

## The sensorimotor slice

Mary M. Rocco<sup>a</sup>, Joshua C. Brumberg<sup>a,b,\*</sup>

<sup>a</sup> *Neuropsychology Ph.D. Subprogram (Psychology), The Graduate Center, CUNY, 365 5th Avenue, New York, NY 10016, United States*

<sup>b</sup> *Department of Psychology, Queens College, CUNY, 65-30 Kissena Boulevard, Flushing, NY 11367, United States*

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### Abstract

The reciprocal connections between primary motor cortex (M1) and primary somatosensory cortex (S1) are hypothesized to play a role in an animal's ability to update its motor plan in response to changes in the sensory periphery. These interactions provide the sensory cortex with anticipatory knowledge of motor plans. In the mouse neocortex there are representations of the body surface within both M1 and S1. Utilizing physiologically targeted micro injections of biotinylated dextran amine into either the whisker representation of M1 (wM1) or S1 (wS1) we characterized the axonal pathways connecting these two areas. We then used this data to determine a plane of section that contained both whisker M1 and whisker S1 and maintained the axonal pathway between these two areas. *In vitro* physiological studies demonstrated that excitatory synaptic connections are maintained in this novel plane of section. The sensorimotor slice is an ideal preparation to study inter-areal cortical connectivity. © 2007 Elsevier B.V. All rights reserved.

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### 1. Introduction

Both sensory and motor cortices contain finely scaled topographic maps (Woolsey, 1952; Brooks et al., 1961; Li and Waters, 1991). These maps allow both primary motor cortex (M1) and primary somatosensory cortex (S1) to have highly specialized responses to changes in the periphery, allowing for precise execution of motor tasks. S1 and M1 are reciprocally connected yet the functional influence of these interactions remains unknown. In primates, S1 inactivation can result in disruption of chewing (Lin et al., 1993, 1998; Hiraba et al., 2000), fine motor coordination, sustained muscle contraction, appropriate grip force and the ability to determine the weight of an object (Rothwell et al., 1982; Johansson and Westling, 1984; Hikosaka et al., 1985; Brochier et al., 1999). These results suggest that motor control can strongly be influenced by S1 inputs. Understanding the pathway between S1 and M1 is an important first step towards comprehending how sensorimotor computations are preformed.

Despite the role that intercortical connections have in cognitive computations, research has traditionally focused on the response properties (*in vivo* studies) or the physiological properties (*in vitro* studies) of neurons found in specific cortical areas. Furthermore, little research has focused on interactions between cortical areas, especially those serving different functions (e.g. sensory and motor). The lack of a model preparation that contains interconnected cortical areas has been a hindrance to this line of research and the development of such a preparation is an important step towards understanding intercortical connectivity.

At present, it is unknown what pathways the axons that connect S1 and M1 traverse, or what their synaptic targets are. In the case of the rodent somatosensory, visual and auditory systems it is possible to cut a slice that contains both the thalamus and cortex with some degree of connectivity maintained (Agmon and Connors, 1991; Cruikshank et al., 2002, MacLean et al., 2006). Despite the fact that motor cortex and somatosensory cortex are adjacent to each other little work has been done to develop a slice which contains these two areas and their reciprocal connections. The advantage of a slice that contains S1 and M1 would be the ability to study the relationship between cortical feedforward and feedback networks in the same slice. A similar approach has been utilized to effectively study the interactions and the spread of epileptic activity between the hippocampus and adjacent cortical areas such as the entorhinal cortex (Breustedt et al., 2002) or the parahippocampal cortex (Bear and Lothman, 1993).

\* Corresponding author at: Department of Psychology, Queens College, CUNY, 65-30 Kissena Boulevard, Flushing, NY 11367, USA.

Tel.: +1 718 997 3541; fax: +1 718 997 3257.

E-mail address: joshua.brumberg@qc.cuny.edu (J.C. Brumberg).

In order to study the interconnectivity between whisker M1 and whisker S1 we have developed an *in vitro* preparation that contains the whisker representations of M1 and S1 and maintains their synaptic interconnectivity.

## 2. Materials and methods

All experiments were performed on white laboratory adult Swiss mice (CD-1, postnatal day 30–60) of either sex (Charles River Laboratories, Wilmington, MA). All experiments were performed in accordance with the Institutional Animal Care and Use Committee guidelines of Queens College, CUNY.

### 2.1. *In vivo* microstimulation and electrophysiology

*In vivo* experiments were done to physiologically identify the precise locations of the whisker representations of M1 (wM1) and barrel cortex (wS1) for both the anatomical and *in vitro* studies.

#### 2.1.1. Electrophysiological mapping and BDA injection

Adult CD-1 mice of either sex were anesthetized through i.p. injection of ketamine/xylazine (153 mg/kg/2.23 mg/kg) until they were unresponsive to a noxious stimulus (toe pinch). All recordings were performed while animals were fixed in place using a small animal stereotaxic apparatus (Kopf Instruments). An approximately 1 mm × 1 mm window was made in the skull to expose the cortex above the whisker representations of S1 and M1 using published stereotaxic coordinates (Franklin and Paxinos, 1997). To confirm the location of M1, a bipolar stimulating electrode (500 μm intertip distance, Frederick Haer Co., Brunswick, ME) was inserted perpendicular to the pial surface to a depth of approximately 300 μm into the cortex of the right hemisphere. Stimulating pulses were applied via a stimulus isolation unit (World Precision Instruments), controlled by a Master-8 (AMPI) beginning at low intensity (0.1 mA) and gradually increasing until isolated contralateral (left) whisker movement was observed. The location of whisker S1 (barrel cortex) was confirmed by inserting a tungsten microelectrode (3–4 MΩ, Frederick Haer Co.) perpendicular to the pial surface and manually driven to a depth of approximately 300 μm. Activity was amplified, digitized and recorded (A-M systems amplifier, Digidata analog-to-digital system (Axon Instruments), Axoscope version 8.0 (Axon Instruments)) while whiskers were being manually deflected contralateral to the recording location. Robust unambiguous responses to contralateral whisker deflection were used as confirmation of barrel cortex.

Following localization of either M1 or S1 appropriate tracers or dyes were injected into their confirmed locations via glass micropipettes. All injections were made in the right hemisphere. Glass micropipettes (O.D./I.D. in mm; 1.0/0.58) were fashioned on a Sutter micro-pipette puller (P-87, Sutter Instruments), and were filled with either biotinylated dextran amine (BDA; 2.5 g/ml in 0.01 M Phosphate Buffered Saline (PBS), Molecular Probes) or cresyl violet (2.5 g/1.5 ml, dissolved in acetate buffer). These substances were pressure injected (Toohey

Company) into the region of interest using several pulses of pressurized N<sub>2</sub> of duration of less than 1 ms. Animals were recovered and survived for at least two days to allow for adequate BDA transport.

