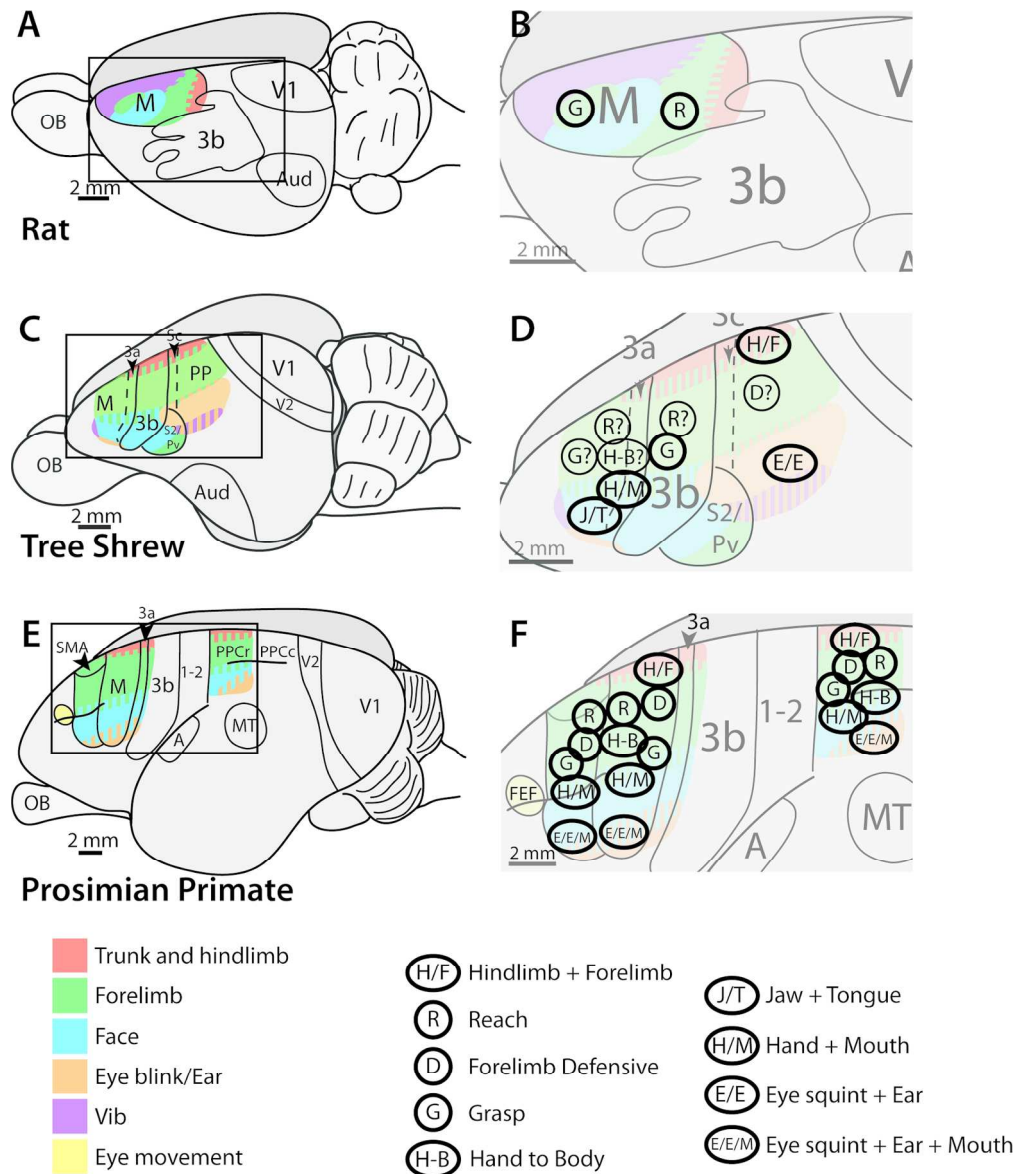


Figure 13. Comparisons of motor maps across different mammals within the Euarchontoglires clade including rats (A, B), tree shrews (C, D), and prosimian galagos (E,F). General color-coded motor maps across all cortical areas are presented on the left column (A, C, E), while the location of possible movement domains within cortical areas are presented to the right (B, D, F). C and D, summarize the current results, with the location of possible movement domains within motor, posterior parietal cortex, as well as primary somatosensory cortex (3b). The rat map is based on results from Brecht et al., 2004; Tandon et al. 2008; and Brown and Teskey 2014. Movements involving the eyes are located along the medial wall (Brecht et al. 2004). Prosimian primate movement domains have been reported in motor, premotor, and posterior parietal cortex by Stepniwska et al. 2005, 2009, 2014.

180x210mm (300 x 300 DPI)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60