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ORIGINAL ARTICLE

Intracortical Microstimulation Maps of Motor, Somatosensory, and Posterior Parietal Cortex in Tree Shrews (*Tupaia belangeri*) Reveal Complex Movement Representations

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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 multijoint and multiple-body-part movements in primary motor, premotor, and PPC. 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.

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

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 and Kaas 2009). However, features of their body morphology are more similar to rodents such as rats and squirrels (Le Gros Clark 1959; Jenkins 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 multijoint and multibody-part movements can be elicited (Graziano, Taylor, and Moore 2002; Graziano, Taylor, Moore, et al. 2002; Graziano et al. 2005; Stepniewska et al. 2005, 2009; Graziano 2008; Gharbawie, Stepniewska, and Kaas 2011; Gharbawie, Stepniewska, Qi, et al. 2011; 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,

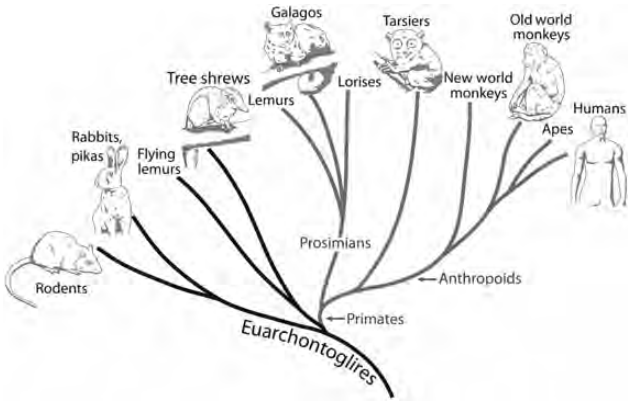

Figure 1. Phylogenetic tree representing the relationship of mammals within the Euarchontoglires clade, which includes rodents, rabbits and pikas, tree shrews, and primates.

complex multijoint (such as wrist + elbow) and multibody-part (such as forelimb + mouth) movements can be elicited in motor, premotor, and posterior parietal cortex (PPC) (Graziano, Taylor, and Moore 2002; Graziano et al. 2005; Stepniewska et al. 2005; 2009; Stepniewska 2009; Gharbawie, Stepniewska, and Kaas 2011; Gharbawie, Stepniewska, Qi, et al. 2011; 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 2009, 2011; Gharbawie, Stepniewska, and Kaas 2011; Gharbawie, Stepniewska, Qi, et al. 2011). 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, Taylor, Moore, et al. 2002; Graziano et al. 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 multijoint movements of the forelimb almost exclusively, with only a few references to the presence of movements involving multiple body parts (Gioanni and Lamarche 1985; Li and Waters 1991; Tandon et al. 2008; Tennant et al. 2011).

To date, multijoint and multibody-part movements have not been observed in tree shrews, and until recently it was not even known whether 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 et al. (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 et al. (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 2009; Stepniewska et al. 2011; Gharbawie, Stepniewska, and Kaas 2011; Gharbawie, Stepniewska, Qi, et al. 2011). 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 whether 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.

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

Materials and Methods

We examined the organization of motor maps using long-train intracortical microstimulation (LT-ICMS) in 10 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

Table 2 Case overview

Case No.	Stimulus duration tested (ms)	Number of sites tested in each brain area (sites with movements/*total sites tested*)						
		M	3a	3b	Sc	PP	S2/Pv	Total
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

Note: Sites that were tested but found to be within V1 or V2 (see Figs 6 and 7) are not included.

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 4 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.

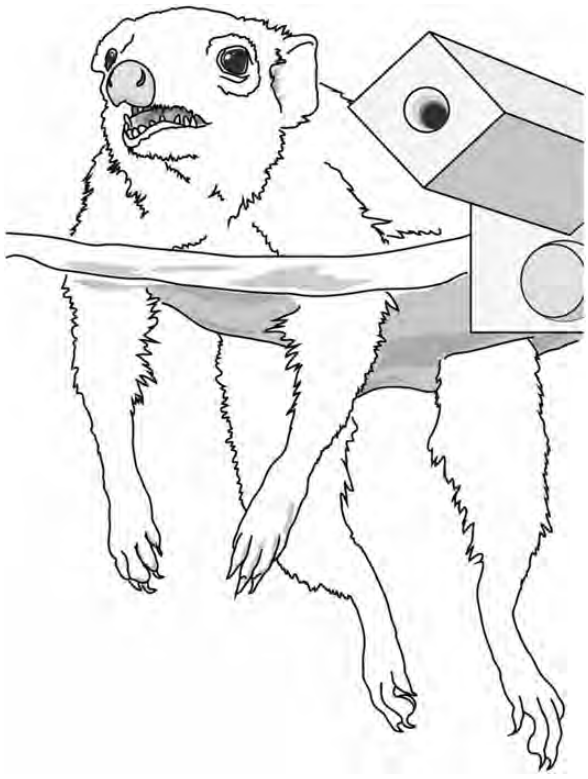

Figure 2. Experimental set-up. Tree shrews were placed in individually designed hammocks such that all 4 limbs could move freely. Their heads were secured with a head post, and the electrode was lowered using a micromanipulator. The hammock, head post, and micromanipulator were all attached to the same stereotax.

ICMS Motor Mapping

ICMS was delivered through low impedance (0.1–1 MΩ) microelectrodes. Trains of pulses were generated using a Grass S88 stimulator and 2 SIU6 stimulus isolation units and measured by the voltage drop across a 10-kΩ 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 3 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, 1920 × 1080 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 h and then placed in a 30% sucrose solution for 24 to 48 h.

Flattened sections were cut into 3 or 4 series of sections, at a thickness of 40 μm (however, the first 4 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. 3*a*). 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 2 independent observers and recorded during the experiment. These characteristics were later confirmed, and movements were further analyzed offline.