#### 2.1.2. Histology

Mice were anesthetized through an i.p. injection of Nembutal (0.1 mg/100 g) and perfused with 0.9% saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB). Tissue was postfixed overnight. Sensorimotor (SM) sections containing identified wM1 and wS1 were cut parasagittally at a 45° angle relative to midline (Ted Pella Vibratome (vt 1000s, Leica; Fig. 1) at 350 μm in a 0.2 M phosphate buffer solution. As shown in Fig. 1, brains were hemisected and the medial edge was glued to an angled agar block. With the rostral end of the brain facing upward, the tissue was sliced at 350 μm.

The staining protocol was adapted from previously published protocols (Alloway et al., 2004; Brumberg et al., 2003; Pucak et al., 1996). Free floating 350 μm sections were rinsed (3 × 10 min) in 0.05 M cold PBS. Sections were then incubated twice (30 min each) in a blocking solution of 0.05 M PBS, 0.4% Triton-X (Sigma) and 0.5 mg/ml Bovine Serum Albumin (Fisher Scientific). The injected and transported BDA was revealed using the avidin–biotin–peroxidase complex method

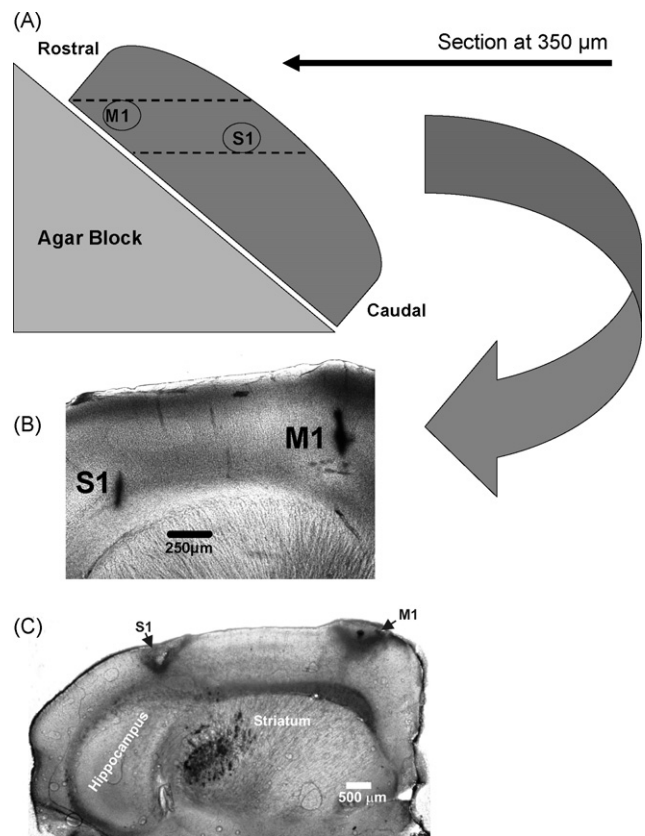


Fig. 1. The sensorimotor slice. (A) The position of the injected hemisphere in the vibratome when creating the sensorimotor slice. 350 μm sections are cut at a 45° angle relative to midline. (B) An example of the sensorimotor slice where cresyl violet was injected *in vivo* into whisker M1 and whisker S1 note both injection sites are recovered in the same plane of section. (C) A low magnification image of the sensorimotor slice with prominent subcortical areas labeled.

(ABC, Vector Laboratories) with 3,3'-diaminobenzidine (DAB; 5 mg/ml in 0.1 M PB Fluka; 0.5% H<sub>2</sub>O<sub>2</sub>) and nickel ammonium sulfate (NAS, 1.10 g/10 ml H<sub>2</sub>O) as the chromagen for the peroxidase reaction. Sections were incubated in the avidin–biotin solution overnight at 4 °C. Sections were then rinsed at room temperature in 0.1 MPB (4 × 10 min). Slices were incubated in DAB/NAS solutions (0.4 ml NAS/20 ml DAB) for 12 min exactly. Hydrogen peroxide was added dropwise until a black precipitate formed. Sections were immediately rinsed in 0.1 MPB (3 × 10 min), dehydrated, mounted and coverslipped using mounting media (Shurmount, Triangle Biomedical Sciences).

Mounted sections were viewed with an Olympus BX51 microscope using 4× (0.1 numerical aperture (NA)), 10× (0.4 NA) and 60× (1.4 NA, oil) objectives. Digital images were taken using an Optronics Microfire camera attached to a dedicated PC via firewire connection. Axons were reconstructed using the NeuroLucida program and analyzed using NeuroExplorer (MicroBrightfield). Axonal trajectories were computed by measuring their tortuosity which compares the actual path length of an axon in three-dimensions to its shortest possible path length; a value of 1.0 indicates a perfectly straight line.

## 2.2. *In vitro* experiments

Live sensorimotor slices were utilized to determine their functional synaptic connectivity and to anatomically confirm the presence of unsevered axons.

## 2.3. Stimulation and recording in sensorimotor slices

M1 and S1 whisker representations were marked *in vivo* with injection of non-toxic food coloring (see Section 2.1) to allow for subsequent identification of the whisker representations *in vitro*. Adult mice of either sex were anesthetized as above. The brain was quickly removed and sectioned in the SM plane in chilled, oxygenated artificial cerebral spinal fluid (ACSF) solution (in mM: 124 NaCl, 2.5 KCl, 2 MgSO<sub>4</sub>, 1.25 NaH<sub>2</sub>PO<sub>4</sub>, 1.2 CaCl<sub>2</sub>, 26 NaHCO<sub>3</sub>, 10 dextrose). Sections were transferred to an interface chamber where they were perfused with oxygenated ACSF and kept at 34 °C for 1 h before the start of the experiment. Bipolar stimulating electrodes similar to those used *in vivo* were placed in either S1 or M1 and a recording electrode was placed opposite to the location of the stimulating electrode (3–4 MΩ, Frederick Haer Co., Brunswick, ME). Recorded data were amplified 1000 fold and filtered at 100 Hz–5 kHz (A-M Systems Model 1700) and digitized using the Digidata system (Axon Instruments) and recorded with Axoscope 8.0 (Axon Instruments). Analysis was done using PClamp software (version 8.0, Axon Instruments).

