MOTOR CORTEX REGULATION OF THALAMIC-CORTICAL ACTIVITY IN THE SOMATOSENSORY SYSTEM

by

SooHyun Lee

BS, Seoul Women's University, 1997
MS, Seoul Women's University, 1999

Submitted to the Graduate Faculty of

The School of Medicine in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

University of Pittsburgh

2007

UNIVERSITY OF PITTSBURGH

School of Medicine

This thesis "Motor cortex regulation of thalamic-cortical activity in the somatosensory system" was presented

by

SooHyun Lee

It was defended on

October 03, 2007

and approved by

George E. Carvell, Ph.D., P.T., Department of Physical Therapy

Neeraj J. Gandhi, Ph.D., Department of Otolaryngology

Karl Kandler, Ph.D., Department of Otolaryngology

Asaf Keller, Ph.D., Anatomy and Neurobiology, University of Maryland

Peter L. Strick, Ph.D., Department of Neurobiology

Thesis Advisor: Daniel J. Simons, Ph.D., Department of Neurobiology

MOTOR CORTEX REGULATION OF THALAMIC-CORTICAL ACTIVITY IN THE SOMATOSENSORY SYSTEM

SooHyun Lee, M.S.

University of Pittsburgh, 2007

A prominent feature of thalamocortical circuitry in sensory systems is the extensive and highly organized feedback projection from the cortex to thalamic neurons that provide input to it. Intriguingly, many corticothalamic (CT) neurons are weakly responsive to peripheral stimuli, or silent altogether. Here using the whisker-to-barrel system, we examine whether the responses of CT neurons and their related thalamic neurons are affected by motor cortex, a prominent source of input to deep layers of the somatosensory cortex. Pharmacological facilitation of motor cortex activity produced using focal, microiontophoresis leads to enhanced whisker-evoked firing of topographically aligned layer 6 neurons, including identified CT cells, and of cells in corresponding regions of the thalamic ventral posterior medial nucleus (VPm barreloids). Together, the findings raise the possibility that cortico-thalamo-cortical circuitry in primary sensory areas is engaged by other functionally related cortical centers, providing a means for context-dependent regulation of information processing within thalamocortical circuits.

We investigated how vMCx influence activity in thalamic VPm nucleus in a freely behaving rat. We examine afferent-evoked thalamic activity in animals that are either alert but voluntarily relatively motionless or actively whisking in the air without object contact. Afferent activity is evoked in VPm by means of electrical microstimulation of a single whisker follicle. In some experiments, neural processing in brainstem trigeminal nuclei was either by-passed by means of medial lemniscus stimulation, or altered by pharmacological intervention. We found

that sensory responses during voluntary whisker movements, when motor cortex is likely to be active, are reduced relative to responses that occur during periods of wakeful quiescence. Enhancement of thalamic activity during whisking can be observed, however, when signal processing in sub-thalamic centers is either by-passed or experimentally altered. Findings suggest that during voluntary movement activity within the lemniscal system is globally diminished, perhaps at early, brainstem levels at the same time that activity within specific thalamocortical neuronal populations is facilitated. Though activity levels are reduced system-wide, activity within some local circuits may be subject to less net suppression. This decrease in suppression may occur on a moment-to-moment basis in a context-dependent manner. Thus, during voluntary whisker movement, sensory transmission in thalamocortical circuits may be modulated according to specific activation patterns distributed across the motor map.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PREFACE

First and foremost, I am grateful to my advisor, Dr. Simons, for his insights as a great scientist and endless support as a mentor. I thank him for teaching me how to do science and more importantly how to do it well. To Dr. George Carvell, I thank him for his scientific advice, patience and enthusiasm. I thank to Dr. Karl Kandler for his attention and encouragement. I also want to acknowledge the other members of my thesis committee, Dr. Neeraj J. Gandhi, Dr. Asaf Keller, Dr. Peter Strick and the late Dr. Peter Land for their guidance and support. I thank my rotation committee, Dr. Steve Meriney, Dr. Paula Monaghan, and Dr. Carl Olson for my exciting experience on rotation projects in their laboratories. I thank to my lab members – Ernest E. Kwegyir-Afful, Vivek Khatri, Tom Prigg, Mish Shoykhet, Simona Temereanca, Randy Bruno and Brad Minnery – for making my life in graduate school more pleasant. I thank Dr. Harold Kyriazi for his technical assistance, attention and support. I thank Patti Argenzio, Joan Blaney and other members of the departmental staff for their excellent assistance.

I thank my friends, Hanmi Lee and Soyun Cho who have made these years enjoyable. I thank my parents, MyungShin Lee and HeaSoon Park, my sisters, SungA, HwaJung and Yunjun, and my aunt, EunSoon Park for always being there and always believing. I am also grateful to my Merriam family, Dena and Arnold Merriam for their support and encouragement. I thank my husband, Eli Merriam for his constant love and faith in me.

1.0 INTRODUCTION

Mammals gain critical sensory information by actively exploring the world. The tight behavioral interdependency between sensory processing and motor output postulates structural and functional integration between external (sensory) and internal (motor) signals. Here we address one aspect of this issue using the rat whisker somatomotor system.

Rats explore their environment by actively moving their whiskers to palpate nearby objects. Anatomical studies show that somatosensory (S1) and vibrissal (whisker) motor areas (vMCx) of the cerebral cortex are interconnected (Zhang and Deshenes, 1998; Alloway et al., 2004). Despite the anatomical evidence of reciprocal projections between whisker-related motor and sensory areas, the functional relationship is not well understood. Interestingly, in infragranular layers of whisker-barrel cortex, which receives major afferent inputs from vMCx, a substantial proportion of neurons project corticothalamic (CT) feedback to thalamic neurons (Erisir et al., 1997; Jones and Powell, 1969; Chmielowska et al., 1989). Previous studies have provided evidence that CT feedback modulates excitability of thalamic neurons with a stimulus-dependent and topographic-specific manner (reviewed in Sillito and Jones, 2002; reviewed in Alitto and Usrey, 2003). CT projections can thus enable the cerebral cortex to regulate dynamically its own inputs from the somatosensory thalamus. Despite the potentially important function of CT feedback, many if not most CT neurons are weakly responsive to whisker stimulation and even some of them appear to be entirely silent. We hypothesize that descending

motor commands from vMCx regulate whisker-related processing of afferent somatosensory signals in the thalamic ventral posterior medial (VPm) nucleus via S1 corticothalamic projections.

1.1 WHISKER-TO-BARREL SYSTEM

The extensive whisker representation in rodent primary somatosensory cortex attests to the critical importance of whiskers as somatosensory organs. Individual whiskers are represented in the granular layers of S1 by discrete, columnar shaped neuronal assemblies termed "barrels" (Woolsey and Van der Loos, 1970; Welker 1971). Each barrel corresponds both anatomically and physiologically in a one-to-one fashion to a single whisker, referred to as that barrel's "principal whisker" (PW). A barrel receives afferent input from its corresponding "barreloid" in the VPm (Van der Loos, 1976; Bernardo and Woolsey, 1987; Land et al., 1995). Barreloids in turn receives input from corresponding "barrelettes" in the nucleus principalis (PrV) of the brainstem trigeminal complex (Belford and Killckey, 1979; Durham and Woolsey, 1984; Ma and Woolsey, 1984; Sikich et al., 1986; Ma 1991). A majority (70-90%), but not all, of PrV neurons project to thalamic VPm (Jacquin et al., 1988; Minnery and Simons, 2003; Veinante and Deschenes, 1999). A small population of PrV neurons, which have large somata, projects to the superior colliculus and other areas (Bruce et al., 1987; Veinante and Deschenes, 1999). PrV receives a dense innervation of low-threshold mechanoreceptor input from the trigeminal ganglion (Gibson and Welker, 1983a, b; Lichtenstein et al., 1990; Shoykhet et al., 2000; Zucker and Welker 1969). The mechanoreceptors of the trigeminal neurons transduce information about deflections of single, individual whiskers (Clarke and Bowsher 1962; Hayashi, 1980; Jacquin et

al., 1993). Together, PrV, VPm and S1 cortex comprise a lemniscal pathway. The lemniscal pathway is precisely topographically organized. Neurons along this pathway are highly responsive to whisker stimuli, well-tuned for deflection angle and respond to only one or a few neighboring whiskers. The lemniscal pathway thus transmits detailed tactile information faithfully from the sensory periphery to barrel cortex.

A paralemniscal pathway, parallel to the lemniscal pathway, is another prominent projection in the ascending somatosensory system (Diamond, 1995; Yu et al., 2006). This pathway involves the medial divisions of the posterior thalamic nucleus (POm). POm receives input from nucleus interpolaris (Veinante et al., 2000), part of the spinal trigeminal complex (SpVi) in the brainstem and projects to layer 4 (L4) septa located between barrels, and to layer5A (L5A) in S1 (Ahissar et al., 2001). Neurons in this pathway are poorly topographically organized, weakly responsive to whisker stimulation and have large receptive fields. Compared to the whisker-to-barrel pathway, less is known about the functional properties of neurons in the paralemnisical system.

Recently, another ascending sensory pathway was found. Neurons from the caudal part of interpolar nucleus (SpVic, see below) project to the ventral lateral part of the VPm (VPmvl). Projections from VPmvl terminate in the secondary somatosensory cortex and in the septal regions of the barrel cortex (Pierret et al., 2000). The functional role of this pathway is not known.

1.2 CORTICO-THALAMIC CIRCUITS

Structure Primary sensory areas of the cortex project back to the thalamic nuclei from which they receive their major afferent inputs. The following anatomical evidence supports a strong influence of CT feedback to thalamic neurons. First, the number of CT synapses far exceeds the number of synapses from brainstem (Liu et al., 1995). Second, there are up to ten times more CT fibers than thalamocortical (TC) afferents (reviewed in Jones, 1985). Third, up to half of the neurons in infragranular layers of primary sensory cortices are thalamic projecting CT neurons (Zhang and Deschenes, 1997).

CT projections arise exclusively from L5 and L6 pyramidal neurons. The laminar location of a CT neuron determines the destination of its axon to thalamic nuclei (Bourassa and Deschenes, 1995; Bourassa et al., 1995; Castman-Berrevoets and Kuypers, 1978; Gilbert and Kelly, 1975; Usrey and Fitzpatrick 1996). Two types of CT neurons are distinguished based on their projection targets, morphology of axon terminals, synaptic locations on dendrites as well as their electrophysiological properties; these are L5 CT neurons and L6 CT neurons (Bourassa and Deschenes, 1995; Bourassa et al., 1995; Hoogland et al., 1991; Ojima, 1994; Rouiller et al., 1991a, b; reviewed in Deschenes et al., 1998).

CT fibers originating from L5 consist of collateral fibers from axons projecting to the brainstem. The collaterals of these neurons terminate in "higher–order" thalamic nuclei, for instance POm (Hoogland et al., 1991; Bourassa et al., 1995; Veinante et al., 2000). These CT neurons synapse on proximal dendrites. Individual axon terminals are large and spatially clustered (Hoogland et al., 1991; Ojima, 1994; Rouiller and Welker, 1991). The synapses of L5 CT neurons are strong, depressing synapses and their axons are rapidly conducting (reviewed in Sherman and Guillery, 1996).

CT neurons from L6 project to both first-order nuclei (VPm) and higher-order nuclei (POm) as well as to the thalamic reticular nucleus (nRT) (Liu et al., 1995; Bourassa et al., 1995; Zhang and Deschenes 1997; Deschenes et al., 1998). In contrast to L5 CT neurons, L6 CT neurons synapse on the distal dendrites of thalamic relay neurons with small axon terminals (Bourassa et al., 1995; Zhang and Deshenes, 1997; Deschenes et al., 1998). The synapses of L6 CT neurons are weak and facilitating (reviewed in Sherman and Guillery, 1996). CT neurons in L6 can be further characterized based on their projection targets and the laminar location of their somata. CT neurons in the upper half of L6 exclusively project to VPm and to nRT (Bourassa and Deschenes, 1995; Bourassa et al., 1995). CT neurons in the lower half of L6 project more broadly, including VPm, PO and nRT (Bourassa et al., 1995; Zhang and Deschenes 1997; Deschenes et al., 1998).

The precisely segregated projections from CT neurons to thalamic nuclei suggest a distinct role of each projection. Interestingly, cortical feedback to thalamus maintains the structural and function segregation with respect to ascending lemniscal and paralemniscal pathways. One idea may be CT-VPm projection may enhance detailed sensory-motor processing (see below *function*), whereas CT-POm pathway maybe more involved with motor-sensory integration (see Guillery and Sherman, 2002). In the present study we focused on how cortical motor signals influence sensory transmission in the lemniscal pathway both in lightly sedated and in freely behaving rats.

Function Although the basic response properties of thalamic neurons are established by feedforward sensory afferents, accumulating evidence from different sensory systems suggests that cortical feedback serves to modulate thalamic sensory processing, in particular enhancing spatial tuning and excitatory responses to preferred vs. non-preferred

stimuli. Most of the studies regarding the role of CT neurons have employed cortical inactivation or ablation as well as focal manipulation of cortical activity (reviewed in Alitto and Usrey, 2003; Baker and Malpeli 1997; Ghosh et al., 1994; Yuan et al., 1985; Ergenzinger et al., 1998; reviewed in Sillito and Jones, 2002; Webb et al., 2002; Rauschecker 1998; Tsumoto et al., 1978; He 1997).

In the visual system, responses of lateral geniculate nucleus (LGN) neurons to visual stimuli restricted to the receptive field of LGN neurons are reduced following cortical inactivation (Przybyszewski et al., 2000), whereas responses to stimuli extending into the surround display a release of suppression in the absence of cortical feedback (Reviewed in Sillito and Jones, 2002; Webb et al., 2002). In addition, CT feedback enhances firing of neurons in LGN selectively when the receptive fields of LGN neurons are aligned within an oriented visual stimulus, one that is effective for activating cortical neurons (Sillito et al., 1994, Wang et al., 2006). Cortical feedback may serve to enhance the excitatory responses of LGN neurons to stimuli restricted to the receptive field, as well as enhance the suppressive effects of stimuli that extend into the surround. Consequently, the CT feedback may serve to sharpen the receptive fields of LGN neurons. Similarly, in auditory cortex, CT projections arising from a given frequency representation tend to facilitate neurons in the medial geniculate nucleus (MGN) that share the same frequency tuning of cortical neurons, while neurons with non-matched frequency receptive fields are inhibited (Zhang and Suga, 2007; Yan and Suga, 1999, Zhang and Suga, 2000). In the somatosensory system, inactivation of S1 expands receptive fields (Diamond et al., 1992; Ergenzinger et al., 1998; Krupa et al., 1999; Ghazanfar et al., 2001), and reduces evoked responses (Yuan et al., 1985; 1986) of thalamic VPm neurons. Focal pharmacological enhancement of L6 activity leads to facilitation of neuronal responses in the topographically

aligned area in VPm and response suppression in adjacent, non-aligned areas (Temereanca and Simons, 2004).

These results suggest that the role of CT projections, in particular those originating from L6 is to selectively enhance thalamic responses to topographically aligned cortical stimuli. So far, however, most studies investigating the function of CT feedback have been carried out by activating or inactivating widespread regions of cortex using cortical ablation, cooling, electrical stimulation or pharmacological intervention. Such experimental manipulations could create unphysiological conditions, making it difficult to interpret findings. One of the main reasons for using relatively large-scale manipulations is that it is not known what stimuli/conditions normally drive CT neurons.

In the somatosensory system as in the visual system (Swadlow, 1988), it has been reported that a large proportion of antidromically identified CT neurons in L6 has very low or no spontaneous firing rates, and the cells are poorly responsive to peripheral sensory stimulation or even silent altogether (Landry and Dykes, 1985; Swadlow 1989, 2000; Swadlow and Hicks, 1996; Kelly et al., 2001). Considering that CT neurons receive monosynaptic thalamic inputs (White and Hersch, 1982), the poor responsiveness of CT neurons is puzzling. *In vitro* studies suggest that CT neurons elicit more spikes to a depolarizing current, indicating that they are more excitable than other neurons in L6. Synapses onto CT neurons from nearby cortical neurons exhibit short term facilitation (Beierlein and Connors, 2002; West et al., 2006). Together, the intrinsic and synaptic properties of the CT neurons do not explain their unresponsiveness.

Taking into account that CT neurons receive subthreshold sensory inputs and neurons in L6 receive afferent inputs from other cortical areas, activity in other brain regions during certain

processing states may be necessary to produce spiking activity of CT neuron (Deschenes, 1998; Zhang 1997). Here, we hypothesize that vMCx input is one of the major driving sources for CT neurons. To test this hypothesis, we examined the excitability of antidromically identified CT neurons in S1 while pharmacologically facilitating activity in vMCx. Further, we studied such vMCx facilitation modulates sensory processing in VPm neurons, the targets of S1 CT cells.

1.3 INTERCONNECTION BETWEEN MOTOR AND SENSORY PATHWAYS

Anatomical studies provide evidence of interconnections between whisker motor and sensory regions at the level of the brainstem, thalamus and cortex (reviewed in Ahissar and Kleinfeld, 2003). For instance, in brainstem, central projections of trigeminal complex neurons can excite whisker-motor neurons in the facial nucleus. These findings raise the possibility that whisker muscle tone may be reflexively enhanced depending upon contact during active touch (Nguyen and Kleinfeld, 2005).

Detailed functional interaction between higher level sensory and motor areas is not well understood. In the cortex, prominent reciprocal connections exist between the somatosensory barrel cortex and vMCx (White and DeAmicis, 1997; Akers and Killackey, 1978; Chapin and Woodward, 1982; Donoghue and Parham, 1983; Carvell and Simons, 1987; Chapin et al., 1987; Koralek et al., 1990; Fabri and Burton, et al., 1991; Miyashita et al., 1994; Izraeli and Porter, 1995). The vast majority of the projections from S1 to vMCx arises from layer 2/3 and layer 5. The projections from barrel cortex originate predominantly from vertical columns of neurons that are aligned with the layer 4 septal regions (Alloway et al., 2004). Some neurons in L6 of the

barrel cortex also contribute to the projections to vMCx (Koralek et al., 1990; Zhang and Deschenes, 1997).

In a study by Veinante and Deschenes, axonal projections of antidromically identified neurons in vMCx that project to the barrel cortex were examined in detail by labeling single neurons in vMCx (2003). The investigators distinguished two groups of neurons on the basis of the final destination of the main axons: callosal neurons (the main axons terminate in the homotopic region of contralateral motor cortex) and corticofugal neurons (the main axons terminate in subcortical areas including thalamus, striatum and brain stem). Axon collaterals of callosal neurons whose somata are located in infragranular layers of vMCx arborize in infragranular layers of barrel cortex. In contrast, the axon collaterals of corticofugal neurons do not give off branches in the infragranular layers of barrel cortex, but rather they terminate in L1 (Veinante and Deschenes, 2003).

Projections to S1 from the infragranular layers of vMCx comprise one of the major input sources to infragranular layers of barrel cortex. It is, however, not known what types of neurons receive these afferents from vMCx (see Rocco and Brumberg 2007). For instance, do motor cortex neurons directly contact CT neurons in barrel cortex? More importantly, because projections from vMCx could target inhibitory as well as excitatory cells, what is the net effect of vMCx activity on neurons in infragranular layers in barrel cortex? Here, we addressed this issue by recording neurons in L6 of barrel cortex while pharmacologically enhancing neuronal activity in identified areas within vMCx. We further examined the effect of vMCx activation on antidromically identified CT neurons.

1.4 MOTOR CORTEX AND WHISKING BEHAVIOR

Mystacial whiskers of rats constitute an array of mechanical sensors adapted specifically for active exploration of the tactile environment. During exploratory behavior a rat acquires tactile information by rhythmically sweeping its whiskers back and forth in motion. This rhythmic motion is called "whisking". The manner in which a rat palpates an object with its vibrissae during whisking is analogous to the way in which primates use their fingertips to scan objects during active touch (Carvell and Simons, 1990).