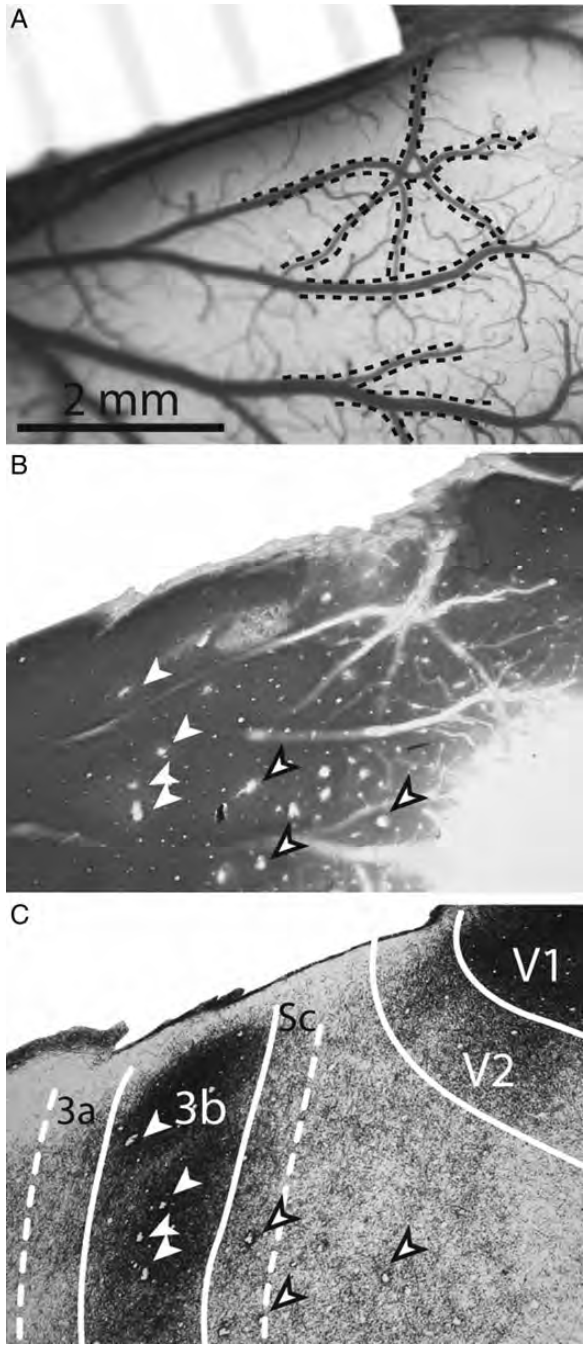

Figure 3. Alignment of motor maps with cortical architecture. Photographs taken during cortical mapping (*A*) were aligned to architectonic sections by matching the surface blood vasculature patterns in the photos with the most superficial tissue sections stained for CO (*B*) to reveal surface blood 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*,*C*) were used to align deeper tissue sections that revealed areal borders.

Movements were characterized as either being "simple," "multijoint," or "multibody-part" depending on whether the observed movement involved muscle contractions around a single joint (simple), multiple joints within the same limb or body part (multijoint), or muscles and joints across multiple body parts (multibody-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.

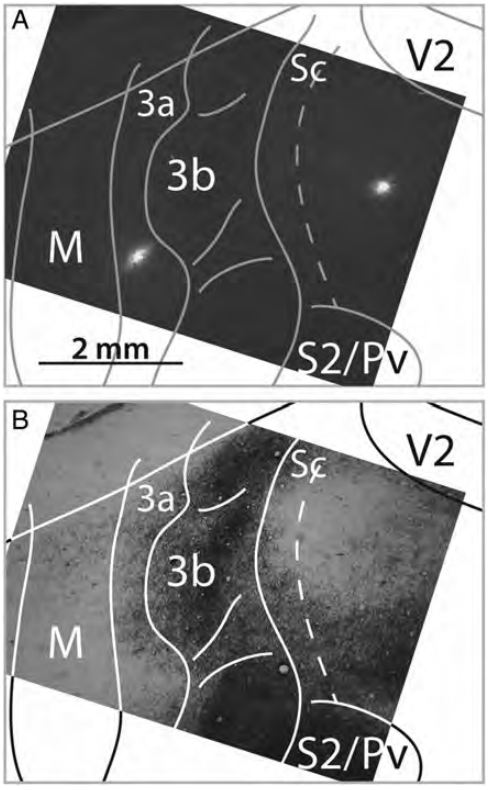

Figure 4. Example of fluorescent fiduciary probes used to help align motor maps with cortical architecture. In this example, fiduciary probes were placed at the end of motor mapping using the anatomical fluorescent tracer, fluoro ruby (FR: 15% in phosphate buffered saline: Thermo Fisher Scientific). (A) Photograph of a myelin section under fluorescent light showing the location of the FR probes (2 white sites within photograph), with the architectonic borders superimposed over the photograph (gray lines). (B) Photograph of the same myelin section under brightfield highlighting the myeloarchitecture used to determine cortical borders (white and black lines).

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 PPC and is thus termed PP (Figs 6, 7, 11, and 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 PPC 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.

Table 3 Complex and simple movements with LT ICMS

Case No.	Total sites Tested	Sites with Movements using LT-ICMS	Complex movements			Total Complex movements
			Multijoint	Multibody-part	Multibody-part + multijoint	
14–14[a]	34	23	1[a] Unknown	10[a]	Unknown	11[a]
14–20[a]	43	28	2[a] Unknown	11	1 Unknown	11
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

Note: [a]Cases where we were unable to determine specific movements (because we did not thoroughly record all movements).