For some experiments, 5 μl of an excitatory synaptic blocking solution of CNQX/APV (Sigma–Aldrich), dissolved in ACSF, was added directly to the slice after recording baseline field potentials. To inactivate S1 or M1 selectively we applied 1 mM Muscimol (Sigma–Aldrich) at the stimulation site which hyperpolarizes the somata without affecting fibers of passage (see

Hirsch, 1995). Stimulation was immediately applied following the application of the pharmacological agents and responses were recorded as stated above.

## 2.4. Axonal labeling in the sensorimotor slice

To confirm the presence of unsevered axons connecting wS1 and wM1 in the sensorimotor slice, crystals of biocytin were placed in the live 350 μm sensorimotor sections. Crystals were placed into either wM1 or wS1 via a 27-gauge needle that was pushed into the slice. Fiduciary marks were made in the gray matter lateral to the identified areas for later identification. Slices were held in an interface chamber for approximately 6–8 h to ensure transport of the biocytin between locations. Slices were carefully removed and post fixed overnight in a 4% paraformaldehyde solution. Slices were then processed, mounted and coverslipped as described above.

## 3. Results

We created an *in vitro* preparation that contains the whisker representations of both primary motor and primary sensory areas while also maintaining their synaptic interconnectivity. The precise locations of wM1 and wS1 were identified via microstimulation and electrophysiological methods, respectively. In our anatomical studies, both areas of interest were marked and the brain was subsequently hemisected and sectioned at a 45° angle relative to the midline. We found this angle of sectioning to optimally contain the whisker representations of M1 and S1. Further anatomical and electrophysiological studies indicate that axonal connectivity and functional connectivity is maintained in this novel slice preparation.

### 3.1. Connectivity in the sensorimotor slice

We initially characterized the pathway that axons took from wS1 to wM1 and vice versa in order to maximize our ability to maintain the axonal pathways and their synaptic targets in the sensorimotor slice. *In vivo* injections of rhodamine conjugated BDA into S1 and/or M1 revealed the axonal pathway that projects via the infragranular layers connecting these two areas (Fig. 2). The labeled axonal pathway extends the entire distance (~3 mm) between S1 and M1 and at higher magnification individual axons can be seen turning from their trajectory parallel to the white matter to a trajectory that is perpendicular to it when they reach their target area (Fig. 2B). Some sparse axonal labeling is present in the supragranular layers. These axons are not included in the analysis because the few that are observed all are transected and do not extend greater than a few hundred microns. These data suggest that wS1 axons which project to wM1 are preserved in our sensorimotor slice.

In order to quantify the connections between whisker M1 and S1, BDA was injected into either area and allowed to transport (Fig. 3). The projection site (e.g. M1 if BDA was injected into S1) was marked with an injection of cresyl violet just prior to perfusing the animal (Fig. 3C (M1), 3D (S1)). Transport across the entire length of the pathway was observed in all animals ( $n = 20$ )

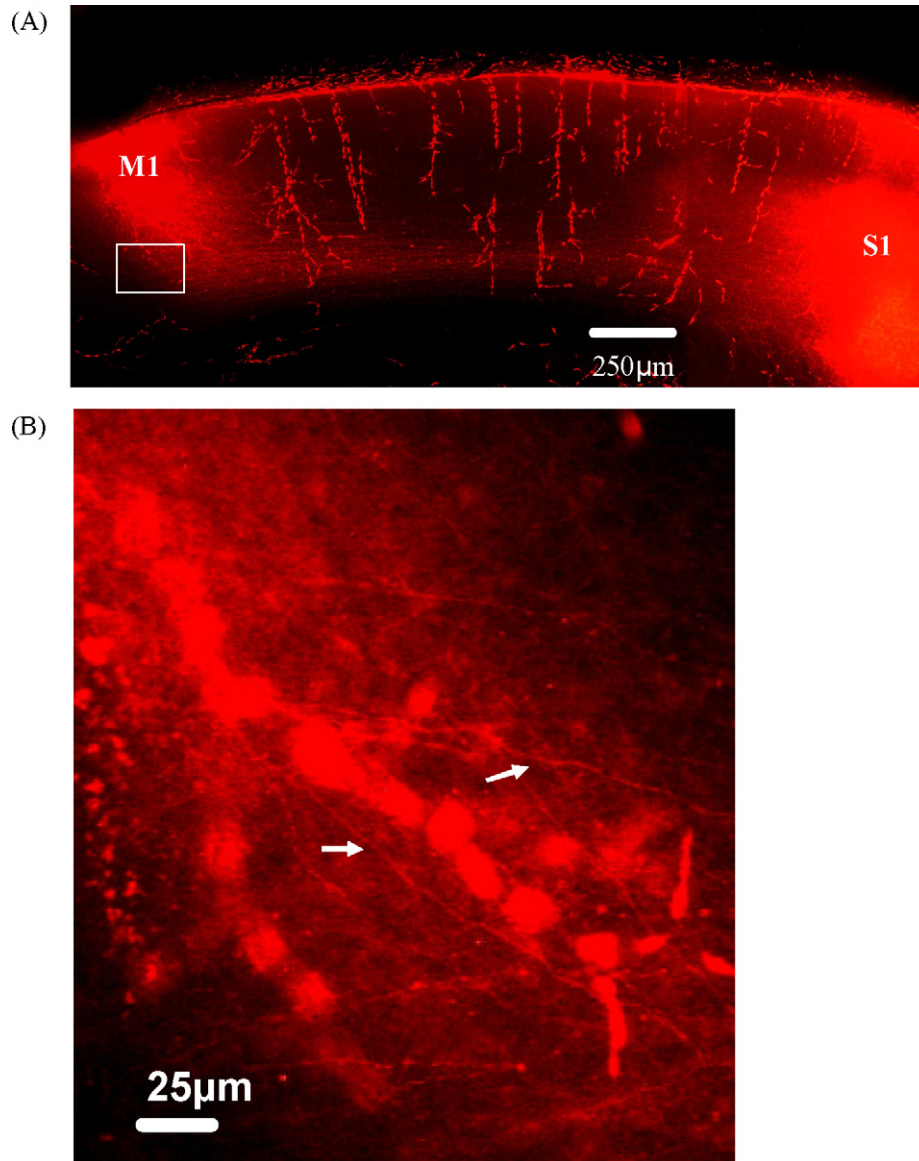


Fig. 2. Visualization of the axonal pathway of the sensorimotor slice. Anterograde axon labeling observed two days after an injection of fluoro-ruby conjugated biotinylated dextran amine in whisker S1. (A) The sensorimotor axon pathway extends horizontally between S1 and M1 and is restricted to deep cortical layers above the white matter (WM). (B) At higher magnification axons can be seen turning upward (white arrows) as they reach their target (M1).