What controls whisker movement? The cortical representation of the whiskers in M1 occupies approximately 35% of the rat's entire motor cortex. The fact that the whiskers are disproportionately represented in the motor cortex indicates the functional significance of whisker movements. Physiological and anatomical evidence indicates that neuronal activity in vMCx is correlated with whisker movement. Neurons in vMCx increase their overall firing rates during active whisking (Carvell et al., 1996). Field potential activity in vMCx often correlates with changes in whisker kinematics measured by EMG activity (Freidman et al., 2005). Individual neurons in infragranular layers of vMCx do not discharge rhythmically in a one-to-one fashion with the periodicity of whisking movements (Carvell et al., 1996). Other experiments, however, suggest that population neuronal activity measured as field potentials in vMCx are matched with each whisking cycle (Ahrens and Kleinfeld, 2004). Considering that vMCx neurons do not discharge precisely with whisking cycle, it is possible that the field potentials in vMCx may reflect a sensory response.

Intracortical microstimulation (ICMS) of vMCx region can evoke whisker movement (Donoghue and Wise, 1982; Weiss and Keller, 1994; Berg and Kleinfeld, 2003; Haiss and Schwarz 2005). Injecting current into even a single neuron in M1 could induce whisker

movements (Brecht et al., 2004). Electrical stimulation effects may be related to the precise location of the stimulation and to the specific nature of the electrical stimuli, e.g., frequency and duration. Abnormal rhythmic and synchronized vMCx activity produced by pharmacological disinhibition is capable of driving whisker movement on a cycle-by-cycle basis (Castro-Alamancos, 2006).

Facial motor neurons produce whisker muscle contractions. Anatomical studies demonstrate that the majority of neurons in vMCx project only indirectly to the facial motor nucleus, with most corticofugal axons terminating in other brainstem nuclei thought to contain "pre-motor" whisking neurons (Miyashita et al., 1994; Hattox et al., 2002). Direct monosynaptic inputs from the vMCx to facial motor neuron, however, exist but they are notably sparse (Grinevich et al., 2005).

Does vMCx directly control whisker movement? Whisking persists after sensory denervation (Welker 1964), cortical ablation of vMCx (Semba and Komisaruk 1984) or decerebration (Lovick 1972), suggesting that rhythmic whisking is generated by subcortical structures and is controlled by a central pattern generator (CPG) (Gao et al. 2001; Hattox et al. 2002; Cramer and Keller, 2006; Cramer et al., 2007). A series of studies by Keller et al. suggested that serotonergic neurons in brainstem are involved in indirect vMCx control of whisking via CPG (Hattox et al. 2002; Hattox et al., 2003; Cramer and Keller, 2006; Cramer et al., 2007). Unilateral ablation of vMCx alters whisking kinematics, even though cortical removal does not abolish whisking. Ablation of vMCx affects both the amplitude and the velocity of whisker movements and the degree of synchrony between whisker movements on both sides of the face (Gao et al. 2003). These findings suggest that vMCx initiates and modulates whisking through its actions on subcortical pattern generators.

Haiss and Schwarz described an additional whisker-related area in vMCx from which prolonged rhythmic whisking, involving both protraction and retraction, can be evoked by trains of electrical stimuli (Haiss and Schwarz, 2005). This non-topographically organized "rhythmic whisking" area is posteromedial to the large vMCx/face area and appears to be located in agranular medial (AG_m) motor cortex. However, how these two areas interact with each other is not clear. More importantly how the two vMCx areas mutually interact with subcortical areas remains unexplored.

Available evidence indicates that on-going activity in the large vMCx area during whisker movement is not temporally patterned in concert with whisking protractions and retractions (Carvell et al., 1996, Freidman et al., 2005). Neuronal activity in large vMCx is, however, related to whisking initiation and termination, and to changes in whisking frequency. Together, it suggests that motor activity in the large vMCx area may not control whisking on a cycle by cycle basis. Rather, the small whisking area in vMCx as described by Haiss and Schwarz may be specialized for commanding stereotypical "rhythmic" whisking.

What does the large vMCx area do in terms of whisker movement? The large vMCx is topographically organized, unlike the small whisking area of vMCx. Topographic organization of vMCx, although it is not as discrete as in sensory/barrel cortex, implies some degree of independent influence of individual whiskers. In this regard, fractionation of whisking has been observed in behaving animals (Sachdev et al., 2002), although rats typically move all their whiskers synchronously (Carvell and Simons, 1990; Fee et al., 1997; Gao et al., 2001; Berg and Kleinfeld, 2003; Hattox et al., 2003; Sachdev et al., 2003). Fine discrimination of an object may require an ability to independently control individual whiskers or, perhaps more likely, to integrate whisking with head and neck movements. Indeed, the traditionally defined vMCx is

part of a larger face area in motor cortex, and whisking is complexly integrated into motor behaviors involving other face, neck and respiratory muscles (Welker et al., 1964; Carvell and Simons, 1990). Anatomical studies provide evidence of a relatively coarse row-dependent projection from barrel cortex to vMCx (Hoffer et al., 2003; Alloway et al., 2004), raising a possibility that vMCx may also exert some topographically organized influence on sensory cortical activity. Here, we examined topographic dependency of vMCx modulation on neurons in L6 of barrel cortex and in thalamic VPm.

1.5 SENSORIMOTOR INTEGRATION

Animals obtain vital information from the world by simultaneously using their sensory and motor systems. Most studies of sensory processing, however, have been done in an experimental framework that is designed to test passive rather than active sensation, thus leaving out one of the major components in the completed sensory process. Recent work in several sensory systems provides evidence on how sensory signals are processed during movements (reviewed in Chapman, 1994; Nelson, 1996; Cullen, 2004; Wurtz and Sommer, 2004). One of the most common mechanisms across different systems is that sensation during voluntary movement involves suppression of sensory-evoked responses during the course of voluntary movements (Chapman, 1994; Nelson, 1996; Cullen, 2004). This phenomenon has been referred as 'gating' (Chapman, 1994). A common hypothesis holds that voluntary movement-related suppression of sensory responses serve to filter out sensory stimuli generated as a by-product of voluntary movement, thus helping to distinguish self-generated sensory inputs from those that arise externally (Chapman, 1994; Nelson, 1996; Cullen, 2004).

In the somatosensory system, functional imaging studies have shown that self-generated tactile stimulation in humans does not result in the same tickling sensation that arises when the stimulation is externally produced (Blakemore et al., 1998, 1999 a, b). Consistent with the perception, self-produced tactile stimuli result in less activation of S1 than externally generated same tactile stimuli. Neurophysiological data from animals show that evoked sensory responses in thalamus and cortex are attenuated during active touch relative to responses evoked by passive stimulation (Ghez and Lenzi, 1971; O'Keefe and Gaffan 1971; Coulter, 1974; Chapin and Woodward, 1982a,b; Chapman et al., 1988; Shin and Chapin 1990a,b; Chapman, 1994). Such movement-related sensory "gating" has also been observed in the rat 'whisker-barrel' system. Fanselow and Nicolelis (1999) showed that electrical stimulation of the infraorbital nerve evoked smaller magnitude responses in VPm and S1 during voluntary whisker movement compared to an awake but non-whisking state. Along the same line, Castro-Alamancos reported that sensory-evoked responses in S1 are suppressed and sensory adaptation is reduced during alert states (2004). A recent study by Schwarz and his colleagues further demonstrated that state-dependent changes in S1 sensory responses occur rapidly and coincide closely with the onset of whisker movement (Hentschke et al., 2006). In addition, by showing that such rapid changes in sensory response are still present when information about whisker kinematics was interrupted by transaction of infraorbital nerve, the investigators suggested that central signals from the motor system may be involved in this process. Together these studies show that movement-related gating of sensory response exists in whisker-barrel system. The detailed underlying mechanisms, however, are not well understood.

As described above, vMCx projects to L6 of barrel cortex and L6 exerts a net excitatory effect on VPm neurons via CT feedback. These two observations lead to a prediction that vMCx

activation may enhance evoked sensory responses in thalamic VPm. This hypothesis, however, is at variance with observations that VPm and S1 responses are attenuated during active whisking. Here, we re-examined how thalamic responses to peripheral stimulation differ depending upon whisking vs. non-whisking states in freely behaving rats. In some experiment we stimulated the medial lemniscus in order to by-pass trigeminal brainstem nuclei and thus more directly assess CT effects.

1.6 BRAINSTEM TRIGEMINAL COMPLEX

Both anatomical and functional evidence supports the role of the brainstem trigeminal complex in the suppression of sensory responses during voluntary whisking.

The PrV receives local inhibitory projections from SpVi (Jacquin at al., 1990, Furuta at al., 2006). The circuitry exists for SpVi to have both excitatory and inhibitory effects on PrV neurons. The brainstem trigeminal complex is a relay station between the trigeminal ganglion and the thalamus in the whisker-to-barrel pathways. It consists of the principal sensory nucleus of the trigeminal nerve (PrV) and the spinal trigeminal nucleus (SpV). SpV can be further subdivided: oralis (SpVo), interpolaris (SpVi) and caudalis (SpVc) (Belford and Killackey, 1979; Durham and Woolsey, 1984; Ma and Woolsey, 1984; Sikich et al., 1986; Ma, 1991). SpVi provides afferent input to the thalamus together with the PrV. PrV neurons project to VPm, whereas neurons in the rostral portion of the SpVi project to POm (Williams et al., 1994; Veinante and Deschenes, 1999; Pierret et al., 2000; Yu et al., 2006). Only a small portion of neurons in the caudal part of the SpVi are thalamic-projecting neurons. Instead most neurons there project locally within the brainstem trigeminal complex, including PrV (Jacquin et al.,

1990; Furuta et al., 2006). Many neurons in caudal SpVi express glutamate-decarboxylase, suggesting that they play an inhibitory function on targeted neurons (Timofeeva et al., 2004; Furuta et al., 2006). It is worth noting that excitatory projections from SpVi provide multi-whisker receptive fields to PrV (Timofeeva et al., 2004; 2005; Kwegyir-Afful et al., 2005).

SpVi appears to be strongly state-dependent, receiving potent cholinergic projections from neurons in the pedunculopontine tegmentum (PPTg), in addition to sensory input from trigeminal ganglion neurons (Timofeeva et al., 2005). Cholinergic modulation from PPTg is highly related to alert/arousal states of the brain (Aston-Jones et al., 1991; Buzsaki et al., 1988). Interestingly, vMCx projects to the PPTg as well as to other nuclei in the brainstem (Hattox et al., 2002). Rats are in a highly alert state during active whisking. Whisking may thus increase cholinergic modulation, thereby driving SpVi neurons. SpVi neurons may in turn inhibit PrV neurons.

Here, we tested the possibility that SpVi is responsible for sensory suppression during whisking. We used two different approaches to examine this possibility. First, we applied electrical stimulation directly to the medial lemniscal tract. This stimulation protocol bypassed the brainstem trigeminal complex. Second we pharmacologically inactivated SpVi.

1.7 OVERVIEW

In the present study, we tested the hypothesis that descending motor commands from vMCx regulate whisker-related somatosensory transmission in VPm via CT projections from barrel cortex. In Chapter 2, we investigate function in a circuit linking vMCx with thalamic barreloids: from vMCx to CT neurons in barrel cortex, from CT to thalamic VPm neurons. In chapter 3, we

examine how cortical activity during whisking modulates sensory processing via the CT pathway in freely behaving rats. Our findings are consistent with the idea that whisking-related increases in motor cortical activity lead to facilitation of neural responses in thalamic and cortical neurons involved in processing afferent somatosensory information. Experiments involving antidromically identified CT cells suggest that the effect of vMCx facilitation on VPm is mediated via these CT neurons. Our findings in freely behaving rats suggest that during voluntary whisking afferent activity is globally suppressed in the brainstem at the same time that it is simultaneously enhanced in the thalamus, perhaps in a topographic and context-specific fashion. In chapter 4, we asked how the input from motor cortex to cortico-thalamo-cortical circuit affects responses in layer 4 barrel circuit which is another major component of this loop.

2.0 PRIMARY MOTOR CORTEX MODULATION OF SOMATOSENSORY CORTICOTHALAMIC PROCESSING

2.1 INTRODUCTION

Descending cortical activity modulates on-going processing of afferent sensory information within hierarchically organized neural systems. One prominent feedback circuit involves the corticothalamic (CT) pathway, which projects from infragranular cortical layers to neurons in the thalamus that provide essential afferent inputs to that same cortical area. CT synapses comprise almost half of the total synaptic inputs to thalamic neurons and is three- to ten-fold greater than the number of synapses from ascending afferent fibers (Jones and Powell, 1969: Liu et al., 1995; Erisir et al., 1997).

Accumulating evidence from the visual, auditory and somatosensory systems suggests that corticothalamic (CT) feedback enhances thalamic response tuning (Baker and Malpeli 1977; Tsumoto et al., 1978; Yuan et al., 1985; Ghosh et al., 1994; He 1997; Ergenzinger et al., 1998; Rauschecker 1998; reviewed in Sillito and Jones, 2002; Webb et al., 2002; reviewed in Alitto and Usrey, 2003). For example, in the visual system CT feedback selectively increases the firing of neurons in the lateral geniculate nucleus (LGN) whose receptive fields are circular but aligned within an oriented stimulus reflective of cortical receptive field properties (Sillito et al., 1994; Wang et al., 2006). Similarly, in auditory cortex, CT projections arising from a given frequency

representation in auditory cortex facilitate neurons in the medial geniculate nucleus (MGN) that share the same frequency tuning, whereas neurons with non-matched frequency preferences are inhibited (Yan and Suga, 1999; Zhang and Suga, 2000). In the somatosensory system, inactivation of S1 expands receptive fields (Diamond et al., 1992; Ergenzinger et al., 1998; Krupa et al., 1999; Ghazanfar et al., 2001), and reduces evoked responses (Yuan et al., 1985; 1986) of thalamic VPm neurons. Conversely, focal pharmacological enhancement of L6 activity leads to facilitation of neuronal responses in the topographically aligned area in VPm and response suppression in adjacent, non-aligned areas (Temereanca and Simons, 2004).

Most studies investigating the functional role of CT neurons have employed cortical ablation, widespread cooling or pharmacological inactivation, or electric shocks to affect CT firing. For example, in a recent study, 500 Hz electrical stimulation was applied to L6 of S1 for 3-30min in order to examine whether CT feedback affects angular tuning of thalamic neurons (Li and Ebner, 2007). Such experimental manipulations create physiological conditions that are unlikely to occur normally, making it difficult to extrapolate findings to behavior. Indeed, an unresolved issue concerns the circumstances during which CT neurons may normally be actively engaged. This issue is particularly important, because a substantial proportion, perhaps even a majority, of CT cells is unresponsive or only weakly responsive to peripheral sensory stimulation under standard experimental conditions (Swadlow 1989; Swadlow and Hicks 1996, Kelly et al., 2001) and even in awake animals (Beloozerova et al., 2003; Sirota et al., 2005). Weakly responsive CT neurons and even some that are otherwise silent are reported not only in somatosensory cortex but also other primary sensory cortices (Swadlow 1988). Weak responsiveness is especially striking considering that CT neurons are known to receive monosynpatic inputs from thalamic relay neurons (White and Hersh, 1982).

The somatosensory system intimately cooperates with motor systems during tactile exploration and active touch. In the whisker sensorimotor system of the rat, anatomical studies have shown extensive interconnections between sensory and motor neural sub-systems including reciprocal connections between vMCx and S1 (Akers and Killackey, 1978; Donoghue and Parham, 1983; Carvell and Simons, 1987; Chapin et al., 1987; Koralek et al., 1990; Fabri and Burton, 1991; Miyashita et al., 1994; Izraeli and Porter, 1995; Zhang and Deschênes, 1998; Alloway et al., 2004). Multiple levels of interaction between sensory and motor areas may integrate sensory input and motor output to optimize sensory transmission and adjust motor responses (Ahissar and Kleinfeld, 2003; Kleinfeld et al., 2006). Mechanisms by which such dynamic processing occurs are not, however, well understood.

An intriguing finding is that deep layers of vMCx project to deep layers of S1 barrel cortex where they can potentially influence CT cells either monosynaptically or by means of local circuit interactions (Fabri and Burton, 1991; Miyashitat et al., 1994; Izraeli and Porter, 1995; Zhang and Deschênes, 1998; Veinante and Deschenes, 2003). In addition, L6 of somatosensory cortex receives inputs from the claustrum as well as from the perirhinal and secondary somatosensory cortex (SII) (Erisir et al., 1997; Jones and Powell, 1969; Carvell and Simons, 1987). Thus, CT neurons in primary somatosensory cortex are strategically positioned to regulate afferent processing in thalamocortical circuits based on activity in other telencephalic centers that are likely to be involved in sensorimotor integration. If so, CT neurons in somatosensory cortex should be influenced by activation of vMCx. In behaving rats, vMCx activity is elevated during whisking. Increased firing rates, whose onset shortly precedes the start of whisking, is tonic (Carvell et al., 1996) and can thus be approximated in acute experiments by pharmacological facilitation of background activity. Here we report that

pharmacologically enhanced vMCx activity facilitates whisker-evoked responses in infragranular neurons located in topographically aligned regions of S1 and in similarly topographically aligned thalamic barreloid neurons. Increased activity observed in antidromically identified CT cells suggests that the effect of vMCx facilitation on thalamic VPm is mediated via these CT neurons.

2.2 METHODS

Surgical procedures

Data were collected from adult female rats (Sprague-Dawley strain; Hill Top, PA) weighting 200-380 g. Rats were prepared for electrophysiological recordings using methods described previously in detail (Simons and Carvell 1989). In brief, under 1.5-2.0% halothane or isoflurane anesthesia, a tracheotomy was performed for artificial respiration, the jugular vein was catheterized for drug delivery, and a cannula was inserted into the femoral artery for monitoring blood pressure. Two small craniotomies were made over the right barrel cortex and the vibrissa representation in motor cortex (vMCx). Following surgery, rats were transferred to a vibration-damping table for neuronal recording. After mapping vMCx (see below), halothane (or isoflurane) was discontinued, and rats were maintained in a lightly sedated state by intravenous infusion of fentanyl, a synthetic opiate (~10 μg/kg/hr). Rats were immobilized with pancuronium bromide (1.6 mg/kg/hr) and artificially respired (~90-100 breath/min) using a positive pressure respirator. A servo-controlled heating pad maintained the animal's body temperature at 37°C. The condition of the rat was monitored throughout the experiment by a custom-written computer program that continually assessed the electroencephalogram (EEG), mean arterial pressure, arterial pulse rate, and tracheal airway pressure waveform. Experiments

were terminated if any of the above indicators could not be maintained within the normal physiological ranges.

vMCx mapping with electrical stimulation

Under halothane or isoflurane anesthesia, the vMCx was identified using intracortical microstimulation delivered through small diameter stainless steel microelectrodes (100-500 kΩ at 1 kHz; Frederick Haer, Brunswick, E). vMCx was found 1-3 mm lateral from the midline and 1-3 mm anterior to Bregma. For detailed mapping of vMCx, 30 ms trains of 0.2 ms duration pulses were delivered at 300 Hz through the stimulation electrode at a depth of 1,400 - 1,600 μm, and evoked whisker movements were observed using a stereo microscope (Kelly et al., 2001). Current intensity was reduced to levels that evoked small but noticeable movements (> 1 mm) of 1-3 whiskers; threshold currents ranged from 50 to 500uA, depending in part on the depth of anesthesia. In most cases electrical stimulation produced whisker retractions, consistent with previous reports (Donoghue and Wise, 1982; Berg and Kleinfeld, 2003; Brecht et a., 2004; Haiss and Schwarz, 2005; Castro-Alamancos, 2006).

BMI Microiontophoresis Microiontophoretic application of the GABA-A antagonist bicuculline methiodide (BMI) was used to enhance the on-going activity of infragranular neurons (1,400 - 1,600 μm depth) in vMCx. Three- or seven-barrel glass microelectrodes having a carbon-fiber recording channel were used to apply BMI and record simultaneously multiunit vMCx activity (MUA). In order to assemble these microelectrodes, a carbon fiber (7μm diameter) was inserted into one of the barrels, the glass was pulled, and the tip was beveled to yield 3~5 μm inner diameters for each barrel (Kyriazi et al., 1996; Temereanca and Simons

2004). Two or three of the barrels were filled with 10mM BMI (Sigma Chemical, Saint Louis, MO) in 0.9 %NaCl, pH 3.0. Immediately after the BMI electrode touched the pial surface, a negative current of 30-50 nA was applied to prevent leakage. Positive current of 15~30 nA for each barrel was used to expel BMI; one or two barrels were used and BMI in the remaining "back-up" barrels was retained with negative (30-40nA) current. In most cases, 5-10 minutes of BMI application was sufficient to induce reproducible and reversible changes in spontaneous neuronal activity. If spontaneous activity was not elevated, the current was increased up to 40nA for 5-10 additional minutes. Typically, a 40-60 minute recovery period was sufficient for the MUA to return to baseline levels. During an individual experiment, BMI was injected no more than 4-8 times. For each application, the micro-iontophoretic electrode was relocated ~100μm up or down within same penetration or a new penetration was made nearby within 100μm. If local seizure-like activity accompanied the BMI, data from S1 or VPm were not collected.