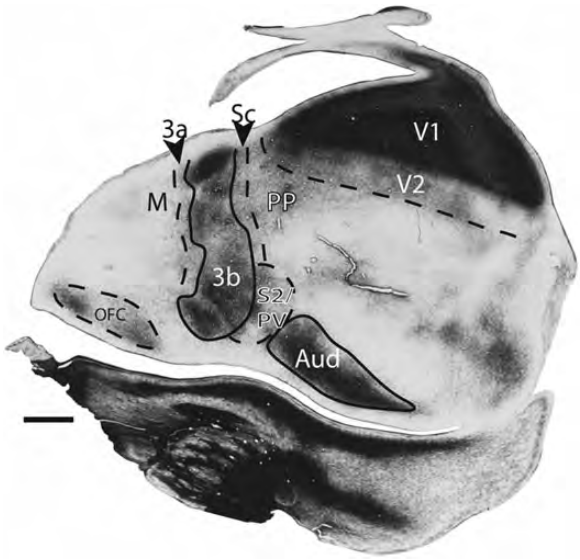

Figure 5. Myeloarchitecture of the tree shrew cortex from a single section of flattened cortical tissue. Areal borders for areas 17/V1, 3b, and auditory cortex are easily identified as darkly staining regions of cortex. Scale bar is 2 mm.

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 and 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 and 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, and 12).

Short-Train versus Long-Train ICMS-Evoked Movements

In 3 animals, we stimulated with 3 different stimulation train durations (Table 2). For all 3 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 and 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 6*C*,*D*; 7*B*,*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 6*E* and 7*D*).

Threshold values were much lower for LT-ICMS than they were for ST-ICMS (Figs 6*E* and 7*D*). For some sites, a 6-fold current increase was required to produce movements using ST-ICMS compared with LT-ICMS. However, the majority of sites only required a 2-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. 7*E*).

Simple and Complex Movements

In 7 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 earlier, LT-ICMS produced complex movements in all areas explored (Figs 6, 7, 11, and 12; Table 3). Most of these movements involved a combination of forelimb joint and facial movements, such as multijoint movements mimicking reaching and grasping behaviors, multibody-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 and 10*B*,*C*,*D*). Complex movements involving multiple body parts (e.g., Fig. 10*B*,*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. 10*C*), defensive postures (Fig. 10*D*), or locomotion (Fig. 10*B*).

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 Remple et al. (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, and 12), except

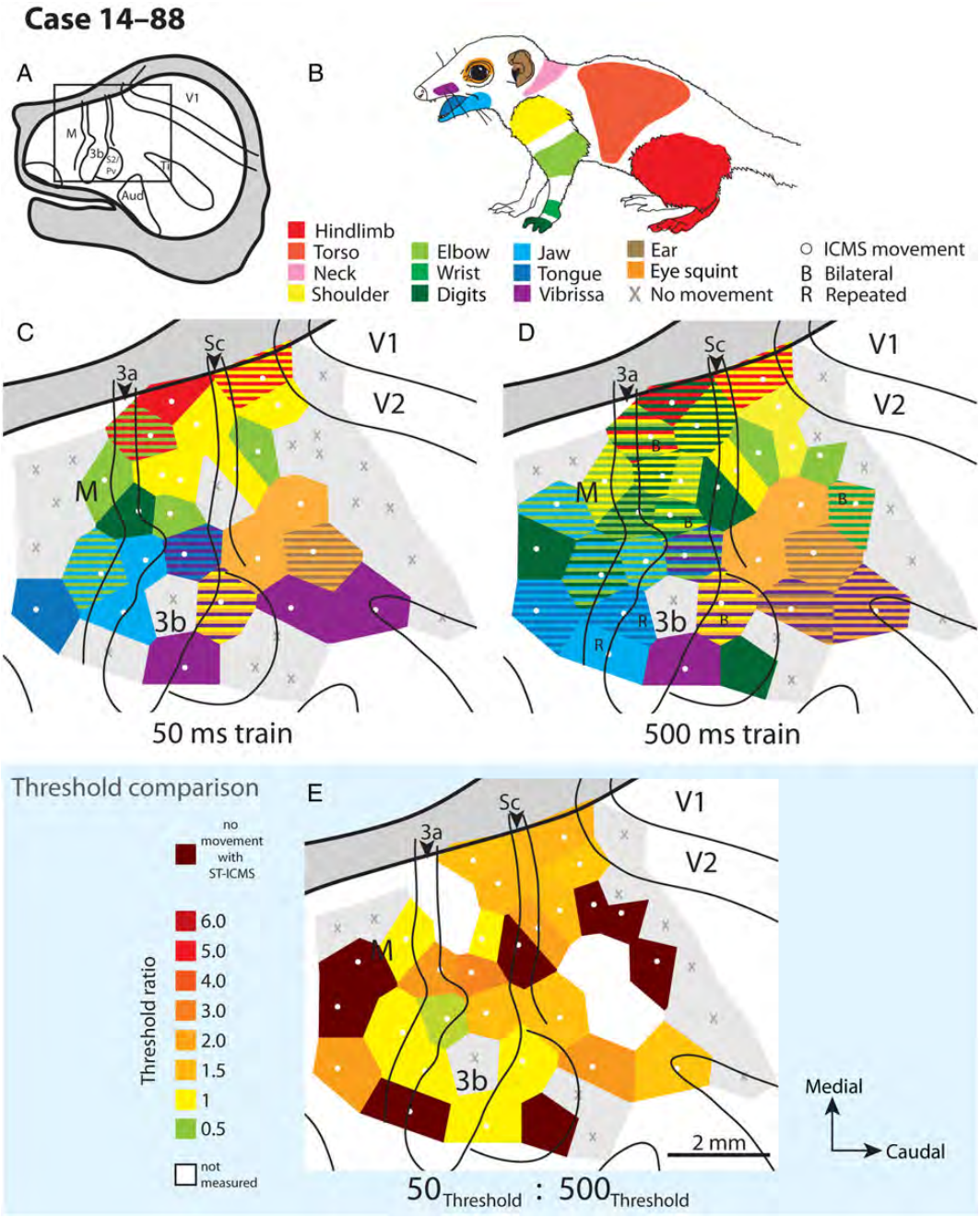

Figure 6. Short-train (ST, 50 ms) and long-train (LT, 500 ms) intracortical microstimulation (ICMS) maps of Case 14–88. (*A*) Entire flattened cortical hemisphere with anatomical borders indicated with solid black lines. The gray region represents tissue that was located along the medial wall prior to 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, and 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 μA, and white tiles indicate sites where threshold values were not measured. For the most part, greater currents were required to elicit movements using ST- versus LT-ICMS (at one site, shown in green, LT threshold > ST threshold).