regardless of injection location. Similar patterns of labeled axons were observed in the S1 injected (Fig. 3B and C) and M1 injected (Fig. 3D and E) animals. Labeled cells (Fig. 3A) were observed at the injection sites and at various locations throughout the pathway. Injections of large volumes of BDA into whisker S1 reveal the outlines of the layer IV barrels (see the unstained areas highlighted by asterisks in Fig. 3C) providing additional confirmation of the correct placement of our BDA injections. The lack of staining of the barrels confirms that neurons involved in the pathway from S1 to M1 do not originate from the barrel. Control experiments were done to determine the specificity of the connections observed in the sensorimotor slice. *In vivo* BDA injections were made into the forepaw representation of S1 and M1 and sectioned in the identical sensorimotor plane (see Section 2). Axons were not maintained for either the S1 forepaw

or M1 forepaw injected animals indicating that this pathway is topographically specified.

It is possible that axons connecting S1 and M1 may be transected during slice preparation and that the observed pathway may be an incomplete representation since the injections were made *in vivo* prior to slice preparation. To address this question injections of biocytin were made during separate *in vitro* experiments in either S1 or M1 in slices maintained in an interface style chamber. After six to eight hours the slices were fixed and reacted for biocytin. Results of these *in vitro* experiments (Fig. 4) revealed analogous patterns of connectivity to *in vivo* BDA experiments, indicating that a significant number of axons are preserved in our plane of section. Further analysis revealed that the axons labeled in the *in vivo* and *in vitro* experiments were found in the same depths of the cortical plate and when

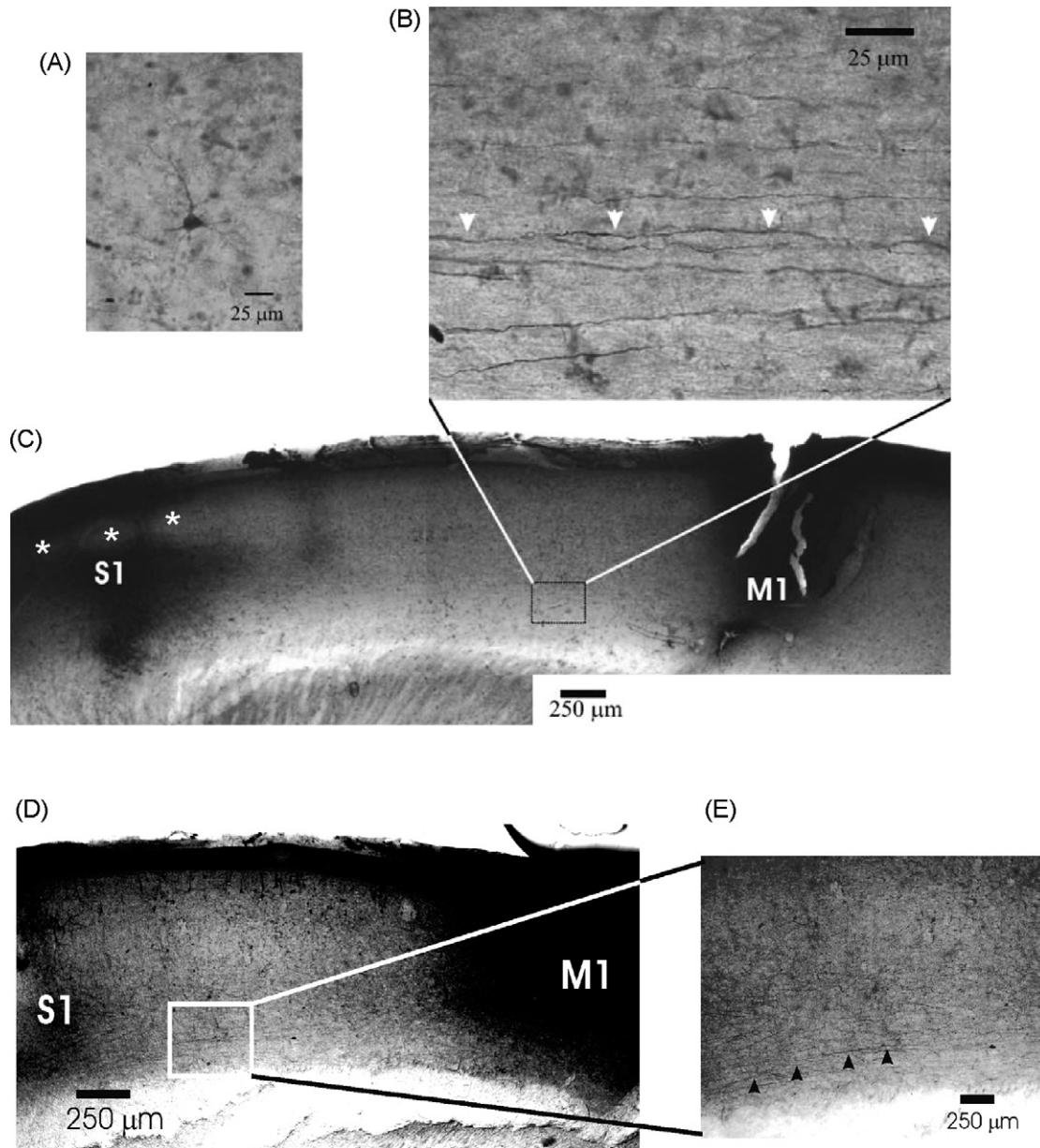


Fig. 3. Axonal labeling in the sensorimotor slice. Anterograde axon labeling observed two days after an injection of biotinylated dextran amine in whisker S1 (A, B, C) and whisker M1 (D, E). (A) Labeled neurons can be seen surrounding the injection site. (B) Axons originating in S1 course primarily through the deeper cortical layers (white arrows). (C) BDA labeled axons (black reaction product) in an S1 injected animal can be seen beginning in S1 and terminating in M1. Whisker M1 was marked *in vivo* with cresyl violet, asterisk denote barrel centers largely devoid of BDA staining. (D) BDA labeled axons in an M1 injected animal can be seen beginning in M1 and terminating in S1. Whisker S1 was marked *in vivo* with cresyl violet. (E) Axons beginning in M1 follow a course similar to those beginning in S1 (black arrows).

their morphological characteristics were compared there were no significant differences.