In one experiment the spatial extent of the BMI effect was evaluated by simultaneously recording MUA using a linear assembly of 4 electrodes spaced 500 μm apart at a depth of 1500 μm (Fig. 2). A three-channel array of individually moveable microelectrodes (Uwe Thomas Recording, Giessen, Germany) and a BMI electrode of the type described above were used for this purpose. Quartz-insulated platinum-tungsten (90 %-10 %) electrodes were used in the three-channel array (1-3 MΩ).

Electrophysiological recordings

Extracellular single-unit recordings were obtained from neurons in infragranular layers of barrel cortex (depths of 1,400-1,800 μm) using high impedance NaCl-filled glass micropipettes (~1 μm tip diameter; 5–10 MΩ impedance at 135 Hz), similar impedance stainless steel microelectrodes,

or electrodes made from pulled and beveled quartz-insulated platinum-tungsten (90 %-10 %) core fibers. Recordings were targeted to infragranular layers deep to barrel centers as determined by prior physiologic mapping, because CT neurons projecting to VPm are preferentially located there (Chmielowska et al., 1989). Electrodes were slowly advanced perpendicular to the pial surface using a hydraulic microdrive (Kopf Instruments, Tujunga, CA). Analog signals were amplified, bandpass-filtered at 300 Hz – 10 kHz and digitized at 32 kHz. Recorded neurons were identified by spike amplitude and waveform criteria using a virtual oscilloscope. Data acquisition was achieved using custom software written in LabVIEW (National Instruments, Austin, TX), which parsed and saved spike waveforms for subsequent analyses. When multiple units were present, only the one showing the largest amplitude was discriminated. Units were further isolated off-line using custom software written in LabVIEW. Units were classified as regular spiking units (RSUs) or fast spiking units (FSUs) on the basis of the shape and duration of spikes (see Simons, 1978; Bruno and Simons 2002). No attempt was made to obtain single unit recordings from vMCx, because the purpose of recording in vMCx was to monitor the BMI effect, and thus MUA recordings were more suitable. At the end of each experiment, electrical lesions were made at selected recording sites.

Whisker stimulation

A hand-held probe was used to identify the whisker most effective at evoking activity in an isolated cell, i.e., that cell's principal whisker (PW). For controlled stimulation, the PW was deflected using a piezoelectric stimulator attached to the whisker hair 12 mm from the skin (Simons and Carvell 1989). Ramp-and-hold stimulus waveforms were similar to those employed in previous studies in our laboratory and were generated via D/A converters using custom

software written in LabVIew (National Instruments, Austin, TX). Whisker deflections were applied ten times in each of eight directions differing in 45-degree increments, for a total of 80 stimuli. Deflections, which occurred every 1.5 seconds, consisted of 1 mm displacements of 200ms duration, with average onset and offset velocities of ~125m/s. Deflection angles were varied in pseudorandom order throughout the stimulus presentation.

Data Analysis

Unit responses were compiled into peri-stimulus time histograms (PSTHs) having 1-ms bin width and quantified as the average number of spikes per stimulus during selected time periods. Responses to stimulus onsets (ON) and offsets (OFF) were computed during a 30 ms period after the beginning of whisker movement away from or back to its resting position. Spontaneous activity was measured during a 100 ms period before stimulus onset. Plateau responses were computed for 100 ms between deflection onset and offset. For direct comparison with ON and OFF responses, plateau responses were normalized to 30 ms.

Data were analyzed using Microsoft Excel/Visual Basic and Matlab (The MathWorks, Natick, MA). Statistical significance was evaluated using paired t-tests for group data and Student's t-tests for assessing changes within individual neurons. Means ± standard deviations are given throughout the text. Results are displayed as means ± standard errors.

Histology

At the termination of the recording session, the rat was deeply anesthetized with pentobarbital sodium (Nembutal, 100 mg/kg) and perfused transcardially for cytochrome oxidase (CO) histochemistry. The barrel cortex was cut tangentially, whereas vMCx and VPm was sectioned

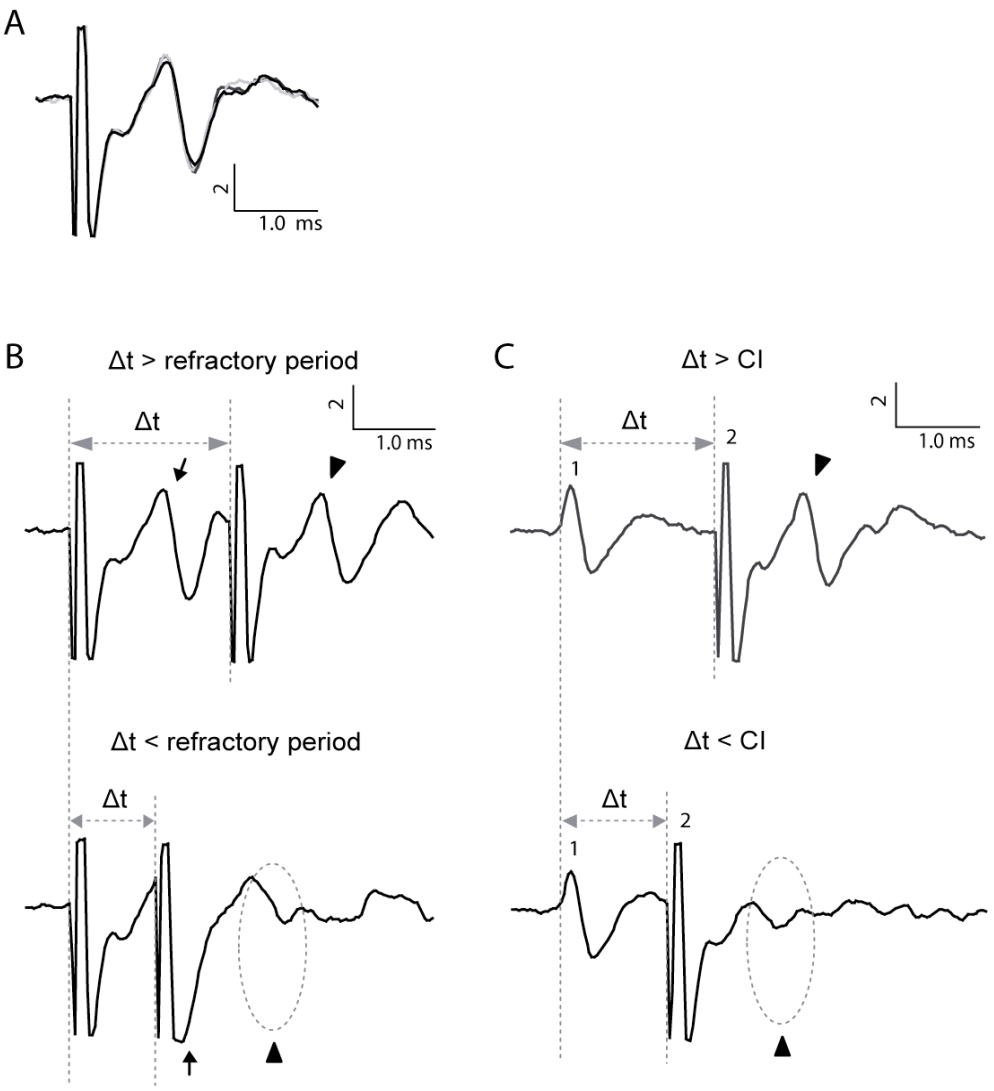


Figure 1. Antidromic identification of CT neurons. (A) Three superimposed traces of antidromic spikes demonstrating little variability in latency in response to electrical pulses delivered to the topologically aligned thalamic barreloid. (B)Measurement of refractory period using paired and temporally separated (Δt) electrical stimuli. In top trace, spikes indicated by the arrow and arrowhead are evoked by the first and second stimulus, respectively. Bottom trace: with short Δt, a second spike is no longer evoked. Arrow indicates expected time of occurrence of first spike which is partially obscured by the stimulus artifact. (C) Collision tests verifying the antidromic activation of the cell shown in B. Spontaneous spike (1) used to trigger the electrical stimulus (2), which in turn evoked the antidromic spike (arrowhead). As Δt becomes smaller, the spontaneous and antidromic spikes eventually collide. This interval is designated as the collision interval (CI). CI is predicted to equal the sum of antidromic latency and the refractory period. Top trace: Δt was longer than CI of the neuron, thus the spike evoked by the trigger stimulus did not collide with the spontaneous spike. Bottom trace: Δt was shorter than CI, thus the spontaneous and antidromic spikes collided somewhere along the axon. Note the absence of the expected antidromic spike (arrowhead).

coronally (70 μm thick sections). Histology was performed for all cortical and thalamic experiments to verify recording sites, but not all motor cortices were processed to examine BMI injection sites.

Antidromic stimulation

In order to identify corticothalamic neurons in L6 of S1, electrical stimulation was applied to the topographically aligned barreloid in thalamic VPm. A search stimulus consisting of brief current pulses (0.05 ms duration, approximately 50μA) was applied through the stimulating electrode (stainless steel microelectrodes, 300-600 kΩ) while the cortical recording electrode was advanced. A spike occurring in response to thalamic stimulation was identified as antidromic if it met the following criteria (see Fig. 1) (Kelly et al., 2001; Minnery and Simons 2003): 1) constant latency response (< 0.1 ms jitter) at suprathreshold stimulation, and 2) the ability to follow a high-frequency stimulus (e.g., a refractory period ≤ 2 ms). We also noted the presence or absence of a supernormal conduction period defined as an increase in conduction velocity in response to the second of two pulses spaced < 10ms apart. We calculated supernormality as the percent change in antidromic latency. If the putative CT neuron fired spontaneously, a collision test was performed. Note, however, that most CT neurons display extremely low or no spontaneous firing. Therefore collision tests were performed on only a subset of neurons.

2.3 RESULTS

vMCx activation by BMI microiontophoresis

Multiple-barrel microelectrodes were used to record and apply microiontophoretically the GABAa antagonist bicuculline methiodide (BMI) to vMCx. BMI application produced a spatially localized and reversible increase in multi-unit activity (MUA). The effect is illustrated for a single recording/drug site in Figure 2A, and mean data for 21 sites/applications are shown in Figure 2B. On average, MUA in vMCx increased more than three-fold and recovered near base-line within 30-60 minutes after BMI injection was terminated. Cortical or thalamic neurons were never tested with vMCx BMI applications spaced less than an hour apart. The spatial extent in vMCx of micro-iontophoretically applied BMI was examined in one experiment by simultaneously recording MUA from 4 electrodes placed in a line ~500μm horizontally apart from each other (Fig. 2C, D). One of the electrodes also delivered BMI. BMI application for 10 min gradually increased MUA ~3.5-fold at the site where it was delivered, while responses at locations 1.0 and 1.5mm away were affected only minimally. Even at a site only 500μm removed, MUA activity increased by a factor of two less than at the application site. Comparable results were obtained using similar BMI microiontophoresis protocols in S1 (Temereanca and Simons, 2004). Therefore, BMI microiontophoresis affects cortical activity within a volume no larger than ~500μm in diameter, corresponding to the size (300-500μm) of a functional cortical column (reviewed in Mountcastle, 2003).

Effect of vMCx activation on neurons in L6 of S1

We recorded neurons in S1 while facilitating activity in vMCx using BMI. When the vMCx and S1 sites were topographically aligned, spontaneous and whisker-evoked activities of neurons in L6 of S1 were enhanced. Note that for these experiments we did not use antidromic stimulation. Instead we sampled neurons that could be identified in extracellular recordings. Because they

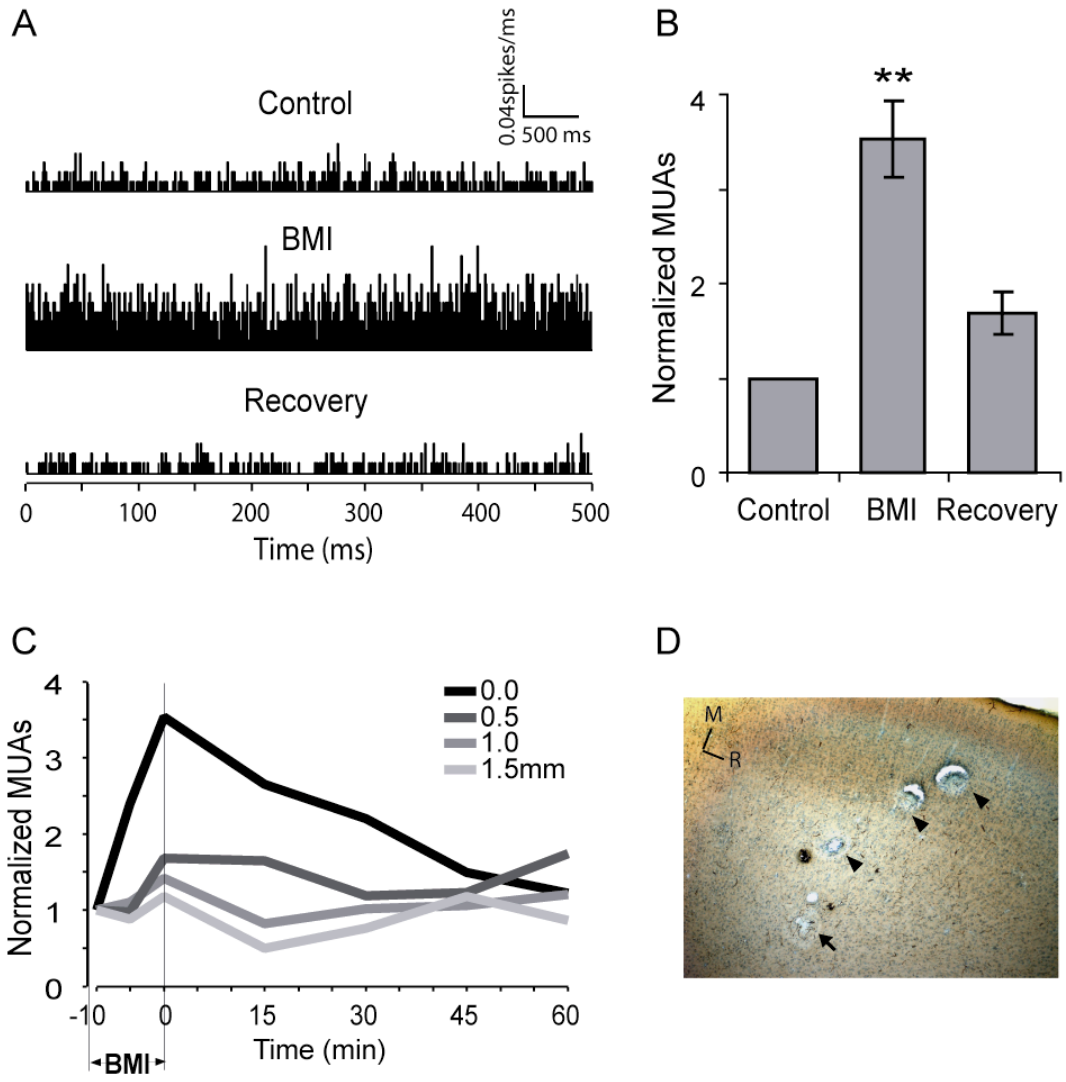


Figure 2. Effect of BMI microiontophoresis on neurons in vMCx. (A) PSTH's show multiunit activity (MUA) recorded from vMCx during eighty 500 ms epochs. Microiontophoretic application of 10 mM BMI for 5 min causes ~ 3X increase in MUA relative to the control level. MUA returns to baseline within 30 min of cessation of BMI application. (B) MUA during BMI application and after recovery was normalized to MUA during control conditions for 21 BMI applications in 4 experiments. Error bars indicate mean ± standard errors. ** *P* value < 0.005 (paired t-test). (C) Lateral spread of BMI effect was evaluated by simultaneously recording MUA from 4 electrodes placed ~500 µm horizontally apart from each other at a depth of 1500 µm. One of the electrodes delivered BMI (black solid line). BMI application for 10 min gradually increased MUA at the site of delivery but only minimally affected responses at other locations. Numbers in the legends indicate distance of recording sites from BMI application site. (D) Histological localization of the 4 recording sites from panel C. A small electrolytic lesion was made at each site (arrowheads). Horizontal sections (70 µm) were reacted for cytochrome oxidase and counter-stained with thionin. Full arrow indicates the site of BMI application.

displayed some spontaneous and/or whisker-evoked firing, the majority of L6 neurons reported here may thus not be thalamic projecting neurons. PSTHs in Figure 3A show responses of a neuron before and during BMI application to vMCx at a site where micro-stimulation evoked movements of caudal row-E whiskers; the whisker evoking the largest response from the S1 neuron was E2 (i.e., the Principal Whisker or PW). Responses to deflection onsets (ON responses) increased significantly from 0.91 to 1.41 spikes/stimulus (Student's t-test, $p<0.05$); during this time MUA in vMCx increased 2.8 fold. The PSTH at the bottom of panel A shows that activity increases were observed also for stimulus offset and pre-stimulus (spontaneous) firing. To test the dependency of these effects on topography, we then repositioned the BMI electrode to a location that was previously identified as producing movement of whiskers B2, B3 and C1. Responses of the same S1 neuron to deflection of E2 were less strongly affected during BMI application here. ON responses increased from 1.1 to 1.27 spikes/stimulus ($p>0.5$) and responses to stimulus offset actually decreased slightly.