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, and 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 multijoint and multibody-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

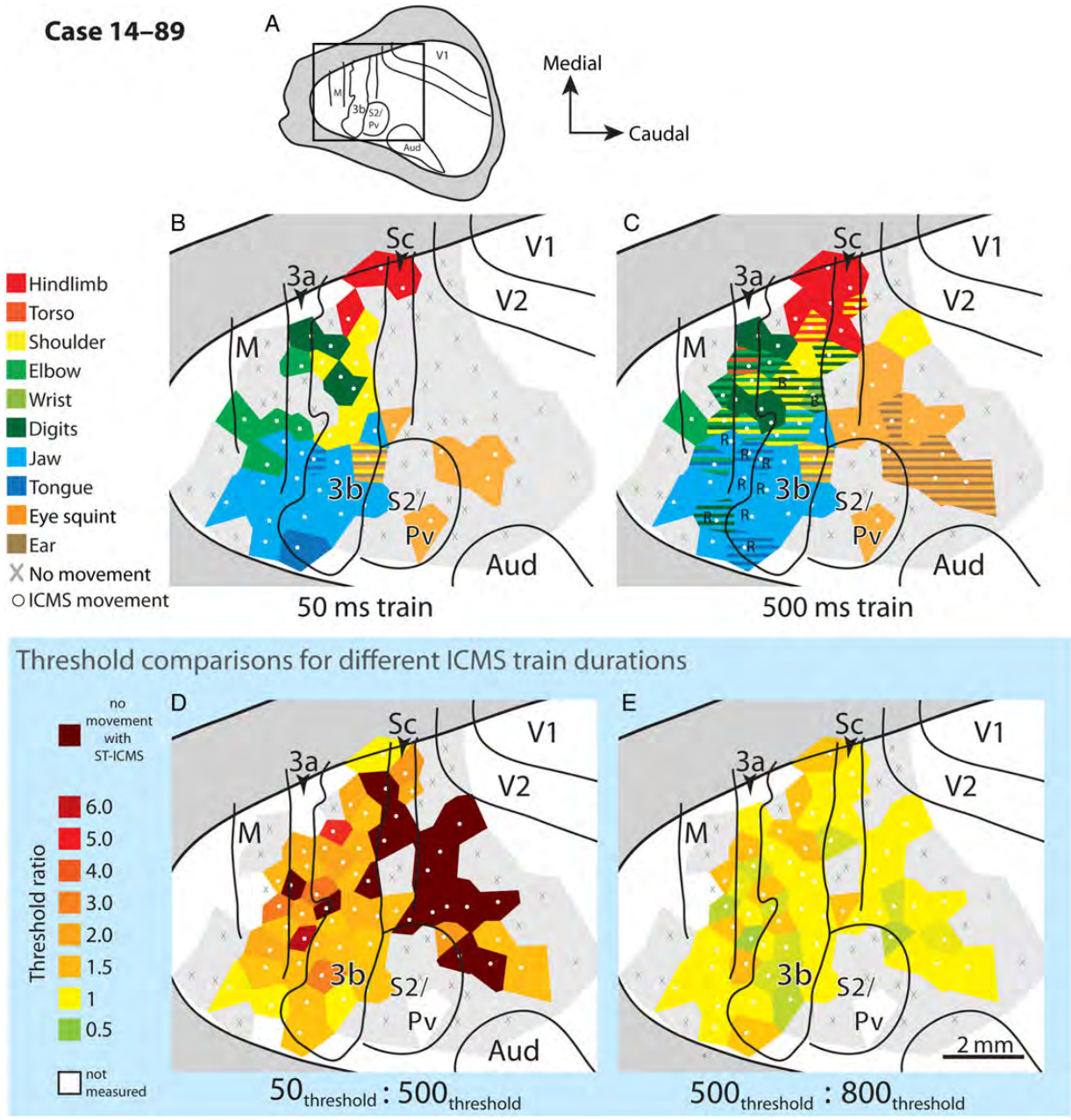

Figure 7. Short-train (ST, 50 ms) and long-train (LT, 500 ms) intracortical microstimulation (ICMS) maps of Case 14–89. (*A*) Flattened cortical hemisphere with anatomical borders indicated. (*B*) ST-ICMS motor map. (*C*) LT ICMS motor map. (*D*) and (*E*) Map of ratios in stimulation current thresholds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

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 toward the mouth (Fig. 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 μA, 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 et al. (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 multijoint forelimb movements and 35% (10/27) involved movements of the jaw and tongue together.

Motor cortex thresholds had a large range (25–475 μA; average of 190 μA). However, it is again important to note that all sites

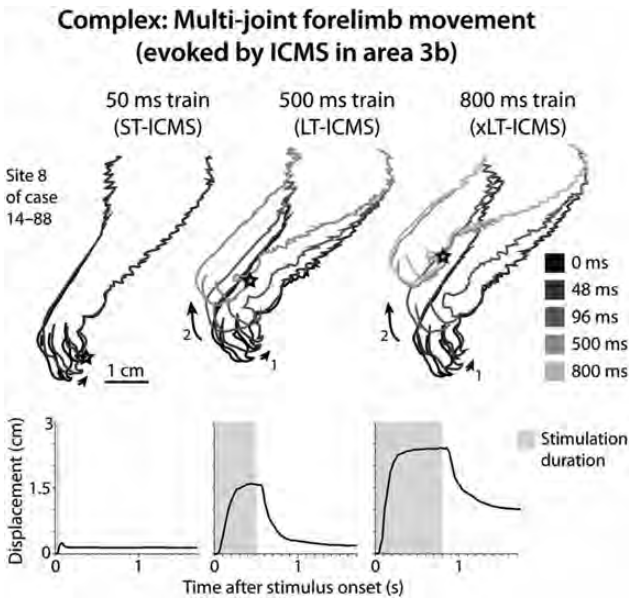

Figure 8. Example of ICMS-elicited movements using short-train (ST: 50 ms), long-train (LT: 500 ms), and extra-long-train (xLT: 800 ms) stimulation parameters. The black traces represent the baseline position of the forelimb. The trace with the darkest shade of gray represents the position of the hand 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 gray shading within the plot represents the period of time stimulation was presented.