### 3.2. Quantitative characterization of sensorimotor axons

Previous studies in the visual cortex have shown variation in the diameters of the axons projecting in the feedforward (from primary visual cortex (V1) to higher order cortical sites) versus feedback (cortico–cortical connections back to V1) directions (Rockland and Virga, 1989). In order to determine if the axonal properties differed in the connection from S1 to M1 versus the reciprocal connection we quantified their diameters and

the directness of their pathway from origin (e.g. M1) to target (e.g. S1). Utilizing the computer assisted morphological system NeuroLucida we were able to quantify these morphological axon properties. Fig. 5 is representative of our data showing a reconstruction of the axons originating in S1 following a BDA injection. Both the BDA injection site (S1) and the marker injection site (M1) are delineated. The distance between the anterior border of S1 and the posterior border of M1 is approximately 3 mm. The reconstruction data show that while many of the axons that connect the two areas are preserved in this plane, a portion of the axons are transected during sectioning. Due to the number of axons labeled and the difficulty in reconstructing

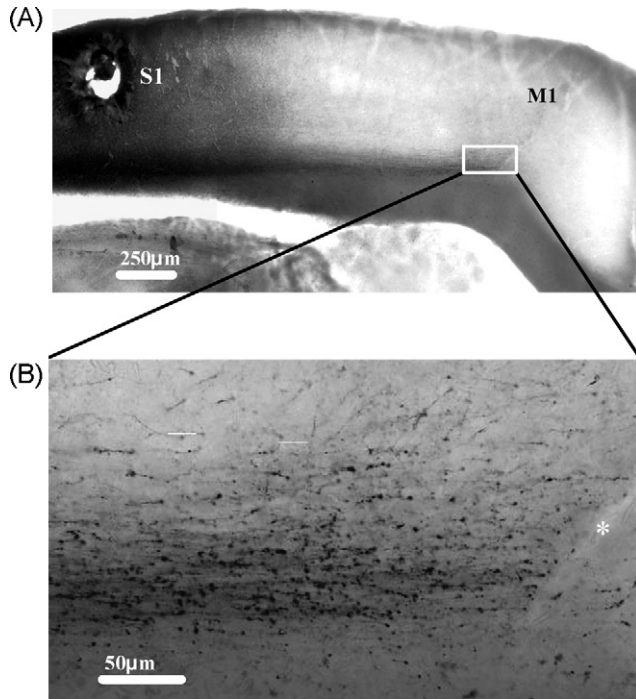


Fig. 4. Axonal connections are maintained post-slice preparation. Biocytin crystals placed in S1 (hole in tissue, panel A) after slice preparation label axons that travel from S1 to M1 and turn up into M1 (panel B, arrows).

them over several millimeters, we are not able to calculate what percentage of the connection is maintained.

We quantified two aspects of the axons that may influence their physiological functioning, their diameter and the directness of the path that they took from their origin to their target (e.g., S1–M1). Using a 60× (1.4 NA) objective the diameters of 52 S1 projecting axons and 38 M1 projecting axons were measured. Axons originating from M1 had an average diameter of  $0.61 \mu\text{m} \pm 0.23$  standard deviation (S.D.) whereas those originating in S1 were significantly smaller in diameter ( $t = 3.06$ ,  $p < .01$ ) with an average of  $0.48 \mu\text{m} \pm 0.12$  S.D. (Fig. 6A). Thus, the feedforward S1 to M1 pathway had smaller diameter axons than the feedback pathway from M1 to S1.

To assess the trajectory of the path that the two axonal populations took we computed their tortuosity (see Section 2.1). Tortuosity is a measure of “straightness”, a value of 1.0 indicates the shortest possible path between the origin and target of the axon. The average tortuosity (Fig. 6B) value for M1 orig-

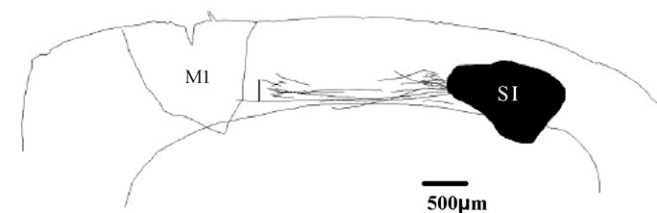


Fig. 5. A neuroLucida reconstruction of the sensorimotor slice. S1 was injected with BDA, M1 was injected with cresyl violet. Injection sites are traced as well as the pial surface and white matter border. Horizontal lines represent all visible anterograde labeled BDA axons that were traced in this section alone. Note that axons can be traced from injection site to projection site (arrow).

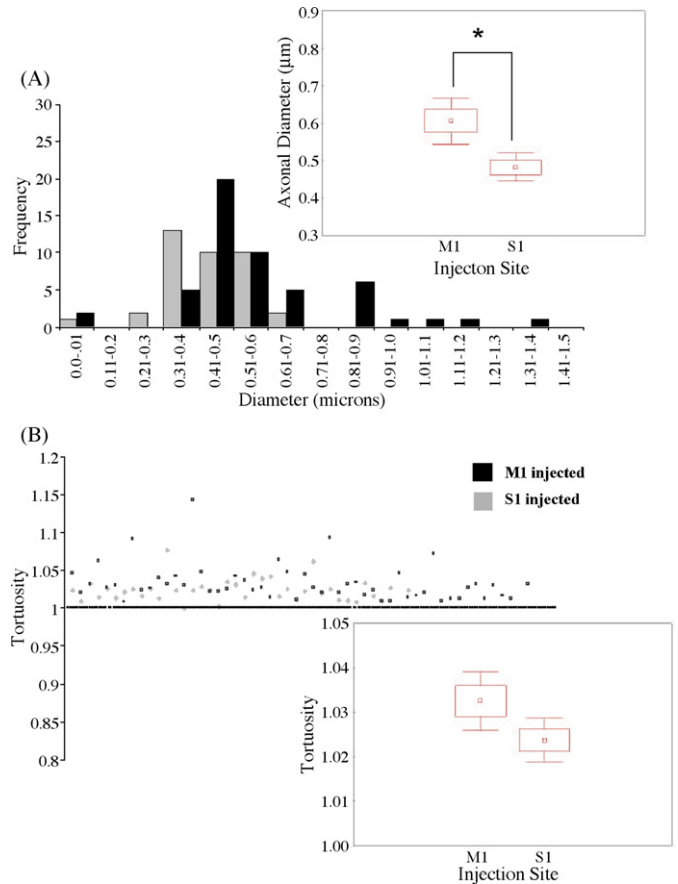


Fig. 6. Characterization of sensorimotor axons. (A) S1 projecting axons had a significantly smaller average diameter than M1 projecting axons ( $t = 3.06$ ,  $p < .01$ ). (B) Tortuosity measurements indicate that axons traveling in both directions travel in straight paths, the x-axis is arbitrary.

inating axons was  $1.03 \pm 0.02$  S.D. (range 1.00–1.14,  $n = 52$ ). The tortuosity values for S1 originating axons did not differ significantly from M1 injected animals with an average value of  $1.02 \pm 0.01$  S.D. (range 1.00–1.07,  $n = 38$ ). These results suggest that axons in the reciprocal sensorimotor pathway take the most direct route between their origin and target.