Quantitative data for 29 aligned and 12 non-aligned S1 infragranular neurons are shown in the scatter plots of Figure 3B. Topographically aligned sites were those in which sensory responses were evoked solely or most strongly from one of the same (two or three) whiskers whose movement was evoked at the outset of the experiment by vMCx microstimulation; non-aligned sites were responsive to whiskers at least two rows removed, e.g. an vMCx site where movements of whiskers D1 and D2 were evoked and a barrel cortex site in which the strongest responses were evoked by B1. As illustrated by the example neuron in Figure 3A, PW-evoked ON responses of aligned L6 neurons in S1 are significantly higher during vMCx activation. On average, responses increased 15 ± 21.4 %. Paired t-tests showed that mean ON responses in control and BMI conditions differed (Control 0.95 ± 0.52, BMI 1.15 ± 0.65 spikes/stimulus,

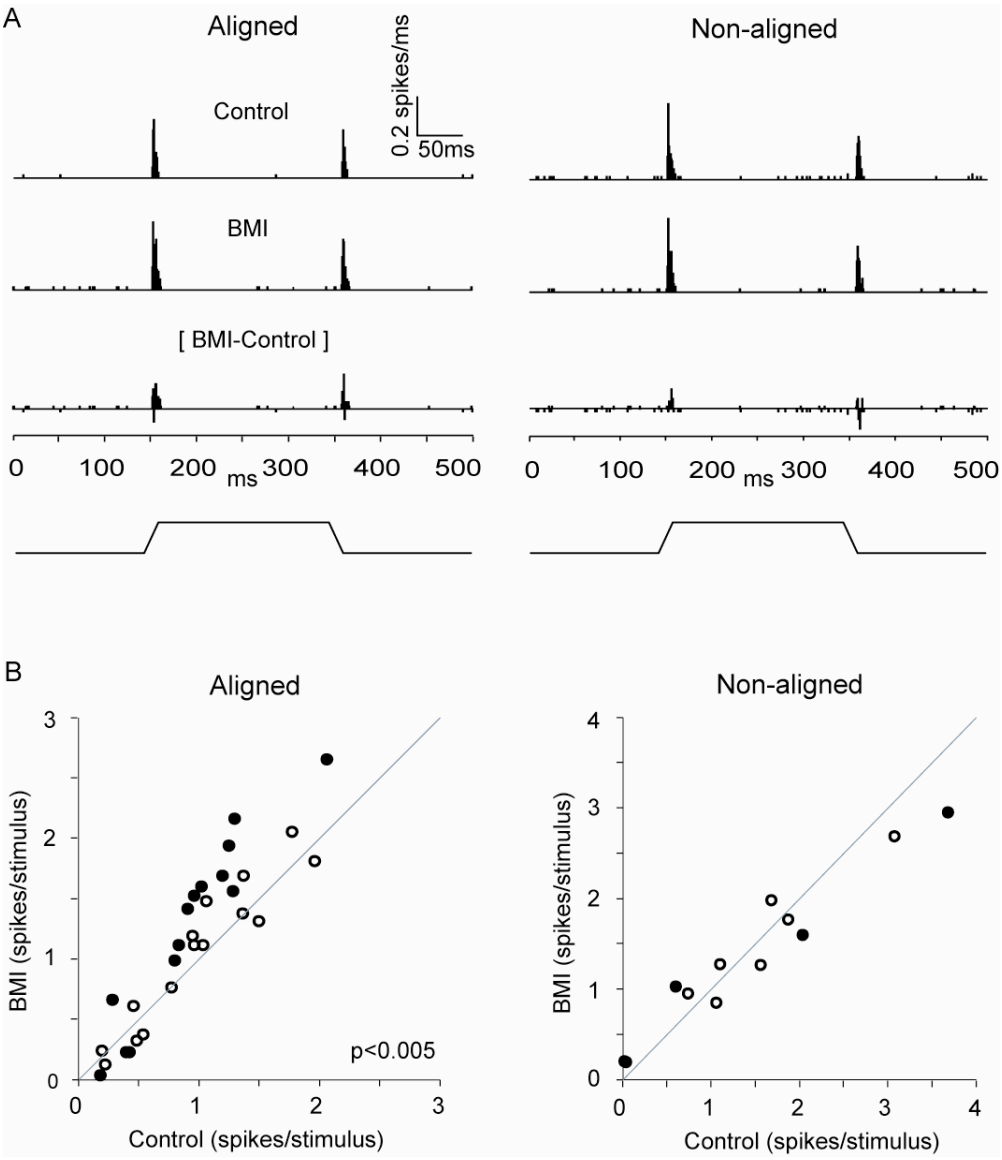


Figure 3. Effect of vMCx activation on L6 neurons in S1. (A) PSTHs show data from a single unit recorded during BMI application to a topographically aligned (left) and a non-aligned (right) region in vMCx. PSTHs (1 ms bins) illustrate accumulated responses (n=80 repetitions) of a neuron to PW deflection before (Control) and after BMI application to vMCx. Bottom PSTH shows response differential ([BMI – Control]) calculated by subtracting the control PSTH from the BMI PSTHS on a bin-by-bin basis. Stimulus waveforms are indicated below PSTHs. Units on vertical scale bar are spike probability per 1-ms bin. (B) Scatter plots show ON response magnitudes for 29 topographically aligned L6 neurons and 12 non-aligned L6 neurons in control and BMI conditions. Individual neurons which show a significant difference are indicated as a closed circle. Gray line is a unity line. *P* value based on paired t-test.

p=0.001). We also analyzed data from each neuron separately. Neurons (14 of 29) which show a significant difference between control and BMI conditions are indicated in the plots (closed circle, Fig 3B). Eleven from these 14 neurons were more responsive to whisker deflection during vMCx activation (Chi-squared test, $p<0.05$). Qualitatively similar results were obtained when comparing population means for spontaneous activity (mean 1.68-fold increase, p=0.03) and OFF responses (mean 1.26-fold increase, p=0.009). Responses during BMI facilitation were larger even when responses were corrected for increased spontaneous activity. As shown in Figure 3B, BMI-associated enhancements were more pronounced in the case of initially larger control responses (see also below). When recordings were obtained from non-topographically aligned regions in S1, no alterations in spontaneous or evoked firing were observed, even for those neurons that were highly responsive in control conditions.

Aligned S1 recordings were obtained from neurons located at microdrive readings of 1400-1750 μm below the pial surface; an additional 4 neurons (also included in our sample) were located slightly below 1200 μm. No effect of recording depth was observed (Fig. 4A). No depth-related effect of BMI application within vMCx was observed either (Fig. 4B). Taken together, the findings suggest that the net effect of tonic facilitation of deep layers of vMCx is enhancement of activity in topographically aligned infragranular S1.

Effect of vMCx activation on antidromically identified corticothalamic neurons

Layer 6 is the source of corticothalamic feedback from S1 barrel cortex to thalamic barreloids, but not all layer 6 cells are CT neurons. The reported L6 neurons above may not necessarily be CT cells. Therefore, in a separate set of experiments, we introduced an additional (third) microelectrode in VPm in order to deliver electrical stimulation for antidromic identification of

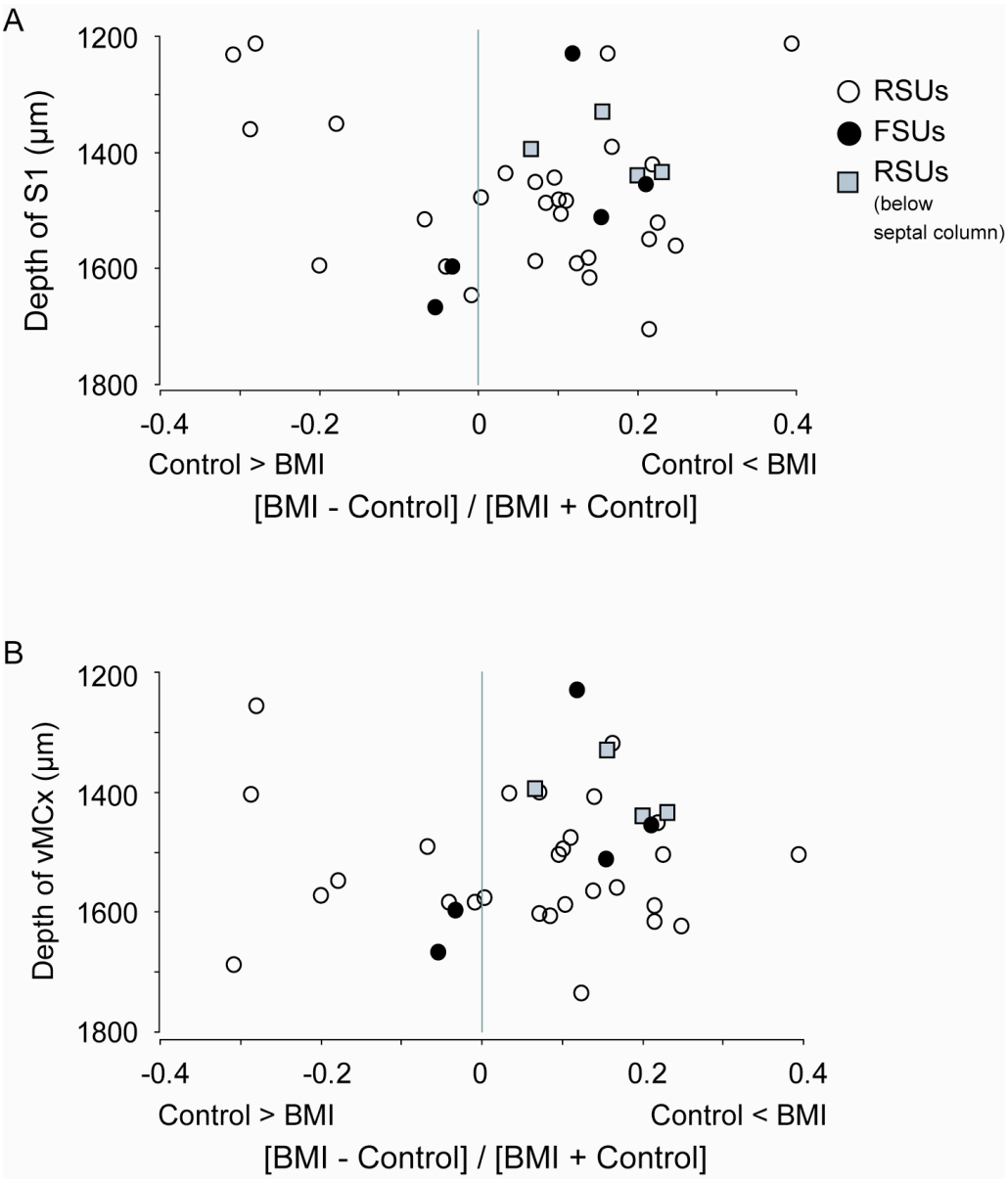


Figure 4. Effects of cortical depth measured from pial surface. (A) The effect of vMCx activation on PW-evoked ON responses of neurons in S1 was quantified as [BMI – Control] / [BMI + Control] ([BMI – Control]; this value is plotted as function of the recorded depth of each cell. Symbols denote location of cell with respect to overlying layer IV barrel field. (B) Effects in S1 as a function of the depth of BMI application in vMCx.

CT cells. Because the vast majority of CT neurons project in highly topographic fashion to VPm (Land et al., 1995), the thalamic and S1 (and vMCx) electrodes were topographically aligned. Thirty-eight antidromically-confirmed CT neurons from 14 rats were recorded at depths of 1400 to 1950 μm in S1. As observed previously, CT neurons varied widely with respect to both axon conduction velocity and the extent to which velocity increased following an action potential (i.e., supernormality) (See Table 2-1) (Swadlow 1989; Swadlow and Hicks 1996; Kelly et al., 2001). Conduction velocities varied from 2.0 to 9.2 m/s (mean 4.4 ± 0.8 m/s, median 4.1 m/s). Increases in conduction velocity in response to the second of two pulses ranged from 0.8 to 19 % (mean 9.2 ± 2.3 %, median 9.4 %). Also consistent with previous reports, most CT neurons (23 out of 38 cells) were silent, displaying no spontaneous activity and no whisker-evoked response (Swadlow 1989; Swadlow and Hicks 1996; Kelly et al., 2001). Neurons having faster axonal conduction velocities were more likely to be responsive to whisker deflection (linear regression, $p<0.05$), similar to CT cells recorded in awake rabbit S1 (Swadlow, 1989).

Activation of vMCx with BMI enhanced the responsiveness of CT neurons and in some cases led to the appearance of whisker-evoked discharges in otherwise silent cells. Figure 5 shows two examples of CT neurons modulated by vMCx activation. The cell shown in Figure 5A fired only one spike for 80 whisker deflections in control conditions, but during vMCx BM application the cell responded in a highly time-locked fashion (29 spikes / 80 stimuli) to whisker movements in a caudal-ventral direction. Interestingly, the one spike evoked during the control condition was in response to a 315° whisker deflection, and the maximum angle response (1.4 spikes/stimulus) during vMCx activation was also 315°. Figure 5B shows another CT neuron that displayed a low level of spontaneous activity but no stimulus-evoked response during 80

Table 1. Summary of CT neurons (n=38)

	Conduction velocity (m/s)	Supernormality (%)	Refractory period (ms)
Mean ± Std. Dev.	4.43 ± 0.86	9.21 ± 2.37	1.25 ± 0.22
Median	4.38	7.22	1.37

whisker deflections. During vMCx activation, this neuron became more spontaneously active and also responded to whisker deflection.

Scatterplots in Figure 6 show quantitative data for each CT neuron before (Control) and during (BMI) vMCx activation. Spike counts in the left panel are ON responses, whereas those to the right are counts of total activity during the entire 500 ms trial, including pre-stimulus spontaneous activity; the total activity measure provides an indication of the change in overall excitability. Both measures show increases during vMCx activation (control vs. BMI, mean ON 1.02±1.11 vs. 2.86±2.97, p=0.02; total activity 2.71±4.18 vs. 5.86±6.65, p=0.004: spikes/80stimuli, paired t-tests). We did not apply statistical tests to individual CT neurons, because of their low firing rates. Nineteen out of 38 CT neurons showed changes in firing rate during vMCx activation. Among these 19 neurons, all but one displayed activity increases. Of the 18 cells that displayed an increased firing rate during vMCx activation, 12 fired at least one spike during the 80 control trials. In contrast, most (17) of the 19 CT neurons that were not affected by vMCx activation were completely silent during the control period. Thus, as in the case for the L6 sample above, it appears that CT neurons are more likely to be affected by vMCx facilitation if they are active to some degree in the control condition. Nevertheless, nearly one-quarter of the silent cells (6 of 23) displayed spontaneous and/or whisker-evoked firing with vMCx BMI application. There was no dependency of changes in firing rates of CT neurons by

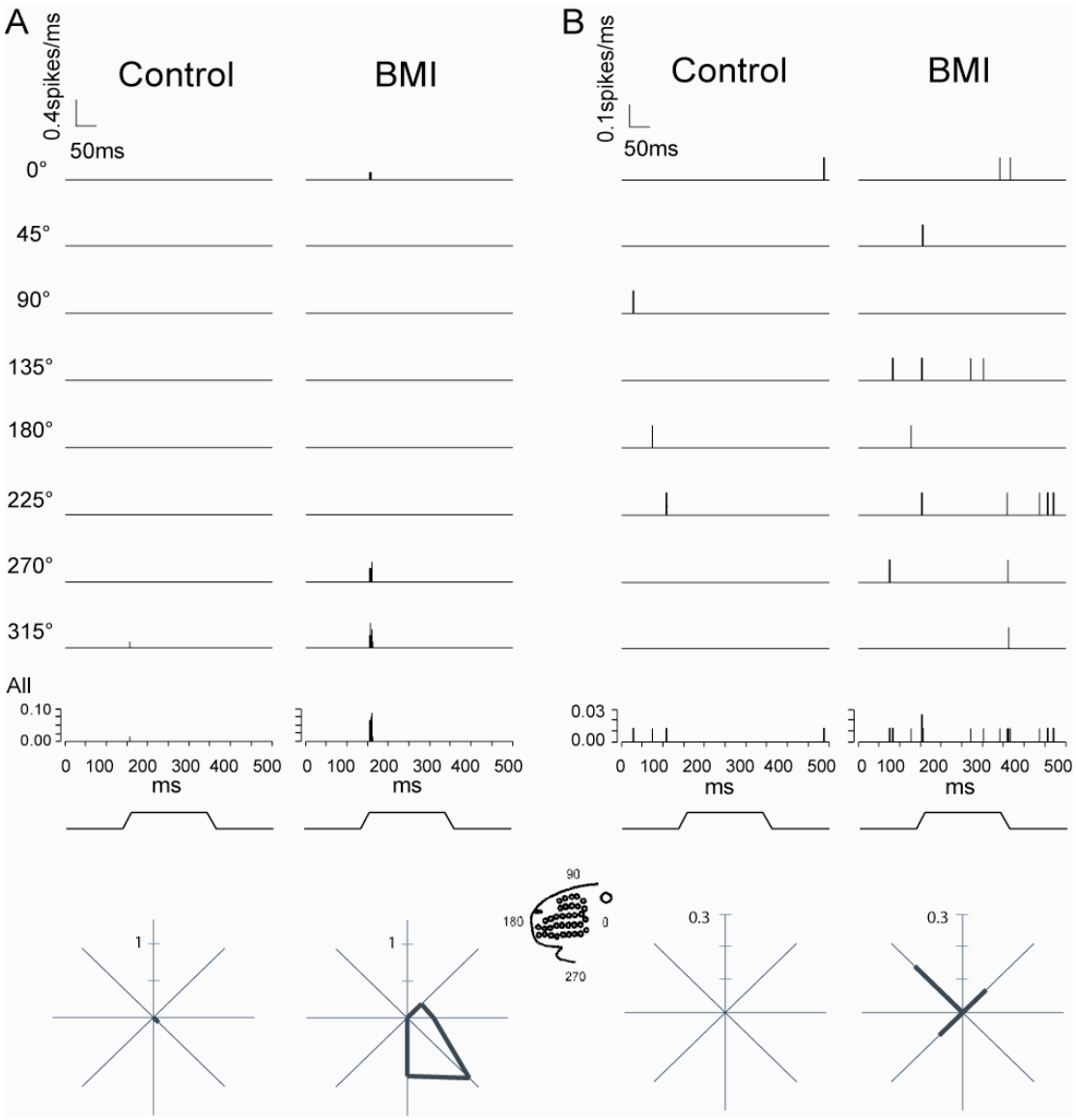


Figure 5. Effects of vMCx activation on two antidromically identified CT neurons. A, B. PSTHs shows accumulated responses of a CT neuron to PW deflection before and during vMCx activation by BMI microiontophoresis. The PW was deflected 10 times at 8 different angles, and the all-angle average responses are shown in the bottom PSTH. Stimulus waveform is indicated below it. Units on the vertical scale bar are spike probability per 1-ms bin. Polar plots illustrate responses to stimulus onsets in polar coordinates. Inset indicates orientation with respect to the face. Units on axes are spikes per stimulus onset (ON responses).

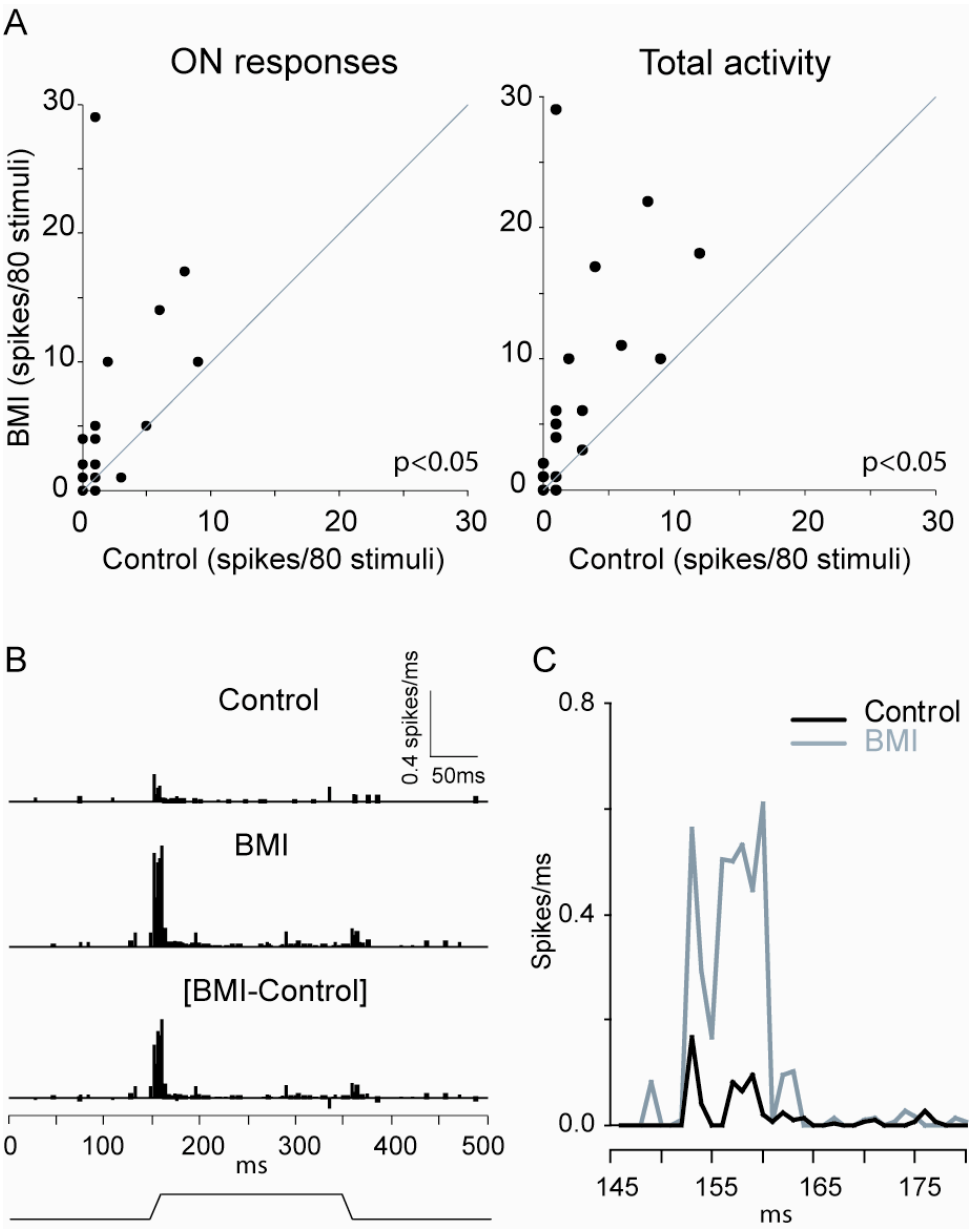


Figure 6. Effects of vMCx activation on antidromically identified CT neurons in S1. (A) vMCx activation by BMI microiontophoresis increases PW-evoked responses of topographically aligned CT neurons (n=38). ON responses (left) are spike counts during a 30 ms period following deflection onsets. Counts of total activity (right) are based on the entire 500 ms trial and thus includes spontaneous activity. Gray line denotes unity; p-values are from paired t-tests. (B) Population PSTHs constructed from all CT neurons. Conventions as in Fig. 3. (C) Overlaid PSTHs of ON responses at expanded time scale. The two peaks reflect contributions of cells having different response latencies; both peaks are enhanced during BMI application to vMCx.