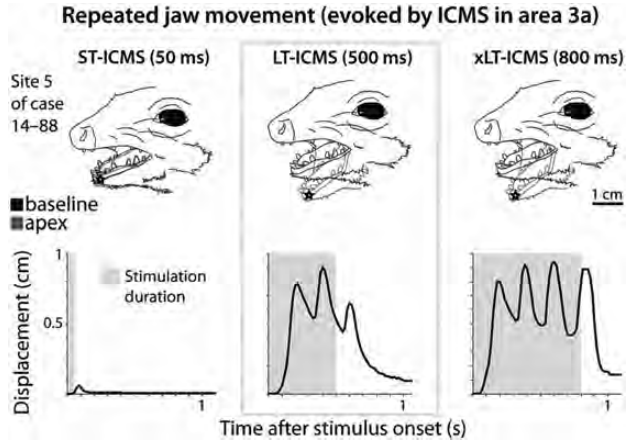

Figure 9. Example of an ICMS-elicited repeated movement using 3 different stimulation train durations at a single penetration site within area 3a of case 14–88 (Fig. 6). The top panel shows the lateral view of the tree shrew face at different points in time during stimulation. Stimulation caused the 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 gray shading within the plot represents the period of time stimulation was presented.

rostral to area 3a were included in our motor cortex analysis. The threshold values that were more caudal within this region (within 1 mm 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, that is, perhaps in Remple's M2) tended to be much higher (see Figs 11 and 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 and 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, and 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 μA. Similar to motor cortex, many of the complex movements elicited from responsive sites involved simultaneous hand and mouth movements (22%: 10/45 sites), and multijoint 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 μA, and the average threshold was 105 μA.

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, and 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, and 12). There were a number of bilateral movements involving the forelimbs, as well as repeated movements involving the jaw and tongue at the most rostrolateral aspect of are 3b.

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

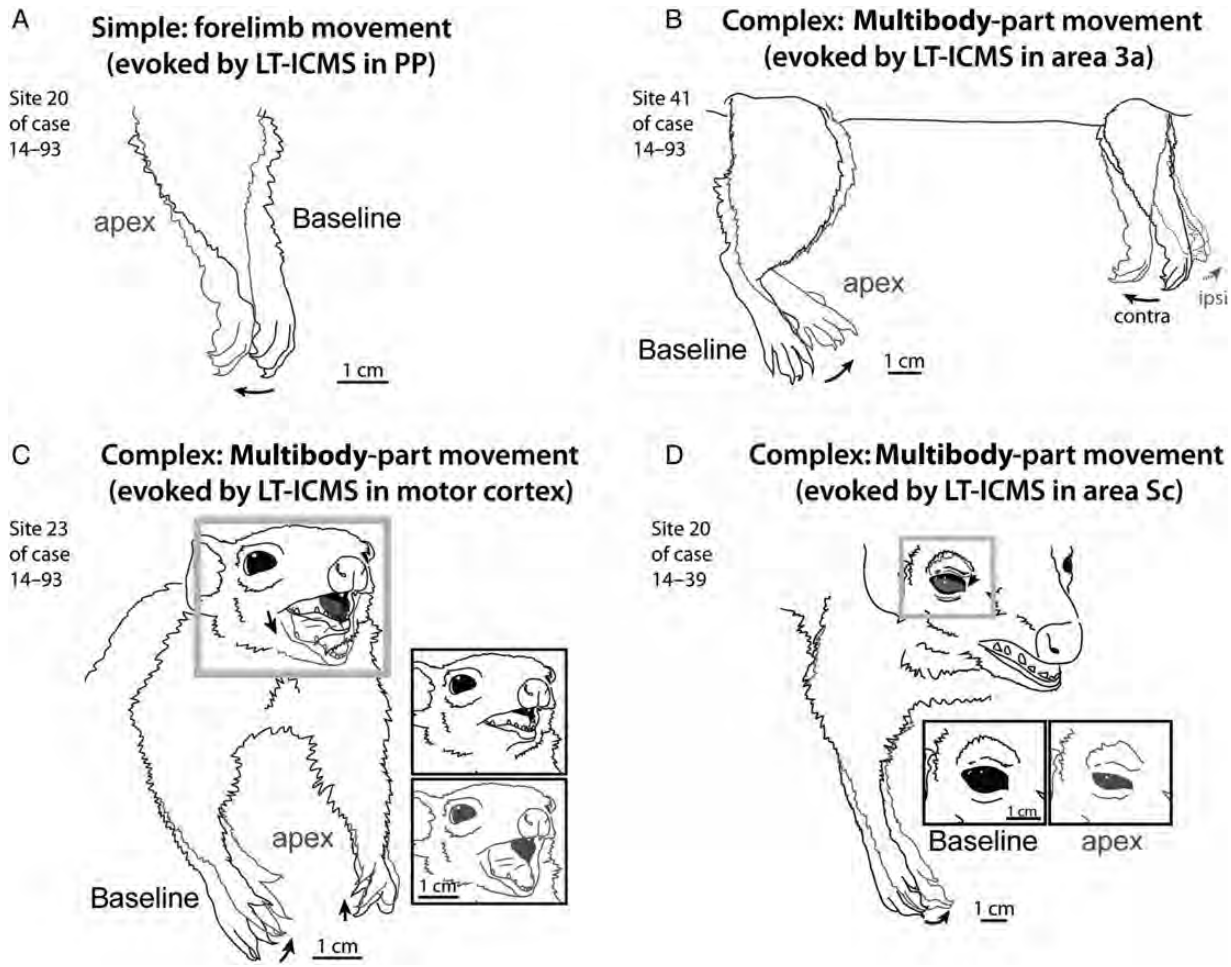

Figure 10. Examples of a simple and complex movements evoked from various cortical fields using long-train intracortical microstimulation. (*A*) An example of a simple movement involving the forelimb evoked during stimulation to a site within the posterior parietal cortex. The black trace represents 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 toward the mouth with elbow flexion, while the jaw opens. (*D*) Elbow flexion with eye squint evoked by LT-ICMS in Sc.