### 3.3. Functional connectivity is maintained in the sensorimotor slice

The existence of an anatomical connection between whisker M1 and S1 does not prove its functionality. Electrophysiological analysis was performed to determine if functional connections were maintained in the sensorimotor slice. Initially, wS1 and wM1 were identified *in vivo* (see *electrophysiological mapping* in the methods section) and non-toxic food coloring was injected into each location. Once slices were placed in the interface chamber, fiduciary marks were made slightly anterior to the M1 injection site and slightly posterior to the S1 injection site to allow for recognition of the two locations since the food coloring washed out in the interface chamber after several minutes. Additionally, subcortical markers such as the relative position of the hippocampus and striatum provide further identifiers for the sensorimotor slice (see Fig. 1C). To test for a functional

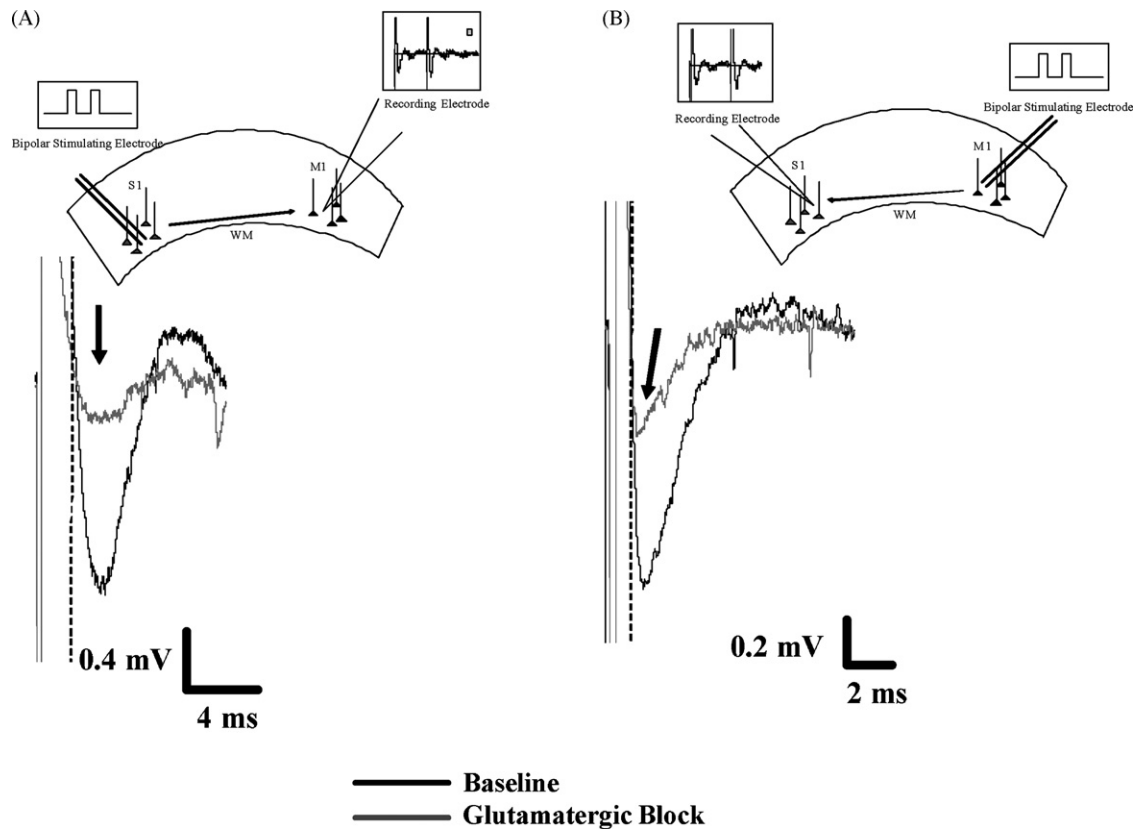


Fig. 7. Functional connectivity is maintained in the sensorimotor slice. Slices were stimulated with a negative current pulse of 200  $\mu$ s duration and 200–500  $\mu$ A of current and resulting field potentials were recorded in the reciprocal location. Application of the glutamatergic antagonists CNQX (40  $\mu$ M) and APV (50  $\mu$ M) lead to a 77% decrease of the field potential in M1 (A) and a 70% decrease in the evoked field potential in S1 (B) following stimulation in the reciprocal area. Arrows point to gray lines which represent field potentials recorded immediately following glutamatergic block. Cartoon drawings shown above depict location of stimulating and recording electrodes.

connection a bipolar stimulating electrode was placed in either identified wS1 or wM1 while a recording electrode was placed in the reciprocal area. We stimulated slices with a negative current pulse of 200  $\mu$ s duration and 200–500  $\mu$ A of current and resulting field potentials were recorded in the reciprocal location. In normal ACSF robust responses were seen in the reciprocal area follow stimulation (e.g. M1 after S1 stimulation, Fig. 7A black line). We next sought to determine if this connection was glutamatergic, to do so we locally applied the glutamatergic antagonists CNQX (40  $\mu$ M) and APV (50  $\mu$ M). CNQX/APV application led to a 70% decrease in the evoked field potential in S1 ( $n=3$ ) and a 77% decrease of the field potential in M1 ( $n=3$ ) following stimulation in the reciprocal area (Fig. 7). The diminution of the response following application of glutamatergic antagonists strongly suggests that some degree of synaptic connectivity is maintained in the SM slice and that the reciprocal connection is mediated by glutamate. In several cases the original amplitude of the field potential could be recovered following washout. These results coupled with the anatomical findings demonstrate the maintenance of synaptic connections between whisker S1 and whisker M1 in the sensorimotor slice. To test for the influence of antidromic activation we inhibited the stimulation site by hyperpolarizing the neurons in S1 or M1 with local application of 1 mM muscimol (GABA<sub>A</sub> agonist)