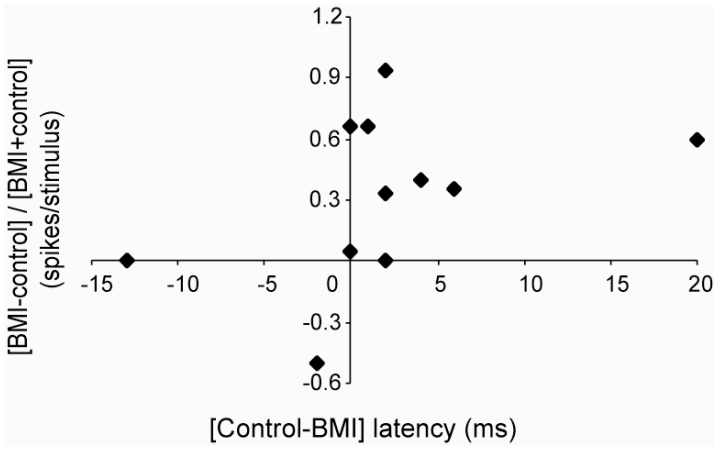


Figure 7. Effects of vMCx activation on CT response latency. Effect of vMCx activation on ON response magnitudes of CT neurons was calculated by: [BMI – Control] / [BMI + Control]. For latency measures, values > 0 indicate that ON response latency is shortened during vMCx activation. Data are based on the subset of CT neurons that fired at least one spike to stimulus onsets in control conditions (n=11).

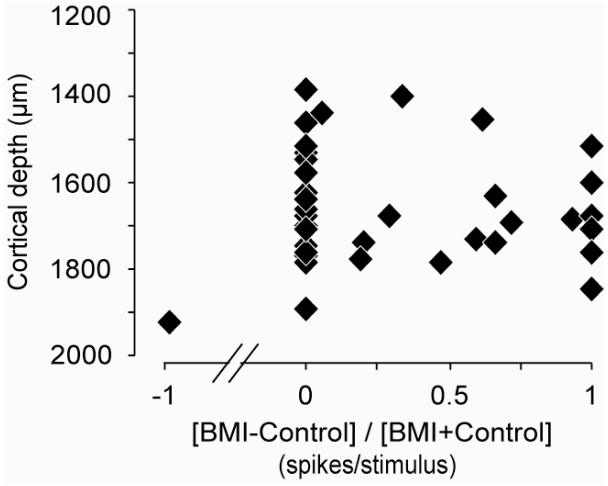


Figure 8. Effects of vMCx activation on CT neurons and their recording depth. Effect of vMCx activation on firing rates (total activity) of CT neurons as a function of their recording depth. Counts of total activity are based on the entire 500 ms trial and thus includes spontaneous activity

vMCx activation with respect to recording depth, conduction velocity, or supernormality (data not shown).

Half of the CT neurons were unaffected by vMCx facilitation. In two cases, we further enhanced MUA in vMCx by using higher BMI microiontophoretic currents (up to 45 nA) and for longer durations (an additional 5-10 min). An additional doubling of MUA in vMCx still did not affect the firing rates of these silent CT neurons (data not shown).

Population PSTHs were constructed for ON responses from the 18 neurons showing increased activity (Figure 6B). The overlaid PSTHs of ON response (Figure 6C) illustrate two peaks that reflect contributions of cells having different response latencies. Note that both peaks were enhanced during BMI application to vMCx.

We further examined effects of vMCx activation on response latency, because increased excitability of neurons is often accompanied by latency shift. Latency was defined as the average time of the first spike in the ON response window. Latency shifts were calculated as the difference between control and vMCx activation; values > 0 reflect shorter response latency during BMI application. Figure 7 plots the proportional change in ON response magnitudes as a function of latency shift. Most CT neurons that respond with more spikes to stimulus onset during vMCx activation also respond earlier. Figure 8 shows changes in CT firing as a function of recording depth. Spike counts are based on the entire 500 ms trial and thus include spontaneous activity. The majority of CT neurons showing activity increases were recorded, between 1600-1800μm. Non-affected CT neurons were distributed more uniformly.

Effect of vMCx activation on thalamic barreloid neurons

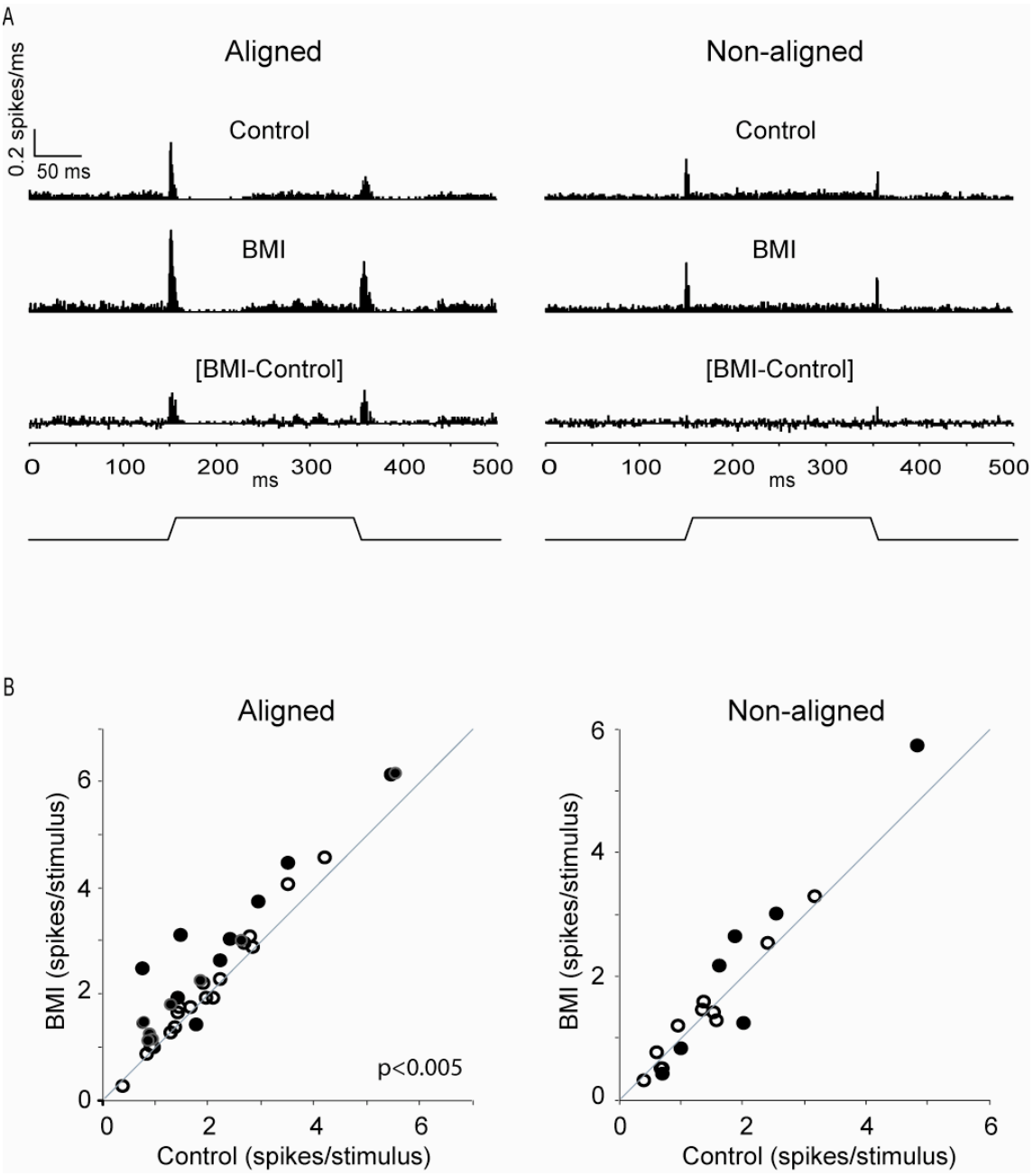


Figure 9. Effect of vMCx activation on thalamic barreloid neurons. (A) PSTHS from a neuron in a topographically aligned barreloid and a neuron in non-aligned barreloid recorded in the same experiment with the same vMCx BMI application site. Each cell's PW was deflected in the caudal direction only (n=100 trials). Other conventions as in Fig. 3. (B) Data from 36 neurons in aligned and 18 neurons in non-aligned barreloids. Individual neurons which show a significant difference in ON response are indicated as a closed circle. Other conventions as in Fig. 3.

Facilitation of vMCx increases the excitability of CT neurons in barrel cortex in topographic fashion. Next, we asked whether such increased firing in turn selectively affects neuronal responses within the thalamic barreloid to which the CT neurons project. To do this we recorded from topographically aligned and non-aligned VPm neurons during vMCx BMI application, as above. Two examples are shown in Figure 9A. PSTHs at the left show responses of a neuron to PW (B2) deflection before and during BMI microiontophoresis in vMCx. Facilitation of vMCx increases the excitability of CT neurons in barrel cortex in topographic fashion. Next, we asked whether such increased firing in turn selectively affects neuronal responses within the thalamic barreloid to which the CT neurons project. To do this we recorded from topographically aligned and non-aligned VPm neurons during vMCx BMI application, as above. Two examples are shown in Figure 9A. PSTHs at the left show responses of a neuron Evoked ON responses were significantly increased (Control vs. BMI, ON 1.49 vs. 3.10 spikes/stimulus, $p<0.05$; spontaneous activity 18.25 vs. 31.50 Hz) when BMI was injected into the motor cortex at a site corresponding to row B whiskers (B1, B2 and A1). Thirty minutes after cessation of BMI microiontophoresis, the VPm recording electrode was moved, and a unit was isolated in the D1 barreloid, while maintaining the BMI electrode at the same row-B vMCx site. BMI application failed to alter the thalamic cell's (D1) whisker-evoked or spontaneous firing rates (Control vs. BMI, ON 1.33, BMI 1.43 spikes/stimulus; spontaneous activity 11.79, BMI 18.20 Hz).

Scatter plots in Figure 9B compare ON responses of individual thalamic barreloid neurons before (Control) and during vMCx activation (BMI). PW-evoked ON responses of neurons in aligned barreloids are on average significantly higher during vMCx activation than those during control condition (Control vs. BMI means, ON 2.01 ± 0.62 vs. 2.37 ± 0.69 spikes/stimulus, $p<0.0005$, paired t-test). Nearly half (18 of 36) showed a significant difference between control and BMI

conditions (indicated by closed circles). Except one neuron, the 17 neurons were significantly more responsive to whisker deflection during vMCx activation (Chi-squared test, $p<0.001$). In addition, neurons displayed greater spontaneous activity, OFF, and plateau responses during BMI application to vMCx (control vs. BMI, OFF 1.48±0.47 vs. 1.79±0.54 spikes/stimulus, $p<0.0005$; plateau 0.36±0.17 vs. 0.46±0.21, $p<0.0005$, paired t-tests). When vMCx BMI application and barreloid recording sites were not topographically aligned, however, vMCx facilitation did not affect spontaneous or whisker-evoked firing rates (control vs. BMI mean, ON1.63±0.55 vs. 1.72±0.68 spikes/stimulus, $p>0.5$; paired t-test). Only four from seven neurons, which showed significant changes during vMCx activation, became more responsive to whisker deflection (Chi-squared test, $p>0.05$).

We further tested the topographic-dependency of vMCx effects on thalamic VPm neurons by simultaneously recording pairs of neurons (n=9 pairs). For each pair, one neuron was topographically aligned and the other was not; BMI was applied at the same single site for testing both neurons. The proportional change in ON response firing rates produced by BMI are shown in the scatter plot of Figure 10A. Most data points lie in the quadrant representing increased responses in aligned barreloids and small or no changes in non-aligned barreloids. Specifically, ON responses of neurons recorded from aligned barreloids increased on average of 57%, whereas those of non-aligned barreloid neurons remained at control levels (Fig. 10B, BMI/Control ratio, aligned 1.57±0.35; vs non-aligned 0.9±0.13; $p<0.05$ paired t-test). We conclude that enhanced activity within vMCx increases the spontaneous activities and whisker-evoked responses of topographically aligned thalamic VPm neurons in a fashion consistent with activation of S1 corticothalamic neurons.

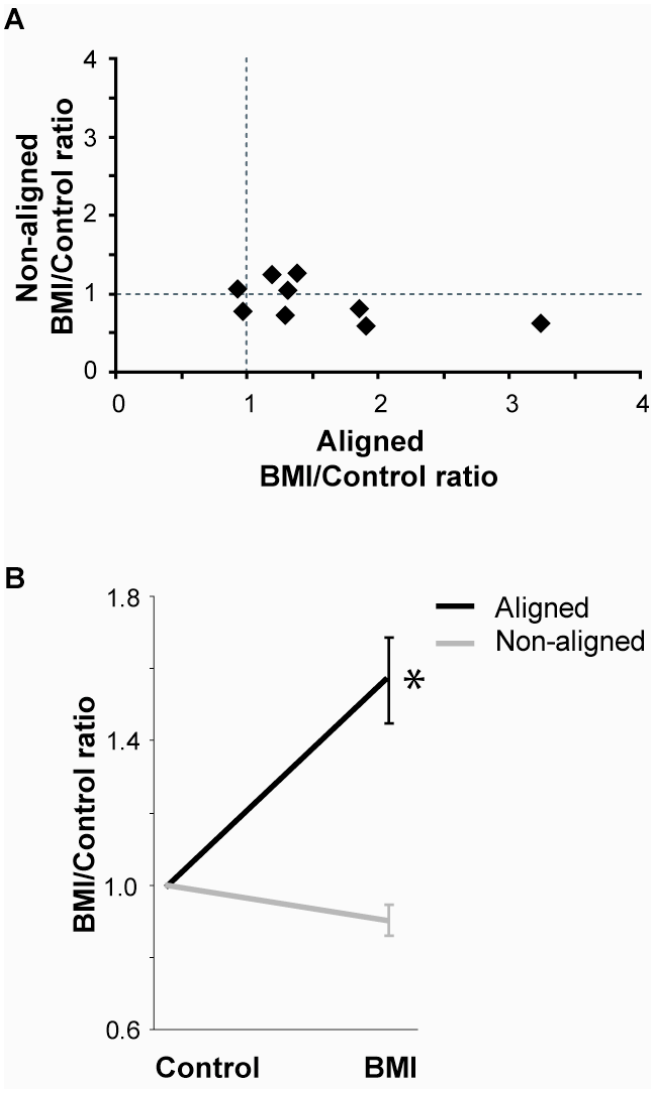


Figure 10. Topographically specific effect of vMCx activation on thalamic barreloid neurons. (A) Proportional changes in ON responses to PW deflection by vMCx activation are compared in simultaneously recorded two neurons (n=9 pairs) from aligned and non-aligned barreloids relative to vMCx activation site. (B) Normalized and averaged thalamic neuronal response during BMI application to that during control condition. BMI application to vMCx significantly increases evoked responses of aligned barreloid neurons but does not affect on non-aligned barreloid neurons (paired t-test, * $p<0.05$).

Effect of vMCx activation on thalamic barreloid neurons in the absence of corticobulbar projections

vMCx facilitation effects on VPm neurons could be mediated by S1 corticothalamic neurons, but also more indirectly by effects on brainstem centers that influence afferent activity to VPm. In order to eliminate potential sub-thalalmic influences, in two experiments we ablated corticobulbar fibers. At the beginning of each experiment we made a series of small electrolytic lesions in the crus cerebri near the caudal end of the diencephalon (Fig. 11C and see also Temereanca and Simons, 2004) and subsequently recorded from VPm neurons, as above. In the absence of corticobulbar fibers, vMCx facilitation still enhanced the firing rates of thalamic neurons. Similar to data obtained in intact animals, effects were topographic. In the case illustrated in Figure 11A, BMI was applied in the row-C area of vMCx and ON responses of a neuron in the C1 barreloid increased from 0.93 to 2.01 spikes/stimulus ($p<0.05$). The thalamic microelectrode was then moved to a non-aligned barreloid (E1), while maintaining the row C vMCx BMI site. The firing of the non-aligned neuron was largely unaffected (ON control 0.99, BMI 0.89 spikes/stimulus).

A scatter plot in Figure 10B shows the effect of vMCx activation on 11 neurons recorded from topographically aligned barreloids in the two experiments. In the absence of corticobulbar fibers, vMCx activation still enhanced evoked ON responses of aligned thalamic barreloid neurons (Control vs. BMI mean; 1.50±0.38 vs. 1.73±0.32 spikes/stimulus, $p=0.05$). To ensure that the lesion of corticobulbar fibers did not itself alter the response properties of barreloid neurons, we compared control ON responses from lesion-bearing animals to those obtained in intact animals and found that they were equivalent ($p=0.13$). These results support the conclusion that CT neurons are responsible for topographically specific transmission of the effects of MCx activation on thalamic barreloids.

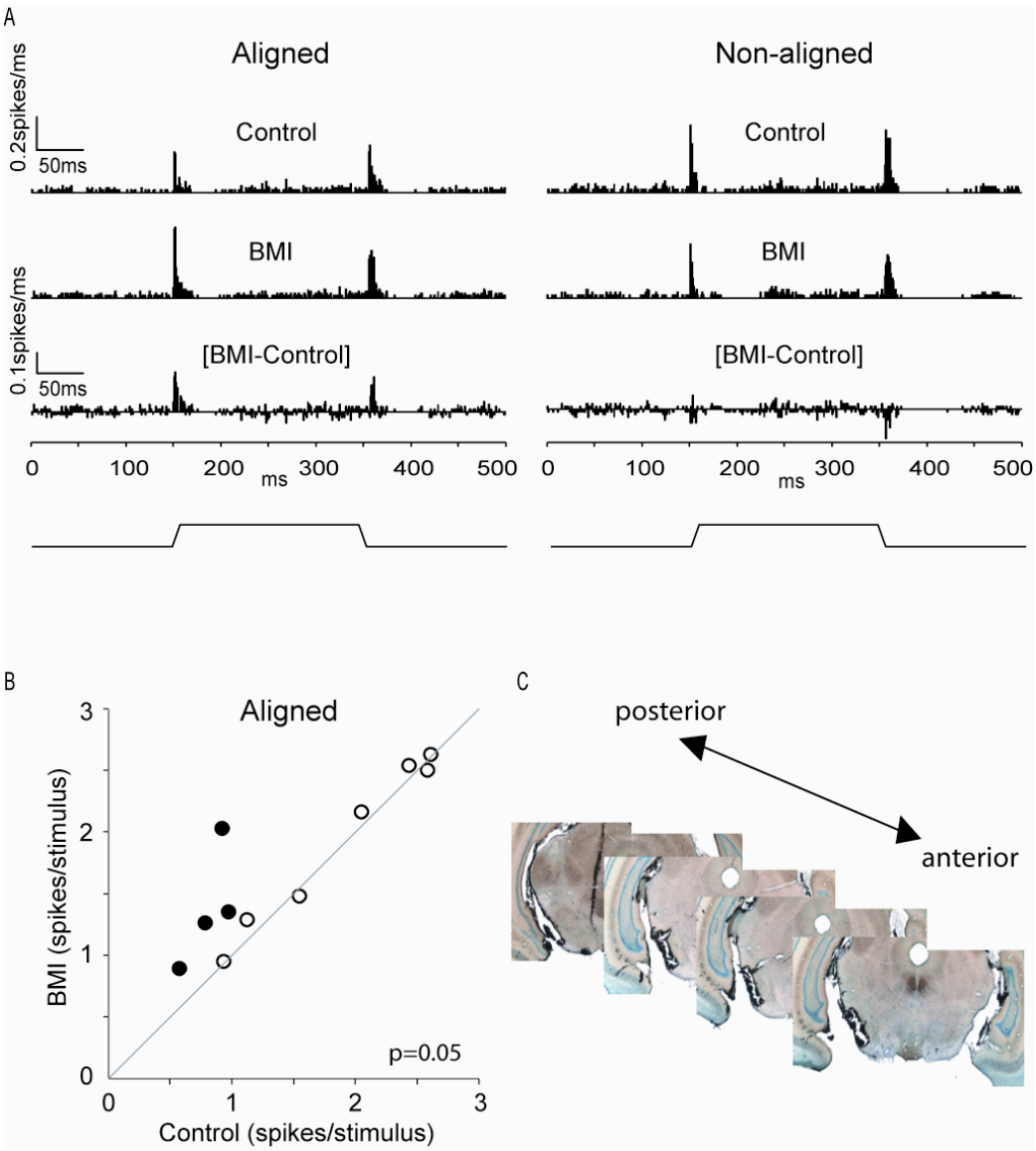


Figure 11. vMCx activation affects thalamic barreloid neurons after ablation of corticobulbar fibers. Multiple, small electrolytic lesions were produced in the right cerebral peduncle prior to recordings. (A) Responses of the cell in the aligned but not the non-aligned barreloid are enhanced. Other conventions as in fig. 3. (B) Scatter plot showing effects in 11 aligned neurons studied in two experiments. Individual neurons which show a significant difference are indicated as a closed circle. Other conventions as in fig. 3. (C) Photomicrographs of sections through the caudal end of the diencephalon; arrows indicate damaged fiber tract in the animal's right hemisphere. Coronally sectioned tissue (70 μm) was reacted for cytochrome oxidase and counter-stained with thionin.