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 with 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 2. 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 2 being multijoint 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 (Figs 10D and 11A), hindlimb and shoulder movements (Fig. 7) or jaw and eye squint movements (Fig. 12).

The average threshold value for Sc was 220 μA, 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, and 12). This region likely included several subdivisions such as PPc, PPd, and PPv as described by Remple et al. (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 2 (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 μA with most sites requiring more than 200 μA of current to elicit a movement.

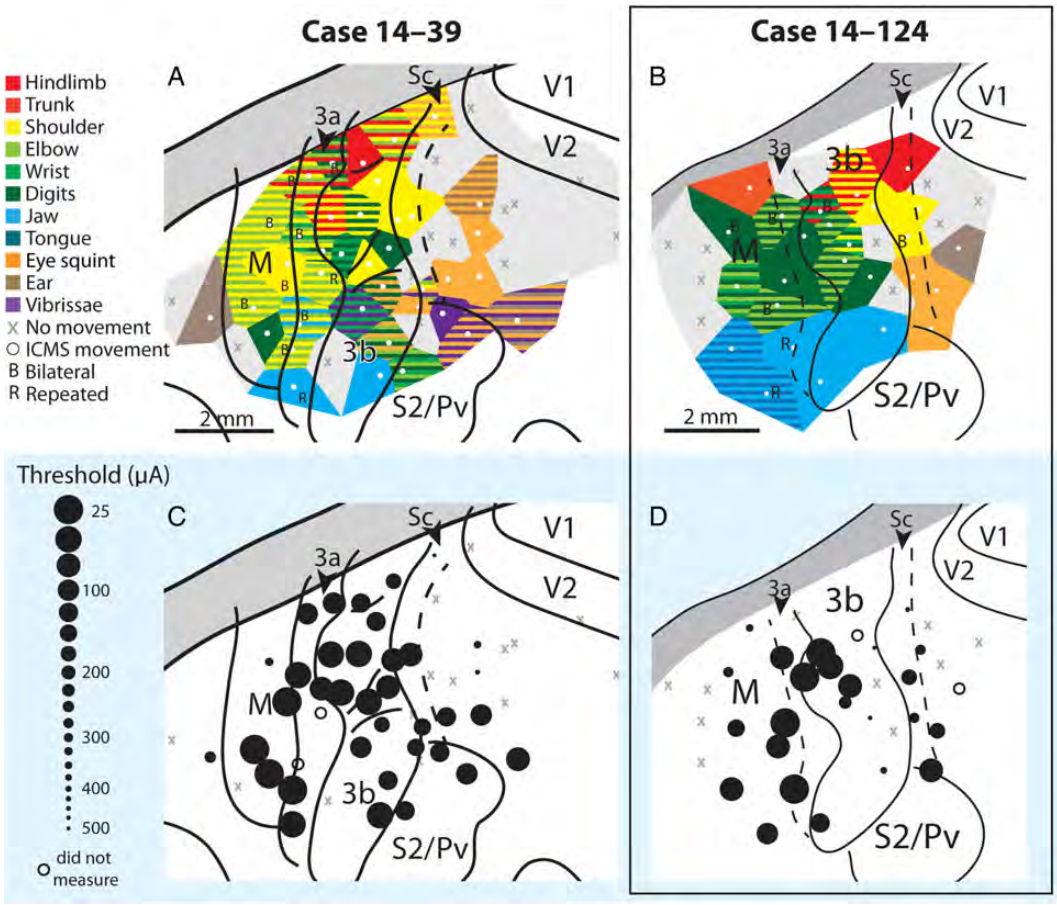

Figure 11. LT-ICMS maps of Case 14–39 (*A*) and Case 14–124 (*B*) with corresponding threshold maps in which larger black circles indicate sites where movements could be evoked with smaller currents (14–39: *C*), (14–124: *D*). Other conventions as in previous figures.

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), whereas 44% (8/18) were simple. Threshold values varied considerably, between 55 and 300 μA with an average threshold value of 159 μA. 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, and 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