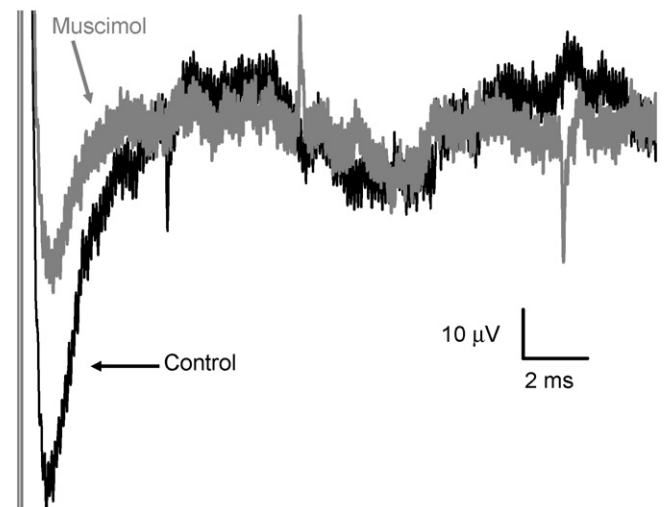


Fig. 8. Muscimol at stimulation site inhibits response at recording site. Application of 1 mM muscimol at the stimulation site in M1 significantly reduced the evoked response in S1. Gray trace response following Muscimol application M1, black trace control response.

and recorded in the reciprocal area. Muscimol application at the stimulation site significantly reduced the evoked field in the reciprocal area (see Fig. 8). Since muscimol should have no effect on axons and the resultant antidromic activity the reduction of the evoked field potential suggests that antidromic activation is being minimized in our preparation.

#### 4. Discussion

The results of the present study indicate that it is possible to create a slicing plane such that the primary motor and primary somatosensory areas are contained within one slice. Furthermore, we demonstrate that the reciprocal synaptic connectivity between these two areas is maintained within this novel plane of section. The sensorimotor slice preparation will be a useful tool for studying sensorimotor integration in specific and inter-areal cortical communication in general.

##### 4.1. Methodological considerations

One limitation of the present study is that we do not know what extent of the whisker maps in M1 and S1 are contained in the sensorimotor slice. At worst our results underestimate the strength of connectivity between the two areas since some axons are severed due to the cutting process. Our *in vitro* biocytin experiments reveal that at least some percentage of the axon pathway that is observed following *in vivo* BDA injection is maintained and the physiological results demonstrate their functional abilities. Another consideration is the specificity of the pathway that we are observing. Injections of BDA were made *in vivo* into the forepaw representation of S1 and M1, which lies adjacent to the whisker representation in both regions. When sectioned in the sensorimotor plane only sparse labeling of axons was observed and these axons were distributed evenly throughout the cortical plate unlike when injections were made into the whisker representations. Furthermore, muscimol inactivation of either S1 or M1 resulted in the significant diminution of the resultant field in the reciprocal area. This suggests that our stimulation procedures are effective in stimulating specifically S1 projecting neurons originating in M1 or M1 projecting neurons that originate from S1. In the corticothalamic slice it has been shown that thalamocortical axons have lower thresholds of activation than corticothalamic axons due to their different diameters, we have found that the axons project from M1 to S1 versus S1 to M1 have different diameters, suggesting that they too may have different thresholds (Hirsch, 1995; Swadlow, 1989). These results strongly suggest that the axonal labeling that we observe in our sensorimotor slice is specific to the reciprocal connections between the motor and sensory whisker representations.

##### 4.2. Identification and characterization of sensorimotor connections

A common goal of many studies investigating cortical connectivity is to understand how information is integrated. Through axonal labeling we have determined that the axonal pathway between S1 and M1 is reciprocal and it is localized to cortical

layers V and VI. These layers are the primary source of descending output pathways to areas such as the thalamus, dorsal column nuclei and spinal cord (Hoffer et al., 2005). It is likely that information that is being sent between M1 and S1 through these output layers is being modulated by these other pathways allowing for gating of sensory input and modulation of motor output (Chapman et al., 1988). These anatomical findings are in agreement with previous studies showing activation in layers V and VI in S1 following arm movements in primates (Prud'homme and Kalaska, 1994).

Previous studies in primates have identified two types of hierarchical cortical processing; feedforward and feedback. Feedforward pathways are those that receive information directly from peripheral inputs (e.g. thalamus onto primary sensory cortices before being relayed to higher cortical levels). In contrast feedback pathways involve higher cortical and subcortical areas impacting primary sensory cortices (Lund et al., 1975; Rockland and Pandya, 1979; Maunsell and Van Essen, 1983; Kennedy and Bullier, 1985; Barone et al., 2000; Falchier et al., 2002). Characterization of these pathways has shown that they can be differentiated by their anatomical and physiological properties (Rockland and Virga, 1989, 1990; Shao and Burkhalter, 1996; Johnson and Burkhalter, 1997). Our results indicate that the axonal diameters of M1 projecting axons (feedforward) are significantly smaller than that of S1 projecting axons (feedback). For both feedback and feedforward pathways the large axon diameter and the directness of their paths suggest that the information between S1 and M1 is being rapidly transmitted. The strong reciprocal connection between M1 and S1 and the anatomical properties of the axonal pathway allow for efficacious and rapid communication which may be necessary for a rodent to alter its whisking strategy as a result of contacting an object in its sensory environment.

##### 4.3. *In vitro* studies utilizing the SM slice

Many studies have utilized *in vivo* techniques to learn more about sensorimotor processing (Smith et al., 1983; Wannier et al., 1991). Though these studies have contributed greatly to our knowledge of this pathway, they do not allow for the direct investigation of how information is processed along the sensorimotor pathway. Through the creation of our novel slice preparation these connections can now be studied for the first time *in vitro*. Similar to how the thalamocortical slice enabled the detailed study of thalamic inputs to the cortex for the first time *in vitro* (e.g. Agmon and Connors, 1991), the sensorimotor slice will, for the first time, allow for similar studies of sensorimotor integration.