2.4 DISCUSSION

The present findings demonstrate that increased motor cortical activity leads to facilitation of neural responses in thalamic and cortical neurons involved in processing afferent somatosensory information. Activity within a small area of motor cortex was facilitated by microiontophoresis of BMI, producing an approximately 3-fold increase in spontaneous firing of nearby motor cortical neurons. We used this pharmacological approach in order to simulate tonic elevations in motor cortical activity observed during whisking in behaving rats (Carvell et al., 1996; Friedman et al., 2006). Results demonstrate that motor cortex facilitates activity in deep layers of primary somatosensory cortex. The affected neural population includes corticothalamic neurons that project to VPm, the primary thalamic relay for discriminative touch. Results are consistent with known anatomical projections from deep layers of vMCx to infragranular laminae of somatosensory barrel cortex (Zhang and Deschênes, 1998; Veinante and Deschênes, 2003). Correspondingly, responses of neurons in VPm were also enhanced. A particularly interesting and potentially important finding is that motor cortex exerts its influence on cortical and thalamic neurons in a topographic fashion. In addition, fast spiking units in S1, presumably inhibitory neurons, were modulated similarly to putative excitatory neurons.

While anatomical projections between vMCx and S1 have been described, the detailed circuitry of the projections within L6 of S1 has not been investigated (Mercer et al., 2005; Rocco and Brumberg 2007). Our findings suggest that motor cortex affects infragranular neurons that project to VPM thalamus as well as those that do not. Whether all of the affected cells comprise a specific functional network or whether the cells belong to different or multiple networks is unclear (Rocco and Brumberg 2007). Barrel cortex also projects to vMCx, and projections are somewhat topographic (Miyashita et al., 1994; Hoffer et al., 2003; Alloway et al., 2004). Taken

together with the present functional results, the findings suggest spatially specific linkages between sensory and motor cortices that can mediate sensorimotor integration at the level of the barrleloid-to-barrel thalamocortical circuit.

Excitability/driving source of CT neurons

Mounting evidence from across different sensory systems suggests that cortical feedback modulates sensory-driven responses of thalamic neurons depending upon both the spatial alignment of receptive fields and response tuning (Baker and Malpeli 1977; Tsumoto et al., 1978; Yuan et al., 1985; Ghosh et al., 1994; He 1997; Ergenzinger et al., 1998; Rauschecker 1998; reviewed in Sillito and Jones, 2002; Webb et al., 2002). In the whisker-to-barrel system, Temereanca and Simons demonstrated that CT projections enhance responses in the topographically aligned VPm barreloids and subtly suppress them in immediately adjacent, non-aligned areas in VPm (2004). Effects are thought to be produced both by direct excitatory corticothalamic feedback and by indirect inhibitory effects mediated by cortical inputs onto neurons in the thalamic reticular nucleus (nRT), the sole source of inhibition within rat VPm. The same circuitry may be engaged by our motor cortex manipulation. Notably, neuronal responses in VPm were enhanced by BMI injection in topographically corresponding regions of vMCx. We did not, however, observe clear suppressive effects in non-aligned barreloids. This may reflect our recording from VPm neurons representing whiskers more distant from the focus of CT activation rather than the immediately adjacent representations studied by Temereanca and Simons (2004).

Alternatively, motor cortex may engage a specific subset of corticothalamic neurons, that is, cells that project to VPm but not nRT. CT neurons in barrel cortex that project to VPm are

heterogeneous. They vary in terms of depth, axon conduction properties (Swadllow 1989, 1991; Kelley et al., 2001) as well as peripheral responsiveness. Landry and Dykes (1985) classified antidromically identified CT neurons in cats as type I or type II, based on the presence or absence of spontaneous activity and responses to tactile stimulation. We found that vMCx facilitation typically increased the firing of both active and normally silent CT neurons, though the latter were less likely to show an effect. Swadlow reported that the responsiveness of CT neurons in awake, restrained rabbits is correlated with axonal conduction velocity; neurons having faster conducting axons are more likely to be responsive to peripheral stimulation (1989). We observed a similar correlation between axonal conduction velocity and responsiveness. Motor cortex effects on identified CT neurons were, however, unrelated to axon conduction velocity, supernormality or depth within the infragranular layers. Our data thus suggest that all components of the CT-VPm-nRT circuitry are engaged by motor cortex.

A striking feature of CT cells is that many, perhaps a majority, are weakly responsive or altogether silent in control conditions; "silent" cells can be observed in extracellular studies only by means of antidromic stimulation (Landry and Dykes 1985; Swadlow 1989; Swadlow 1994; Swadlow and Hicks 1996; Kelly et al., 2001). Large proportions of silent cells, as many as 90% in some studies, have been observed in sensory cortex even in awake animals (Swadlow 1989; Swadlow and Hicks 1996) and in motor cortex during complex locomotion (Beloozerova et al., 2003; Sirota et al., 2005). The poor responsiveness of CT neurons seems incongruous with anatomical findings that CT neurons are located within an infragranular thalamorecipient zone and that many receive monosynaptic thalamic inputs (White and Keller, 1987; Chmielowska et al., 1989; Land et al., 1995). The present finding demonstrates that, in rat primary somatosensory cortex, weakly responsive CT neurons, and even some that are otherwise silent,

become more responsive to whisker stimulation during pharmacologically induced facilitation of the topographically corresponding area in vMCx. Thus inputs from other, functionally related cortical areas can influence the excitability of S1 CT neurons and hence the processing of afferent, sensory signals in thalamocortical circuits. Similarly, secondary somatosensory cortex (SII) may engage CT cells in barrel cortex, as suggested by anatomical studies demonstrating abundant projections from SII to deep layers of S1 (Zhang and Deschênes, 1998; Chakrabarti and Alloway, 2006).

Organization of vMCx

We applied BMI focally within the region of motor cortex previously found to be tonically active during whisking (Carvell et al., 1996; Friedman et al., 2006). Such activity is thought to contribute to rhythmic whisking by the engagement of central pattern generating mechanisms within the brainstem (Gao et al., 2001; Hattox et al., 2002; Hatttox et al., 2003; Cramer and Keller 2006; Cramer et al., 2007). This area of motor cortex, lateral agranular frontal cortex (but see Neafsey et al., 1986 and Brecht et al., 2004b), has been considered to be part of the larger face representation within the primary motor cortex map (Donoghue and Wise, 1982; Gioanni and Lamarche, 1985). Low-threshold electrical stimulation here can produce movements of a small number of neighboring whiskers organized in a loose topographic manner along whisker rows (Hoffer et al., 2003). Evoked whisker movements are retractive, and larger stimulating currents can also produce more generalized facial movements.

Topographic organization, albeit sparse, implies some degree of independent control of single whiskers or groups of neighboring whiskers. Though rats typically move all their whiskers synchronously (Carvell and Simons, 1990; Fee et al., 1997; Gao et al., 2001; Berg and

Kleinfeld, 2003; Hattox et al., 2003; Sachdev et al., 2003), fractionation of whisking has been observed in behaving animals (Sachdev et al., 2003). In this regard, our finding of topographic-dependent vMCx facilitation effects on cortical and thalamic activity raises the interesting possibility that spatial patterns of motor cortex activity can differentially enhance the processing of afferent information within the sensory thalamocortical system in a context-dependent fashion. Indeed, whisking is only one component of a constellation of complexly integrated motor behaviors involving face, neck and respiratory muscles (Welker et al., 1964; Carvell and Simons, 1990). During exploratory or discriminative behaviors, animals may orient the head in order to bring particular sensory receptors (e.g., mouth and ventral whiskers) to bear on objects of interest. Spatially organized sensorimotor systems, such as that described here involving motor cortex and afferent thalamocortical systems, could function to enhance sensitivity of relevant sensory processes.

A recent study demonstrated an additional non-topographically organized whisker related area in vMCx from which rhythmic whisking, involving both protraction and retraction, can be evoked by trains of electrical stimuli (Haiss and Schwarz, 2005). This 'rhythmic whisking' area is posteromedial to the larger vMCx/face area and appears to be located in agranular medial (AG_m) motor cortex. Current injections into single neurons in AG_m motor cortex or immediately nearby can evoke whisker retraction with some degree of topography (Brecht et al., 2004a,b). The interrelationships between these more medial areas and the lateral zone remain to be clarified. Nonetheless, we performed several experiments in which we applied BMI in the "rhythmic area", defined functionally in the same manner as Haiss and Schwartz (2005). Preliminary data showed facilitation of layer 6 cells in barrel cortex and of VPm neurons, as observed for the more lateral whisker/face area.

The present finding suggests that vMCx can modulate thalamic sensory processing via the corticothalamic pathway with topographic manner in whisker-barrel system.

3.0 WHISKER MOVEMENT-RELATED SUPPRESSION AND FACILITAITON OF THALAMIC ACTIVITY IN THE WHISKER-BARREL SYSTEM

3.1 INTRODUCTION

Experiments described in Chapter 2 demonstrate that motor cortex facilitates activity in deep layers of primary somatosensory cortex, including corticothalamic neurons that project to VPm, the primary thalamic nucleus for discriminative touch. Correspondingly, responses of neurons in VPm were also enhanced. This modulation by motor cortex of cortical and thalamic neurons is topographically specific. The findings raise the possibility that CT neurons in S1 may be involved in regulating afferent thalamic activity during voluntary movement.

Findings in Chapter 2 appear, at first glance, to be at variance with results of many studies showing that sensory transmission through sub-cortical nuclei is suppressed or "gated" during movement (see Chapter 1) (Fanselow and Nicolelis 1999; Castro-Alamancos 2004; Hentschke et al., 2006). For example, Fanselow and NIcolelis described reduced firing of rat VPm neurons to electrical stimulation of the trigeminal nerve during whisking vs. non-whisking states. However, in that study animals moved freely about their cage, incidentally contacting objects with their whiskers and evoking afferent activity that may have interfered (e.g., occluded) experimentally produced afferent volleys evoked by electrical stimulation of the nerve. In addition, the electrical stimulation was delivered proximally in the nerve, likely activating large

numbers of somatopically intermingled fibers, including perhaps small diameter axons, that might not normally be engaged synchronously or near-synchronously during behavior. In contrast to the Fanselow and Nicolelis findings, Yuan et al. (1985, 1986) provided evidence that thalamic activity may be facilitated during voluntary movement. In their study, thalamic activity was evoked by electrical stimulation of the medial lemniscus, thus by-passing integration centers in the brainstem trigeminal nuclei.

Experiments in this Chapter examine afferent-evoked thalamic activity in animals that are either alert but voluntarily relatively motionless or actively whisking in the air without object contact. Afferent activity is evoked in VPm by means of electrical microstimulation of a single whisker follicle. In some experiments, neural processing in brainstem trigeminal nuclei was either by-passed by means of medial lemniscus stimulation, as in the studies of Yuan et al. (1985, 1986), or altered by pharmacological intervention. As reported by others, whisker-evoked thalamic activity is reduced during whisking and under conditions where brainstem circuitry is functioning normally. Thalamic activity is enhanced, on the other hand, when trigeminothalamic signals are either by-passed or experimentally altered. Together with results from the studies of Chapter 2, the findings suggest that during movement afferent activity is globally suppressed in the brainstem while it is simultaneously enhanced in the thalamus, perhaps in topographic and context-specific fashion.

3.2 METHODS

Surgical procedures

Data were collected from seven adult female rats (Sprague-Dawley strain; Hill Top, PA) weighing initially 200-300g. Rats underwent a series of aseptic surgeries under isoflurane anesthesia. During surgery, rats were placed in a stereotaxic apparatus. During the first surgery, 7-9 stainless steel screws were inserted into the skull to anchor a dental acrylic cap which would later serve to secure recording electrodes and an electrical connector. Ground and reference wires were implanted in the brain (Prigg et al., 2002).

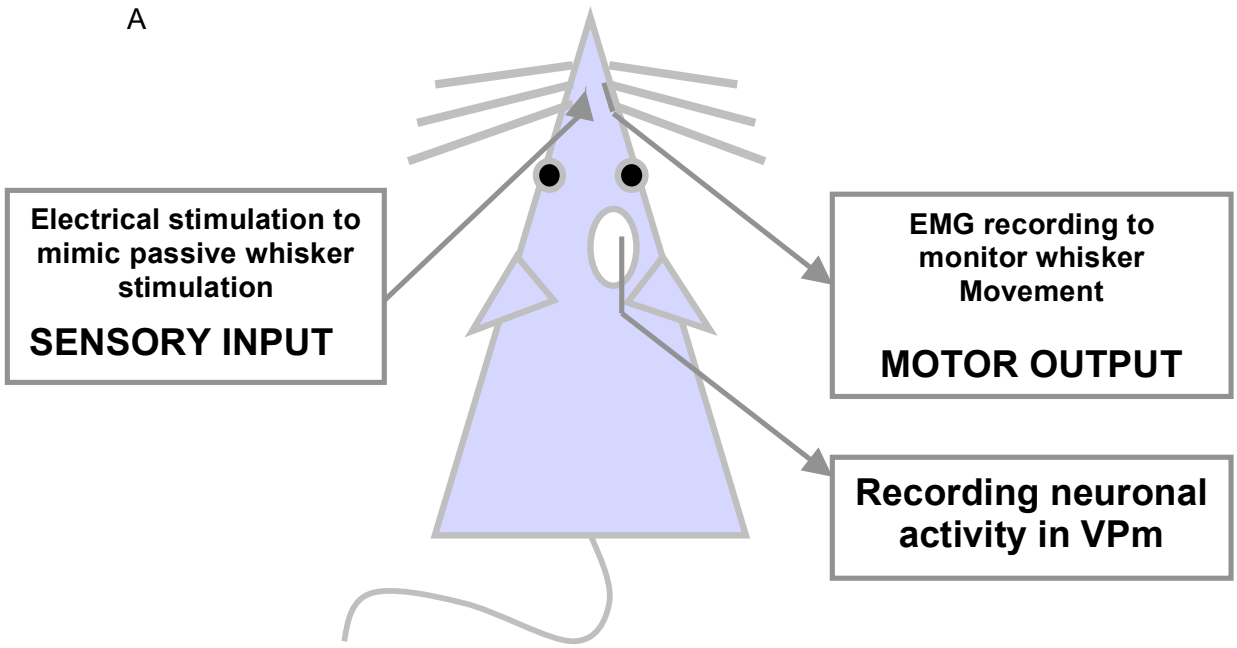
Within a week, a second surgery was performed to implant microelectrodes and to affix the electrical connector. A small craniotomy was made over thalamic VPm (3.0mm posterior to bregma, 3.0mm lateral to the midline). Depending on the experiment, another craniotomy was made over the medial lemniscus (6.0mm posterior to bregma, 1.2mm lateral to the midline). An epoxylite-coated platinum-iridium wire (0.8-3 MΩ at 1000 Hz) was slowly advanced into VPm while a hand-held probe was used to identify a cluster of neurons that clearly responded to a caudal whisker (e.g., D2). The microwire was fixed to the skull by means of the screw-anchored dental acryl cap. Similarly, a stainless steel electrode (0.2-0.5 MΩ at 1000 Hz) was implanted within the medial lemniscus, identified physiologically by whisker-evoked responses (see Yuan et al., 1986).

Following several days of recovery whisker follicle stimulating wires and EMG recording wires were implanted in the face. For stimulating a whisker follicle, a pair of Teflon-coated stainless steel 0.003" wires was tunneled under the skin to the mystacial pad contralateral to the VPm recording site. The ends of the wires, whose terminal 1 mm ends were stripped of insulation, were led out from the skin immediately adjacent to the whisker corresponding physiologically to the site of implantation of the VPm electrode. The stimulating wire was bent back on itself and inserted into the whisker follicle, and the other wire was inserted nearby into

the skin to serve as the indifferent electrode. If necessary, the stimulating wire was sutured to the skin where it looped back on itself. In order to monitor whisking, EMG electrodes were inserted into the opposite mystacial pad, ipsilateral to the VPM recording site. Whisking is typically bilateral, so ipsilateral EMG records are good indicators of whisker motor output. A small incision was made in the cheek, and, as above, a pair of stripped Teflon-coated stainless steel wires (0.003") was tunneled under the skin. The distal ends of the wires were threaded into a hypodermic needle, and the stripped ends were bent back over the bevel. The needle was then inserted subcutaneously deep into the middle of the mystacial pad and withdrawn, leaving the wires hooked into the muscle. The viability of the electrodes was verified by using low-intensity electrical stimulation to elicit protraction of a small number of nearby whiskers (for details, see Carvell et al., 1991). The rats were allowed to recover from this last surgery for 4-5 days prior to recording.

Behavior

Behavioral training was initiated 1-2 weeks prior to the first surgery. The rat was trained to stand on a small (5 x 7.5 cm) elevated platform 34cm in height (Fig. 12B). A T-shaped bar of similar width was placed in front of the platform at the same height. Rats were trained to hold this T-shaped bar with the forelimbs by leaning forward. This position ensured that whiskers did not make contact with any surrounding objects so that during the experimental trials animals "whisked-in-air". The rat was water-deprived between experimental sessions. During a trial a plastic tube connected to a syringe containing sweetened water was moved within a field close to the animal's face. Rats would search for the tube by whisking; the animal was allowed to contact the tube and receive a water reward after several seconds of searching/whisking.



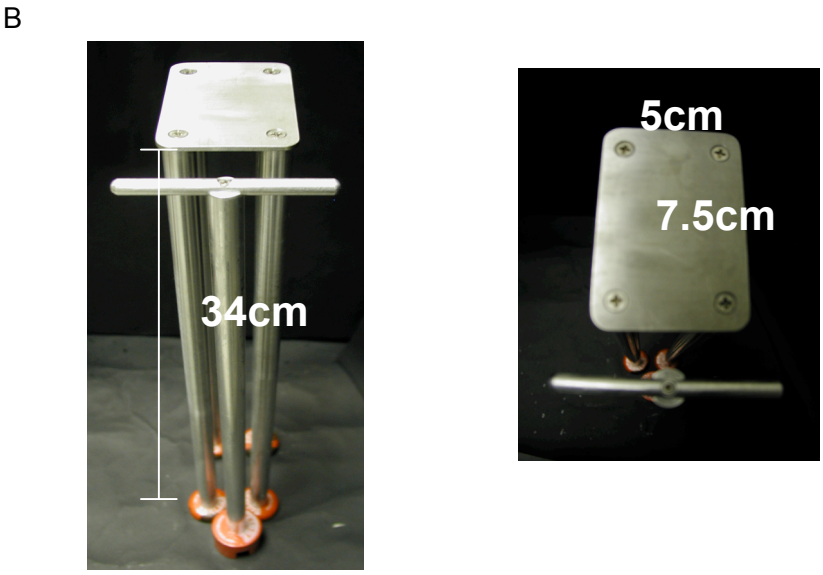


Figure 12. Behavior experimental paradigm. (A) Schematic illustration of experimental paradigm. Electrical stimulation applied to either whisker follicle or medial. Whisker movement was monitored with an EMG wires. An electrode was implanted into thalamic VPm to record neuronal activity. (B) Behavior experimental setup. Animal was placed on an elevated small platform. Measurements in each panel indicate measurement of actual size.