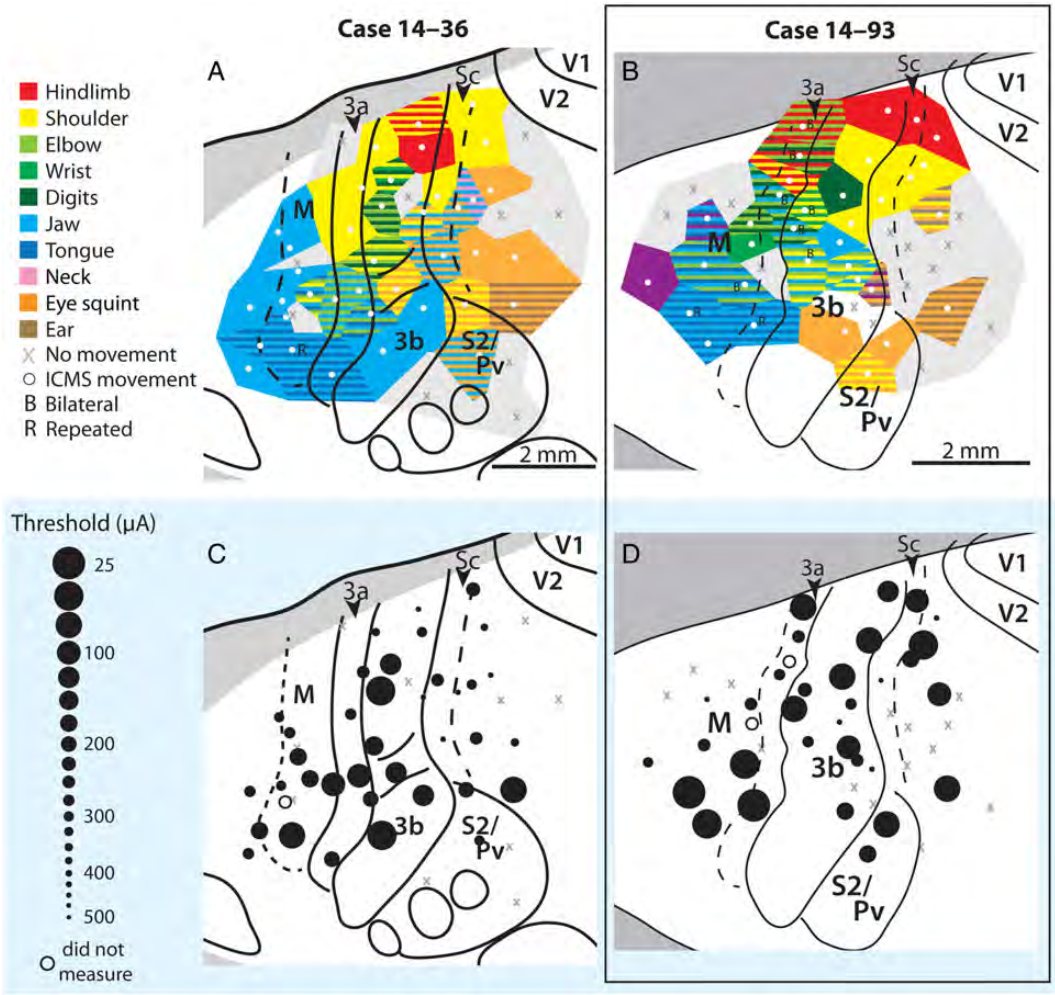

Figure 12. LT-ICMS maps for Cases 14–36 (*A*) and 14–93 (*B*) with corresponding threshold maps (14–36: *C*), (14–93: *D*). Other figure conventions are the same as those described in previous figures.

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, and 12). However, the movements elicited using ST-ICMS were truncated, simple versions of those movements elicited using LT- and xLT-ICMS (Figs 8 and 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 and 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 PPC, than the map generated using ST-ICMS parameters (Figs 6 and 7). This extent of "excitable" cortex is significantly larger than that reported in any other small brained mammal. Finally, complex, multijoint 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 et al. (2006, 2007) were able to differentiate 2 motor areas (M1 and M2) that were separate from somatosensory cortex using differences in threshold values in the different motor 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 2 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

Table 4 Average thresholds across areas and across cases (μA) with LT-ICMS

Case No.	Average threshold (μA) with LT-ICMS/brain area					
	M	3a	3b	Sc	PP	S2/Pv
14–14	n/a	89	107	78	155	n/a
		$n = 4$	$n = 11$	$n = 2$	$n = 5$	
14–20	nm	nm	nm	nm	nm	nm
14–34	205	75	157	n/a	232	n/a
	$n = 2$	$n = 2$	$n = 7$		$n = 2$	
14–36	212	202	192	308	293	250
	$n = 10$	$n = 5$	$n = 9$	$n = 3$	$n = 6$	$n = 2$
14–39	136	68	83	193	221	108
	$n = 5$	$n = 5$	$n = 12$	$n = 4$	$n = 5$	$n = 3$
14–49	138	53	83	85	253	163
	$n = 3$	$n = 3$	$n = 9$	$n = 2$	$n = 12$	$n = 2$
14–88	165	16	85	129	277	125
	$n = 4$	$n = 4$	$n = 5$	$n = 2$	$n = 9$	$n = 2$
14–89	219	111	154	279	364	215
	$n = 8$	$n = 8$	$n = 22$	$n = 6$	$n = 10$	$n = 2$
14–93	222	180	212	290	168	120
	$n = 7$	$n = 4$	$n = 12$	$n = 2$	$n = 5$	$n = 2$
14–124	134	65	237	241	225	n/a
	$n = 6$	$n = 1$	$n = 8$	$n = 4$	$n = 2$	
Average	190	106	148	220	261	159
	$N = 45$	$N = 36$	$N = 95$	$N = 25$	$N = 56$	$N = 13$

Note: nm indicates cases where the thresholds were not measured.

(Figs 11*C*,*D* and 12*C*,*D*), suggesting the presence of the 2 motor fields described by Remple et al. (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. 6*C*). The reason for this difference in the overall size of our maps compared with 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 et al. (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 3 studies, such as the size of the forelimb representation. For instance, though Remple et al. (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, and 12). We did not find any sites at which eye movements could be elicited, like Remple et al. (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; Sanderson et al. 1983; Gioanni and Lamarche 1985; 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 and Kaas 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; Gharbawie, Stepniewska, and Kaas 2011; Stepniewska et al. 2014), and Old World monkeys (Kwan et al. 1978; Sessle and Wiesendanger 1982; Graziano, Taylor, and Moore 2002; Graziano, Taylor, Moore et al. 2002; Graziano et al. 2005; Cooke et al. 2003; Gharbawie, Stepniewska, Qi, et al. 2011; Overduin et al. 2012). 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. Nonhuman 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 8 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, multijoint and multibody part movements have only been described within motor cortex (Li and Waters 1991; Ramanathan et al. 2006; Tandon et al. 2008; Tennant et al. 2011; Harrison et al. 2012; Bonazzi et al. 2013; Brown and Teskey 2014; Hira et al. 2015); however, the presence of evoked movements has not been well explored in posterior parietal cortex of rodents using LT-ICMS. The most studied movements within the rodent literature pertain to 2 multijoint 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 Gioanni 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 often includes movements of both the forelimb and mouth, lies between the region of motor cortex representing the forelimb and mouth