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## References

- Agmon A, Connors BW. Thalamocortical responses of mouse somatosensory (barrel) cortex in vitro. *Neuroscience* 1991;41(2–3):365–79.
- Alloway KD, Zhang M, Chakrabarti S. Septal columns in rodent barrel cortex: functional circuits for modulating whisking behavior. *J Comp Neurol* 2004;480(3):299–309.
- Barone P, Batardiere A, Knoblauch K, Kennedy H. Laminar distribution of neurons in extrastriate areas projecting to visual areas V1 and V4 correlates with the hierarchical rank and indicates the operation of a distance rule. *J Neurosci* 2000;20(9):3263–81.
- Bear J, Lothman EW. An in vitro study of focal epileptogenesis in combined hippocampal-parahippocampal slices. *Epilepsy Res* 1993;14(3):183–93.
- Breustedt J, Gloveli T, Heinemann U. Far field effects of seizure like events induced by application of 4-AP in combined entorhinal cortex hippocampal slices. *Brain Res* 2002;956(1):173–7.
- Brochier T, Boudreau MJ, Pare M, Smith AM. The effects of muscimol inactivation of small regions of motor and somatosensory cortex on independent finger movements and force control in the precision grip. *Exp Brain Res* 1999;128(1–2):31–40.
- Brooks VB, Rudomin P, Slayman CL. Peripheral receptive fields of neurons in the cat's cerebral cortex. *J Neurophysiol* 1961;24:302–25.
- Brumberg JC, Hamzei-Sichani F, Yuste R. Morphological and physiological characterization of layer VI corticofugal neurons of mouse primary visual cortex. *J Neurophysiol* 2003;89(5):2854–67.
- Chapman CE, Jiang W, Lamarre Y. Modulation of lemniscal input during conditioned arm movements in the monkey. *Exp Brain Res* 1988;72(2):316–34.
- Cruikshank SJ, Rose HJ, Metherate R. Auditory thalamocortical synaptic transmission in vitro. *J Neurophysiol* 2002;87(1):361–84.
- Falchier A, Clavagnier S, Barone P, Kennedy H. Anatomical evidence of multimodal integration in primate striate cortex. *J Neurosci* 2002;22(13):5749–59.
- Franklin KBJ, Paxinos G. *The mouse brain in stereotaxic coordinates*. San Diego: Academic Press; 1997.
- Hikosaka O, Tanaka M, Sakamoto M, Iwamura Y. Deficits in manipulative behaviors induced by local injections of muscimol in the first somatosensory cortex of the conscious monkey. *Brain Res* 1985;325(1–2):375–80.
- Hiraba H, Yamaguchi Y, Satoh H, Ishibashi Y, Iwamura Y. Deficits of masticatory movements caused by lesions in the orofacial somatosensory cortex of the awake cat. *Somatosens Mot Res* 2000;17(4):361–72.
- Hirsch JA. Synaptic integration in layer IV of the ferret striate cortex. *J Physiol* 1995;483:183–99.
- Hoffer ZS, Arantes HB, Roth RL, Alloway KD. Functional circuits mediating sensorimotor integration: quantitative comparisons of projections from rodent barrel cortex to primary motor cortex, neostriatum, superior colliculus, and the pons. *J Comp Neurol* 2005;488(1):82–100.
- Johnson RR, Burkhalter A. A polysynaptic feedback circuit in rat visual cortex. *J Neurosci* 1997;17(18):7129–40.
- Johansson RS, Westling G. Roles of glabrous skin receptors and sensorimotor memory in automatic control of precision grip when lifting rougher or more slippery objects. *Exp Brain Res* 1984;56(3):550–64.
- Kennedy H, Bullier J. A double-labeling investigation of the afferent connectivity to cortical areas V1 and V2 of the macaque monkey. *J Neurosci* 1985;5(10):2815–30.
- Li CX, Waters RS. Organization of the mouse motor cortex studied by retrograde tracing and intracortical microstimulation (ICMS) mapping. *Can J Neurol Sci* 1991;18(1):28–38.
- Lin LD, Murray GM, Sessle BJ. The effect of bilateral cold block of the primate face primary somatosensory cortex on trained tongue protrusion task and biting tasks. *J Neurophysiol* 1993;70(3):985–96.
- Lund JS, Lund RD, Hendrickson AE, Bunt AH, Fuchs AF. The origin of efferent pathways from the primary visual cortex, area 17, of the macaque monkey as shown by retrograde transport of horseradish peroxidase. *J Comp Neurol* 1975;164(3):287–303.
- MacLean JN, Fenstermaker V, Watson BO, Yuste R. A visual thalamocortical slice. *Nat Methods* 2006;3:129–34.
- Maunsell JH, Van Essen DC. Functional properties of neurons in middle temporal visual area of the macaque monkey. I. Selectivity for stimulus direction, speed, and orientation. *J Neurophysiol* 1983;49(5):1127–47.
- Prud'homme MJ, Kalaska JF. Proprioceptive activity in primate primary somatosensory cortex during active arm reaching movements. *J Neurophysiol* 1994;72(5):2280–301.
- Pucak ML, Levitt JB, Lund JS, Lewis DA. Patterns of intrinsic and associational circuitry in monkey prefrontal cortex. *J Comp Neurol* 1996;376(4):614–30.
- Rockland KS, Pandya DN. Laminar origins and terminations of cortical connections of the occipital lobe in the rhesus monkey. *Brain Res* 1979;179(1):3–20.
- Rockland KS, Virga A. Terminal arbors of individual “feedback” axons projecting from area V2 to V1 in the macaque monkey: a study using immunohistochemistry of anterogradely transported *Phaseolus vulgaris*-leucoagglutinin. *J Comp Neurol* 1989;285(1):54–72.
- Rockland KS, Virga A. Organization of individual cortical axons projecting from area V1 (area 17) to V2 (area 18) in the macaque monkey. *Vis Neurosci* 1990;4(1):11–28.
- Rothwell JC, Traub MM, Day BL, Obeso JA, Thomas PK, Marsden CD. Manual motor performance in a deafferented man. *Brain* 1982;105(Pt 3):515–42.
- Shao Z, Burkhalter A. Different balance of excitation and inhibition in forward and feedback circuits of rat visual cortex. *J Neurosci* 1996;16(22):7353–65.
- Smith AM, Frysinger RC, Bourbonnais D. Interaction between motor commands and somatosensory afferents in the control of prehension. *Adv Neurol* 1983;39:373–85.
- Swadlow HA. Efferent neurons and suspected interneurons in S-1 vibrissa cortex of the awake rabbit: receptive fields and axonal properties. *J Neurophysiol* 1989;62:288–308.
- Wannier TM, Maier MA, Hepp-Reymond MC. Contrasting properties of monkey somatosensory and motor cortex neurons activated during the control of force in precision grip. *J Neurophysiol* 1991;65(3):572–89.
- Woolsey CN. Patterns of localization in sensory and motor areas of the cerebral cortex. In: *Milbank memorial fund: the biology of mental health and disease*. New York: Hoeber; 1952, 192–206.