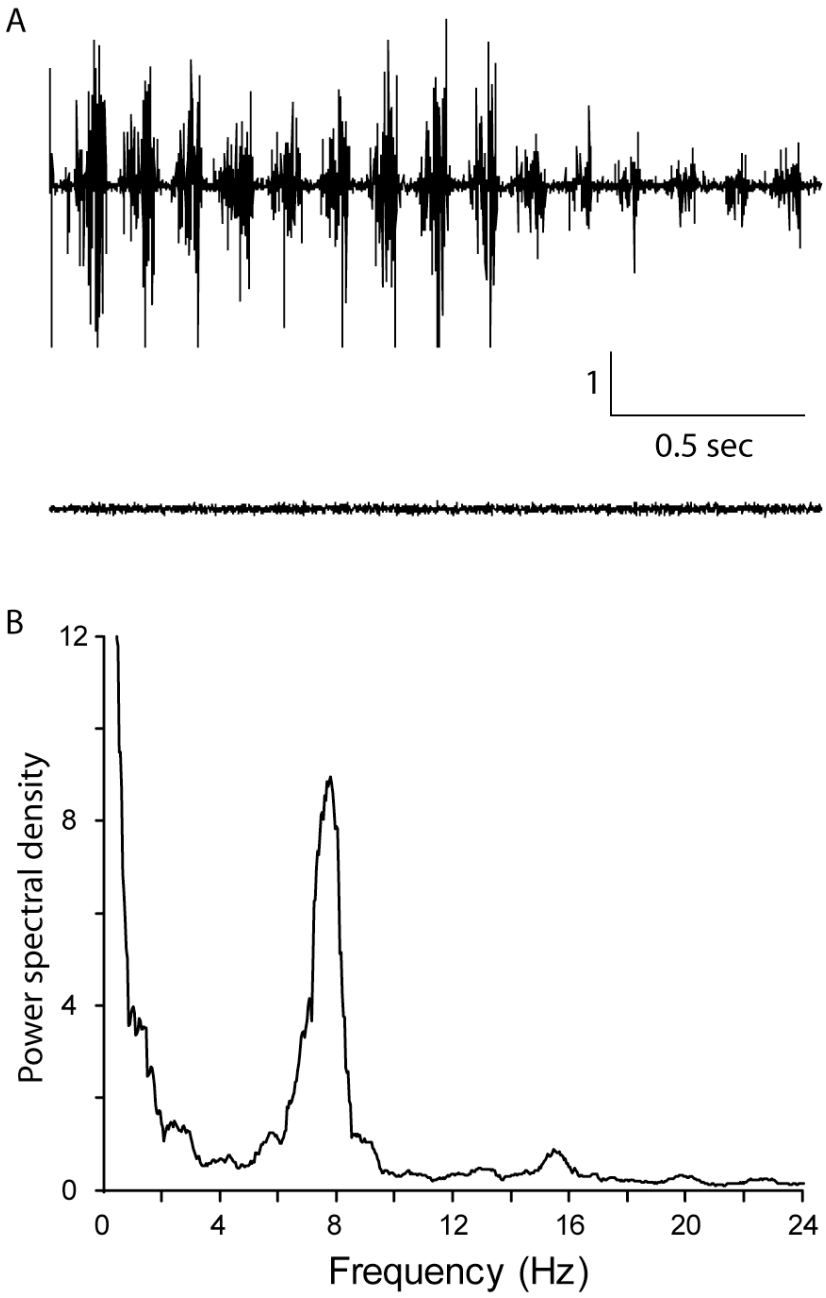


Figure 13. EMG activity. (A) Example of EMG activity during whisking (top) and non-whisking (bottom) over a 2 sec period. (B) Power spectral density of rectified EMG showing peak at 6-9Hz.

Daily recording sessions lasted for 30-90 minutes. A visual record was made of the animal's behavior using a Super VHS video camera equipped with a macro lens and a high-

speed electronic shutter that opens 60 times per second for 1 ms. In addition, we recorded VPm and EMG activity on the audio tracks of the VHS tape. After the recording session, the rat was given unrestricted access to water for one hour. Offline, data recorded on VHS tapes were replayed through the data recorder to identify sustained periods of whisking and non-whisking. These whisking and non-whisking periods were confirmed by reference to recorded EMG activity.

Electrical stimulation

Whisker-afferent pathways were stimulated electrically during periods of whisking and non-whisking behavior. For whisker stimulation, a single whisker follicle was stimulated via the inserted stainless steel wire. Whisker-follicle stimulation (WF) was delivered through a Grass physiological stimulator and a constant-current isolator. Stimuli consisted of 200-800μA pulses of 0.04-0.05ms duration at 1 Hz; the follicle stimulating wire was connected to the negative pole of the isolator. Stimulating current was set 1.5-2.0 times the level needed to evoke small but reliable VPm responses. Thresholds were tested at the beginning of each daily recording session. We found that the follicle-stimulating wire remained stably in place for about 7-10 days.

Two rats were implanted with medial lemniscal stimulating electrodes; one of the animals also had a whisker follicle stimulating wire. Medial lemniscus stimulation consisted of negative 40-80μA pulses of 0.03-0.05 ms duration delivered at 1 Hz. As in the case of WF, current intensity was set 1.5 - 2.0 times above threshold. In some recording sessions, paired-pulse stimulation (inter pulse interval = 25ms) was used. We did not observe any disturbance of the rats' behavior due to electrical stimulation of either the follicle or the medial lemniscus.

Inactivation of SpVi

In three rats we implanted a stainless steel cannula (outer diameter 0.042", inner diameter 0.033") targeting the caudal aspect of the interpolaris subdivision of the spinal trigeminal nucleus (SpVi); this subnucleus is a major source of inhibitory input to cells in the Principal Sensory nucleus (PrV) in brainstem (Timofeeva et al., 2003), the second-order lemniscal relay in the whisker-to-barrel pathway. SpVi was found using stereotaxic coordinates and confirmed by physiological recordings; SpVi coordinates were 13-14 mm caudal to bregma and 3.2-3.6 mm from the midline with a depth of 6.5-7 mm (see Timofeeva et al., 2004; Timofeeva et al., 2005; Kwegyir-Afful et al., 2005). The cannula was implanted during the same surgery in which the VPm electrode and electrical stimulating wires were implanted, and animals recovered for one week. The cannula was used to infuse 8-10µl bupivacane (5mg/ ml, Hospira, IL), Na^+ channel blocker, using a syringe pump (0.8µm/min, Sage Instruments, CA). Five minutes after termination of drug infusion, we collected another data set. The recording session lasted 30-45min. We did not record data after drug wash-out during that recording session.

Electrophysiological recordings

EMG and neural (VPm) signals were recorded using custom-made, high-input impedance, differential amplifiers embedded in a cable that connected the animal to secondary amplifiers and filters (Grass P15 amplifiers). EMG signals were filtered at 0.3-3 kHz, the signal was digitized at 10 kHz using a PC-based National Instruments (Austin, TX) analog data collection board and saved on computer disk. Further quantitative analysis of the EMG data was performed with software written in National Instruments LabView and in MatLab. For neural recordings of local field potentials (LFP) and multiunit activity in VPm (MUA) were recorded with the

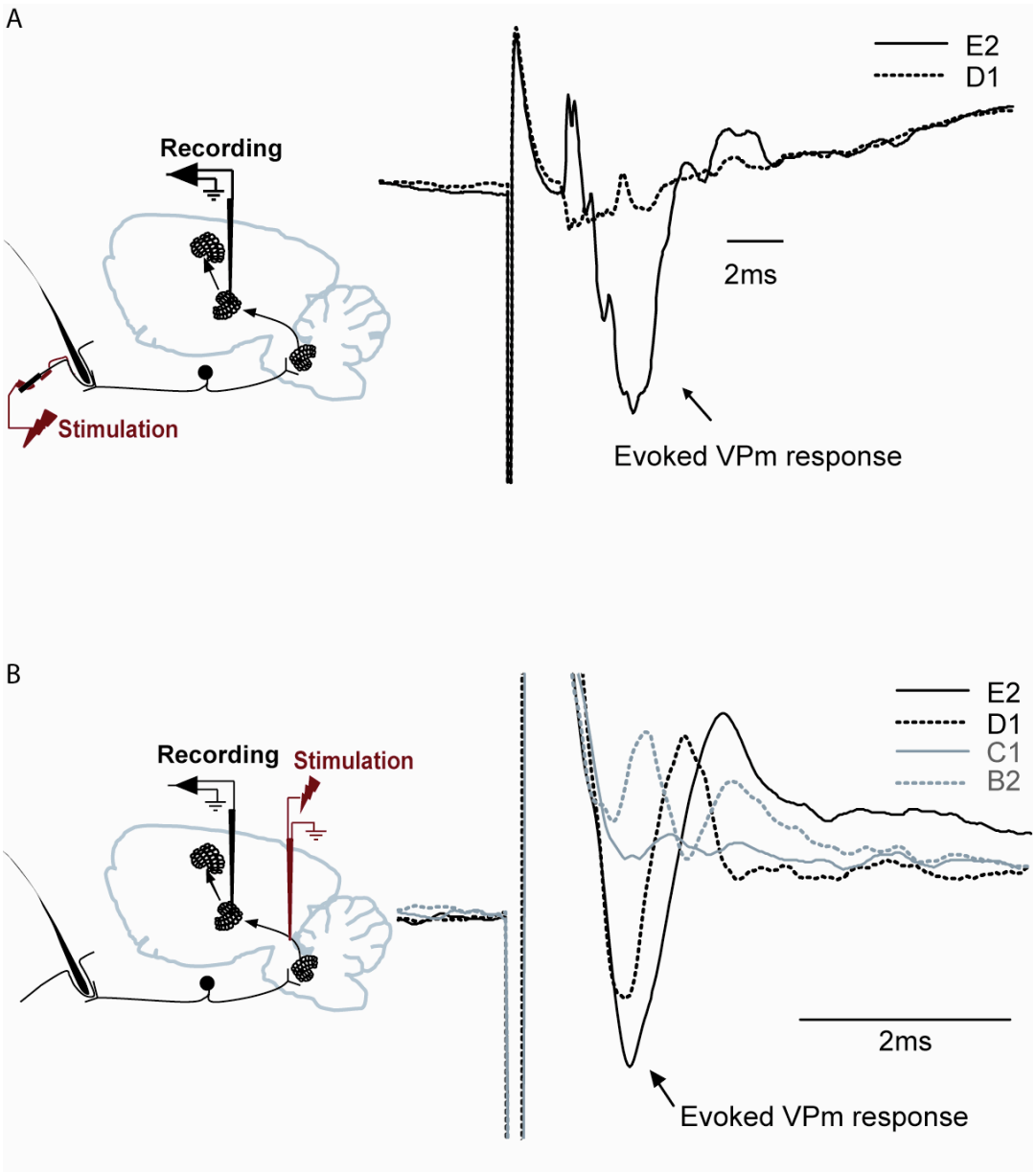


Figure 14. Schematic diagram of sensory stimulation methods; Whisker follicle (A) and medial lemniscal (B) stimulation. (A) Recording electrode was implanted at thalamic barreloid E2 for both cases. A stimulating wire was implanted in the corresponding E2 whisker follicle. Follicle stimulation-evoked responses are shown in the right. Note that in the D1 barreloid, E2 whisker follicle stimulation hardly evoked any response. (B) A wire was implanted in the medial lemniscus axon fibers. Evoked LFP were dramatically decreased, as a recording electrode moved from E2 to B2 barreloid. The data was obtained from two anesthetized animals.

miniature pre-amplifiers, led to a second Grass Instruments amplifier and filtered at 1 Hz – 10 kHz. An additional stage of amplification and filtering was used for LFP recordings (1Hz-

500Hz) and/or MUA recordings (300Hz-10kHz; BAK Electronics). Recorded MUA were identified on-line by spike amplitude and waveform criteria using a virtual oscilloscope, and digitized data were saved on disk along with the EMG records (see Fig. 13).

Data analysis

Behavior. We identified two behavioral states, whisking and non-whisking using both videographic and EMG recordings. *Whisking* epochs were characterized by the occurrence of any whisker movements provided that the whiskers did not contact any object (i.e., the platform). Whisking-in-air usually consisted of relatively large amplitude sweeps at ~8 Hz but we also included periods when whisking was smaller in amplitude or at different frequencies. Finer parcellation of the whisking epochs was precluded due to the need to collect data during extended periods of time when the animal was actively seeking the water probe. Whisker movements were typically associated with head movements, though these did not contaminate the EMG records. An epoch was classified as *Non-whisking* when there were no observable whisker movements or elevated EMG activity and no incidental contact of the whisker with the platform. Periods involving grooming and jaw movements associated with licking were excluded from the analysis.

Neuronal data Data were analyzed offline. We examined the videographic and EMG records to identify periods of whisking and non-whisking, and then quantified the neural (VPm) signals separately for the two behavioral conditions. Throughout the recording session electrical stimuli were applied to the whisker follicle or medial lemniscus, and neural data were analyzed with respect to the times of occurrence of these stimuli. For LFPs, analog signals were averaged across all stimuli and trials. For MUA, spike occurrences were accumulated into peri-

stimulus time histograms (PSTHs) having 0.1ms bins. Responses to stimulus onsets were computed during a certain period after the beginning of electrical stimulation. We calculated the stimulus-evoked MUA during a 2ms window starting 2.5ms after WF stimulation. Similarly, we analyzed the stimulus-evoked MUA data in a 3ms time window starting 1.5ms after the onset of ML stimulation. Note that earlier spikes, if they occurred, were obscured by the stimulus artifact, thus time window does not start with the onset of electrical stimulation. Data from each day's recording session were analyzed separately, because we could not be assured that the VPm electrode remained precisely in the same location from one day to the next. The number of averaged trials varied depending upon the animal's behavior/motivation on any given day with a range of 30-300 trials. Within a given recording day, the numbers of trials of whisking and non-whisking trials were not necessarily equal. Finally, the experiments required that the electrically stimulated whisker follicle and the VPm electrode were topographically matched. To maximize this, we fixed the VPm electrode in place and also used a relatively large-tipped electrode that would reliably record local field potentials. Thus, though LFPs were routinely obtained, MUA recordings were acquired from only a subset of recording sessions.

The thalamic LFP obtained from behaving animals in response to electrical stimulation of a whisker follicle is similar to that reported previously in sedated animals using whisker deflections (Temereanca and Simons 2003). The waveform consists of a prominent negative wave of several ms duration followed by a longer-duration positivity. With electrical stimulation, which synchronously activates afferent fibers, the negative wave is sometimes immediately preceded by a small positive inflection; the thalamic spiking response first occurs during the negative-going slope of the LFP (see Fig. 15). An even earlier positivity can also be observed, though its occurrence was variable; this potential may represent the incoming afferent

volley along trigeminothalamic axons. Previous study by Temereanca and Simons showed that waveform component of LFP can be almost equally well quantified by peak magnitude, slope and the area under the curve of negativity (2003). We chose to measure magnitude of negative peak: the magnitude of the negative wave from the height of the immediately preceding positive-to-negative transition to the nadir of the negativity. The Stimulus-averaged responses were examined to identify these points. These points were also used for computing mean and variance values for trial-by-trial responses. Latencies are based on the onset of the initial positive-to-negative transition. Other details of the analyses are described in appropriate sections of the Results. Throughout, LFP measures are presented in arbitrary units.

Data were analyzed using software written in Labview, Matlab and Microsoft Excel/Visual Basic. Statistical tests were used to compare neural data obtained in whisking and non-whisking epochs during individual recording sessions. Due to deviations from normality in the distributions of data, statistical significance was tested with non-parametric tests. Data were analyzed for each recording session by measuring values (e.g, peak ampitudes in LFPs, spike counts in MUA) from each trial, and testing differences between whisking and non-whisking conditions using Mann-Whitney tests. For analyses of group data, results were pooled across rats and recording sessions and analyzed using a Wilcoxon signed rank test. Throughout the text, findings are reported as means ± standard deviations are given throughout the text and are displayed graphically as means ± standard errors.

Histology

After the final day of recording for an individual animal, the rat was deeply anesthetized with pentobarbital sodium (100mg/kg ip) and perfused for cytochrome oxidase and Nissl staining. An

electrolytic lesion was made through the VPm recording electrode, and in cases where medial lemniscus stimulation was employed a second lesion was made there using the stimulating electrode. The brain was coronally sectioned at 70 μm, and the locations of recording and stimulating sites were identified and confirmed as being in VPm or the medial lemsicus. In rats in which we implanted a cannula targeting the SpVi, the brainstem was additionally sectioned and stained in order to visualize the location of the cannula.

3.3 RESULTS

Whisker follicle stimulation

We recorded thalamic VPm responses evoked by WF stimulation in three behaving rats. We found that thalamic responses evoked by electric stimulation of the corresponding whisker follicle were delayed and attenuated during periods of voluntary whisking relative to periods of non-whisking. This attenuation is evident in the examples of averaged local field potentials (LFP) from an individual recording session (Fig. 3-4A). WF stimulation evoked two pronounced negative peaks. The first peak occurs just after stimulation onset (1.7-1.9 ms); because of its short latency, the signal likely represents activity in the brainstem, distal to the recording site in the VPm. A second, later peak occurs at 1.7-2.8 ms, and corresponds to the evoked thalamic response, inasmuch as its latency range and shape are consistent with previous LFP recordings from VPm in our laboratory (Temereanca and Simons, 2003). Additionally, we were often able to detect a third, smaller negative peak which occurs between the presumed PrV and the VPm responses. This intermediate peak may represent a population axon terminal potential of

incoming trigeminothalamic spikes. The latencies of peaks varied depending upon the strength of the applied current. There was also variability in amplitudes across animals.

In the example shown in Figure 15A, we averaged the stimulus-triggered LFPs over 175 trials in the whisking condition and 211 trials in the non-whisking condition. The magnitude of the stimulus-evoked response during whisking was significantly smaller than during non-whisking (response amplitude was 0.23±0.08 in the non-whisking state; and 0.19±0.06 in the whisking state, Mann-Whitney test $p=0.004$). The whisking state also had an effect on response latency. Stimulus-evoked responses occurred later during whisking (2.78 ms) than non-whisking (2.65 ms). Multi-unit activity (MUA) was consistent with the LFPs (Fig. 15B). MUA corresponds in time to the initial negative slope of the VPm LFP. We calculated the stimulus-evoked MUA during a 2ms window starting 2.5ms after stimulation. Follicle stimulation evoked fewer spikes at trend level during whisking periods, as shown in the PSTHs (Fig. 15B; 0.24±0.25 spikes/stimulus during whisking, 0.29±0.24 spikes/stimulus during non-whisking, $p=0.07$). A recording site in thalamic VPm is shown in Figure 15C.