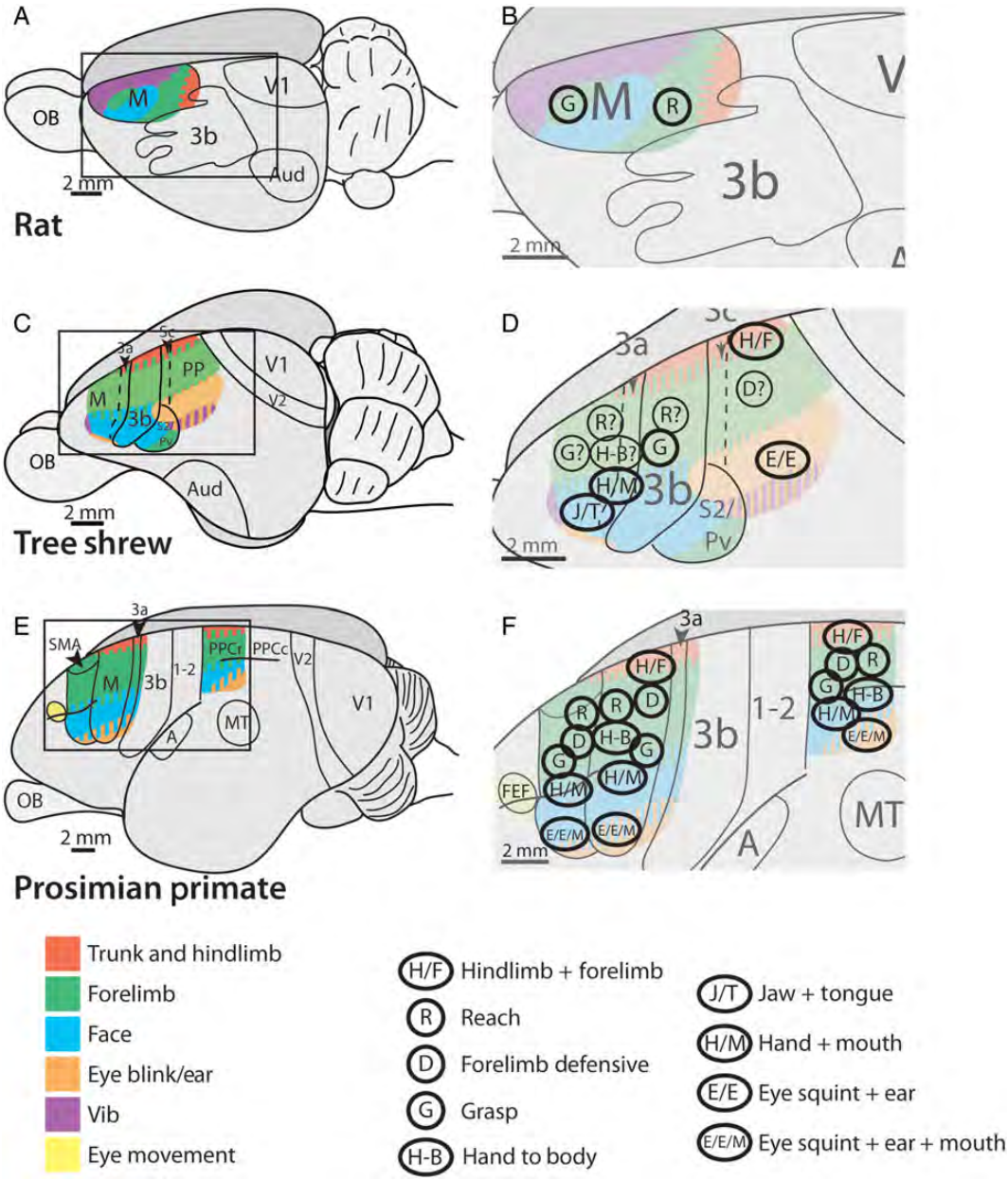

Figure 13. Comparisons of motor maps across different mammals within the Euarchontoglires clade including rats (*A*,*B*), tree shrews (*C*,*D*), and prosimian galagos (*E*,*F*). General color-coded motor maps across all cortical areas are presented on the left column (*A*,*C*,*E*), while the location of possible movement domains within cortical areas is presented to the right (*B*,*D*,*F*). (*C*,*D*), 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.

(Graziano, Taylor, and Moore 2002; Stepniewska et al. 2009), similar to our current results in tree shrews (Figs 6, 7, 11, and 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 6 stimulation sites per subject were evaluated.

The fact that such complex multijoint and multibody-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 8 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 homolog of the primate supplementary or premotor cortex, while CFA may be within the homolog 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, Taylor, and Moore 2002; Graziano et al. 2005; Gharbawie, Stepniewska, and Kaas 2011; Gharbawie, Stepniewska, Qi, et al. 2011; Stepniewska et al. 2011; 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. 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, and 12), but we did find possible movement domains associated with grasping within primary somatosensory cortex (Figs 8 and 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 Fritsch and Hitzig (1870) and Ferrier (1975), 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 and Boldrey 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) or that the resultant movements are an artifact of a stimulus driving muscle "length-tension" equilibrium (Van Acker et al. 2013, 2014; Griffin et al. 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, and 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 and 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 into 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 and Boldrey 1937), nonhuman primates (Welker et al. 1957), and a number of other eutherian mammals (Lende and Woolsey 1956; Saraiva and Magalhaes Castro 1975; Sanderson et al. 1983). 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 and Kaas 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 and Boldrey 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, was likely present in the ancestor of tree shrews, and primates.

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