We recorded thalamic LFPs in a total of 8 recording sessions from three rats (Fig. 16). Figure 16A shows data for each session where average peak magnitudes during whisking are normalized to the average non-whisking values; ratios < 1.0 indicate proportionately smaller peak response amplitudes during whisking. We performed two analyses on these data. In the first, we pooled data across rats and recording sessions and found that the magnitude of the thalamic LFP was reduced by an average of 20% during whisking. Comparisons of actual values showed statistically significant decreases during whisking (Wilcoxon signed rank test, $p<0.05$). In a second analysis, we analyzed data from each recording day separately. Four out of 8 data sets show significantly smaller responses during whisking. Days in which there was a significant

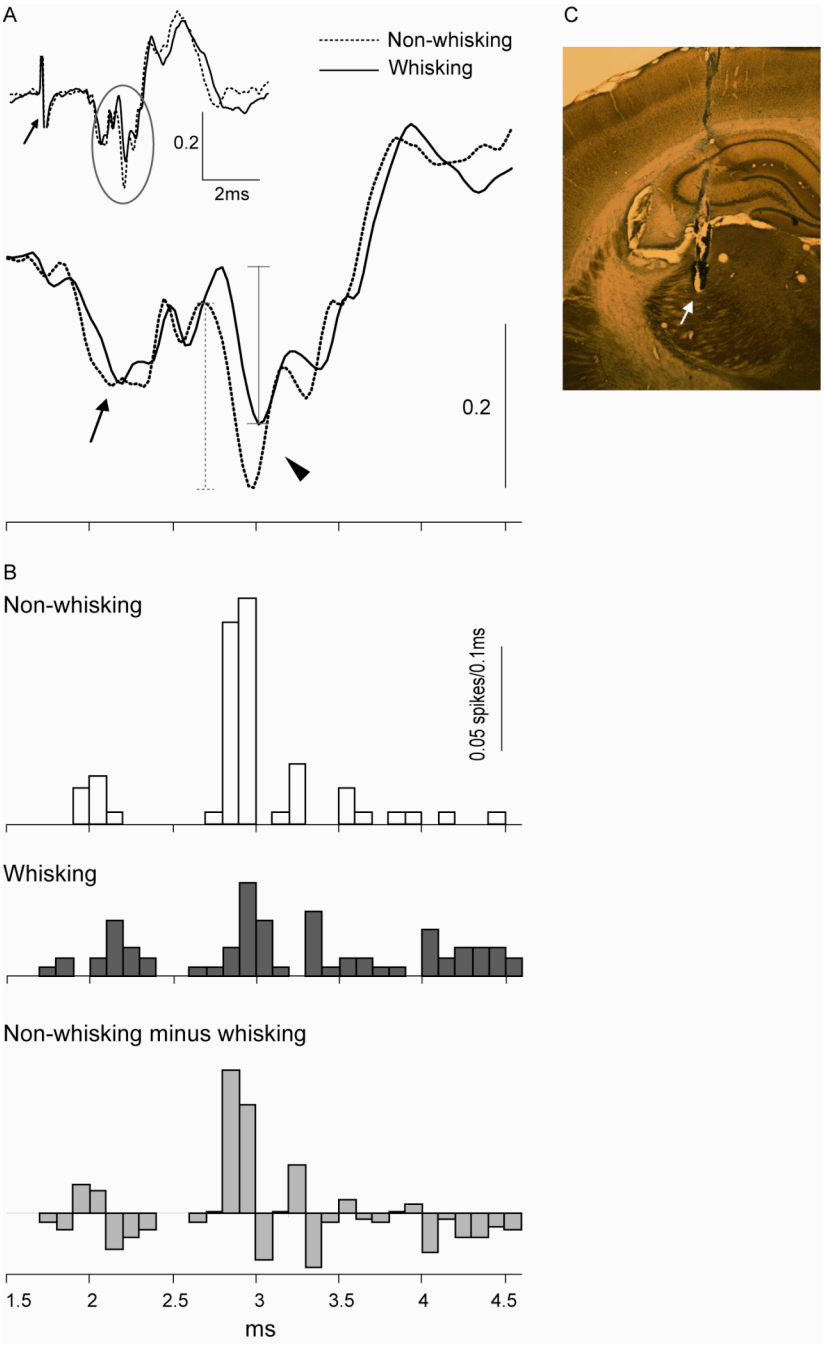


Figure 15. Example of thalamic barreloid responses to whisker follicle stimulation during whisking vs. non-whisking periods. (A) Evoked LFP is bigger during non-whisking (dotted line) than whisking (solid line). Inset shows full traces including stimulus artifact (arrow) and the evoked responses (gray circle). Arrow indicates response evoked in brainstem, arrowhead indicates VPm response. (B) PSTHs of simultaneously recorded MUA (bin=0.1ms) with LFP (above). The bottom PSTH was generated by subtracting MUA during whisking from non-whisking. LFP traces (A) and PSTHs (B) are temporally aligned. (C) Histological localization of a recording site from thalamic VPm (arrow). Coronal sections (70 μm) were reacted for cytochrome oxidase and counter-stained with thionin.

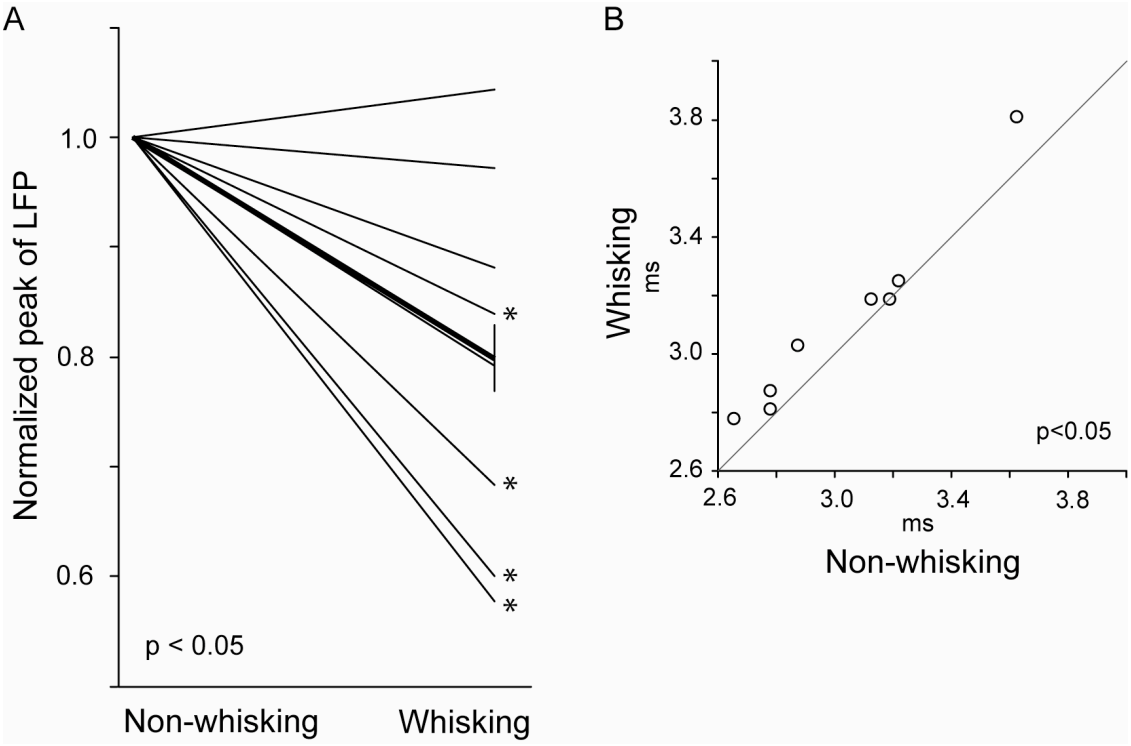


Figure 16. Whisker follicle stimulation-evoked LFP in thalamic barreloid during non-whisking vs. whisking. A) Whisker follicle stimulation-evoked responses are decreased during whisking. Peak of LFP during whisking were normalized to the peak LFP during non-whisking for each recording day. Data from each recording day in which there was a significant difference between whisking and non-whisking are indicated as *. Thick solid lines represent means of CT ratio with standard errors. B) Latency of evoked response is longer during whisking. Stimulus evoked response latencies of individual recording data are plotted for both whisking and non-whisking periods. Gray line is the unity line.

difference are indicated in Figure 16A by asterisks. Response latency was consistently longer during whisking (Fig. 16B, 3.12±0.16 vs 3.03±0.16 ms, p=0.009). Our findings thus indicate that, during active whisking, when activity of vMCx is presumably elevated, peripheral sensory evoked responses in a thalamic barreloids are both reduced in magnitude and delayed. Results are consistent with the earlier report by Fanselow and Nicolelis (1999).

Medial lemniscus stimulation

Studies in Chapter 2 indicate that pharmacologically elevated vMCx activity enhances sensory transmission of thalamic VPm neurons via topographically corresponding CT neurons. The converse effect – decreased VPm responses – was observed during whisking when vMCx is presumably active. One possibility is that the observed reductions in thalamic responses to WF stimulation during active whisking reflect activity-related processes in the brainstem. In order to evaluate more directly the cortical contribution of vMCx during natural whisking, we electrically stimulated ML fibers, thus by-passing the trigeminal brainstem nuclei (Fig. 14B).

We found that ML stimulation elicits a larger response in thalamic VPm during active whisking than during the non-whisking period. This is the converse effect observed with WF stimulation. We recorded thalamic responses to ML stimulation in 17 recording sessions from two freely behaving rats. Figure 17A shows data from one recording session. The averaged LFPs demonstrate that ML stimulation evoked two pronounced negative components, an initial peak occurring 0.7-0.9 ms after stimulation onset and a second peak occurring ~1.7 ms later. We denote these two negative peaks as 'early' and 'late' components, respectively. Note that WF stimulation evokes only one negative peak, corresponding to the 'early' component of ML-evoked response. The second component likely reflects a second burst of firing corresponding to the end of the refractory period of the VPm neurons (see Discussion). In the example of Figure 17A, stimulus-triggered LFPs were obtained from 298 non-whisking and 128 whisking trials. The whisking state of the animal had a pronounced effect on the magnitude of the early component: Evoked responses during whisking were significantly larger than that during non-whisking (0.24±0.06 vs. 0.50±0.08, Mann-Whitney test $p<0.0005$). ML stimulation also evoked two-fold larger peak responses in the late component during whisking. The location of the stimulating electrode in the medial lemniscus is shown in Figure 17C.

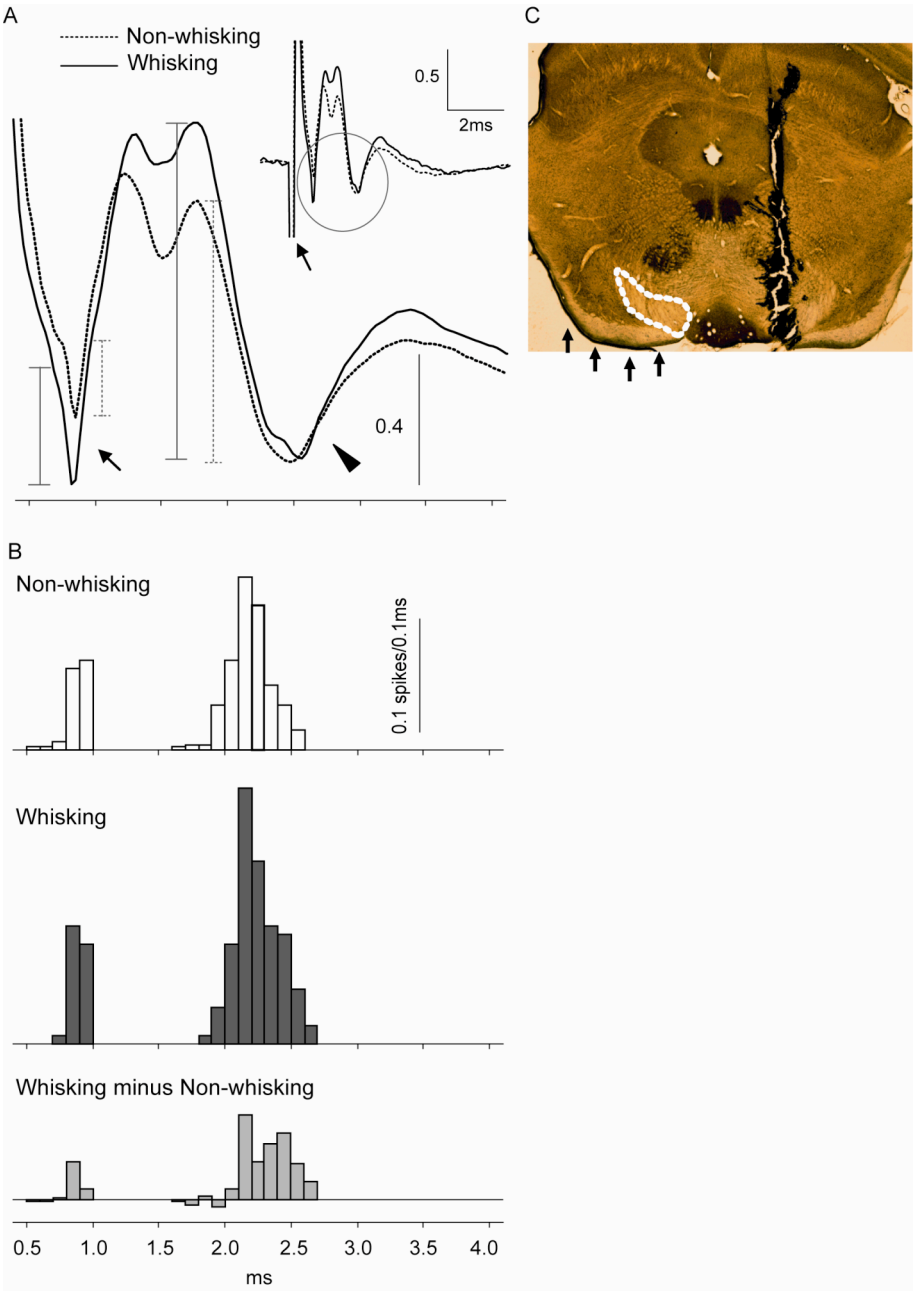


Figure 17. Example of thalamic barreloid responses to medial lemniscal stimulation during whisking vs. non-whisking periods. (A) Evoked LFP is bigger during whisking (solid line) than non-whisking (dotted line) period. Arrow indicates early and arrowhead indicates late components. The inset shows full traces including stimulus artifact (arrow) and evoked responses (gray circle). (B) PSTHs of simultaneously recorded MUA (bin=0.1ms) with LFP (above). The bottom PSTH was generated by subtracting MUA during non-whisking from whisking. LFP traces (A) and PSTHs (B) are temporally aligned. Other conventions as in Fig. 15. (C) Photomicrograph of a section through the caudal end of the diencephalon; electrode tract indicates ML stimulating site. Contralateral side of medial lemniscal tract is indicated with dotted line. Arrows indicate corticobulbar fibers. Coronally sectioned tissue (70 μm) was reacted for cytochrome oxidase and counter-stained with thionin.

In this experiment, we were also able to record MUA. Because of the highly synchronous afferent activity evoked by the ML stimuli, the evoked thalamic spiking response was brief, lasting less than 3ms. As shown in Figure 17, spikes occur during the positive-to-negative slope of the LFP. We analyzed the MUA data in a 3ms time window starting 1.5ms after the onset of electrical stimulation; earlier spikes, if they occurred, were obscured by the stimulus artifact. Consistent with LFP recordings, ML stimulation evoked more spikes during periods of whisking vs. non-whisking (Fig. 17B; 0.80±0.22 vs 0.54±0.25 spikes/stimulus, $p<0.0005$).

We normalized the amplitude of the peak LFP during whisking to that of the LFP during non-whisking for each recording day for each animal. LFP amplitudes were measured at the first- and second peak negativities (arrows and arrowhead, respectively in Fig. 17A). On average, the amplitude of the first negativity was increased by 26% during whisking (Fig. 18A, 1.26±0.08, $p<0.0005$). The second negativity was larger, too (Fig. 18B, 1.26±0.06, $p<0.005$). We also analyzed data from each recording day separately. Seventy percent of data sets (12/17 sessions) showed significantly larger responses during whisking. These data sets are indicated in Figure 18 by asterisks. Unlike the response to WF stimulation, response latencies were equivalent for both conditions (early negativity, non-whisking 0.60±0.05ms, whisking 0.59±0.05, $p>0.5$; late negativity, non-whisking 1.93±0.11ms, whisking 1.91±0.10, $p>0.5$).

Direct electric stimulation of presynaptic axons (ML) induces a larger response in VPm thalamus during whisking, whereas WF stimulation induces a smaller response. These findings suggest that cortical activity that emerges during voluntary whisking enhances sensory transmission in VPm. Our results also imply that the suppression of whisker-evoked responses

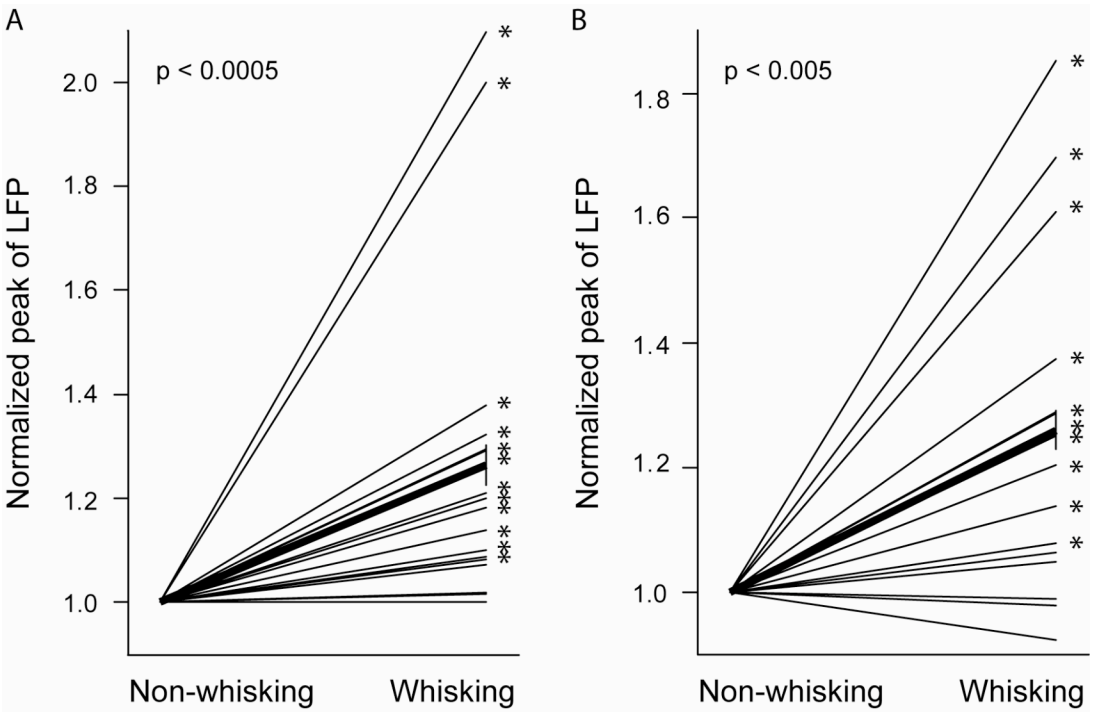


Figure 18. Medial lemniscal stimulation-evoked LFP in thalamic barreloid during non-whisking vs. whisking. Medial lemniscal stimulation-evoked responses are increased during whisking in both early (A) and late (B) responses. Peak of LFP during whisking were normalized to the peak of LFP during non-whisking for each recording day. Data from each recording day in which there was a significant difference between whisking and non-whisking are indicated as *. Thick solid lines represent means of CT ratio with standard errors. Wilcoxon signed rank test was performed. Other conventions as in Figure 16.

observed in VPm during active whisking is a reflection of processes that occur earlier, at the level of the brainstem trigeminal complex.

Paired-pulse stimulation (ML)

In a subset of the ML stimulation experiments, we used paired-pulse ML stimulation as one approach for controlling potential effects of trigeminothalamic synaptic depression. The two pulses likely activated most, though perhaps not all, of the fibers terminating in the recorded thalamic barreloid. The following pulse with short interval will occur when the

trigeminothalamic synapses were near-maximally, or at least substantially, depressed (Yuan et al., 1985, 1986; Fansenlow and Nicolelis, 1999; Castro-Alamancos, 2002). Thus, any differences in the evoked thalamic response to the second pulse likely reflect effects other than trigeminothalamic synaptic depression or other activity-related mechanisms in PrV. We applied paired pulses having 25 ms intervals at 1Hz. Similar to the single pulse protocol, we used a current of 1.5 to 2.0 times threshold (pulse duration = 0.03-0.05ms). Previous studies have demonstrated suppression in the response to the second in a pair of stimuli that closely follow one another (Simons, 1985; Simons and Carvell, 1989; Castro-Alamancos and Connors, 1996; Fanselow and Nicolelis, 1999). The amount of suppression varies with the interval between the first and second stimuli with the strongest suppression produced by a 20-30 ms interval.

An example of LFPs obtained during paired-pulse ML stimulation is shown in Figure 19. During non-whisking, both early and late response components to the second stimulus were noticeably reduced compared to those evoked by the first pulse (early component 1^{st} pulse 0.85, 2^{nd} pulse 0.61; late component 1^{st} pulse 0.12, 2^{nd} pulse undetectable). Note that the late component to the second stimulus is barely detectable (dotted gray circle in Fig. 19B). During whisking, however, the response to the second stimulus was reduced much less (early component 1^{st} pulse 0.92, 2^{nd} pulse 0.80; late component 1^{st} pulse 0.16, 2^{nd} pulse 0.19). Consistent with the LFP recordings, MUA recorded in this experiment also showed paired-pulse suppression during non-whisking. During non-whisking, MUA was reduced by half (from 0.64 to 0.32 spikes/stimulus), whereas during whisking, MUA remained constant or even slightly increased (from 0.68 to 0.71 spikes/stimulus).

We quantified the degree of adaptation based on MUA, because LFPs evoked by the second pulse were often too small to measure accurately, especially during non-whisking (see

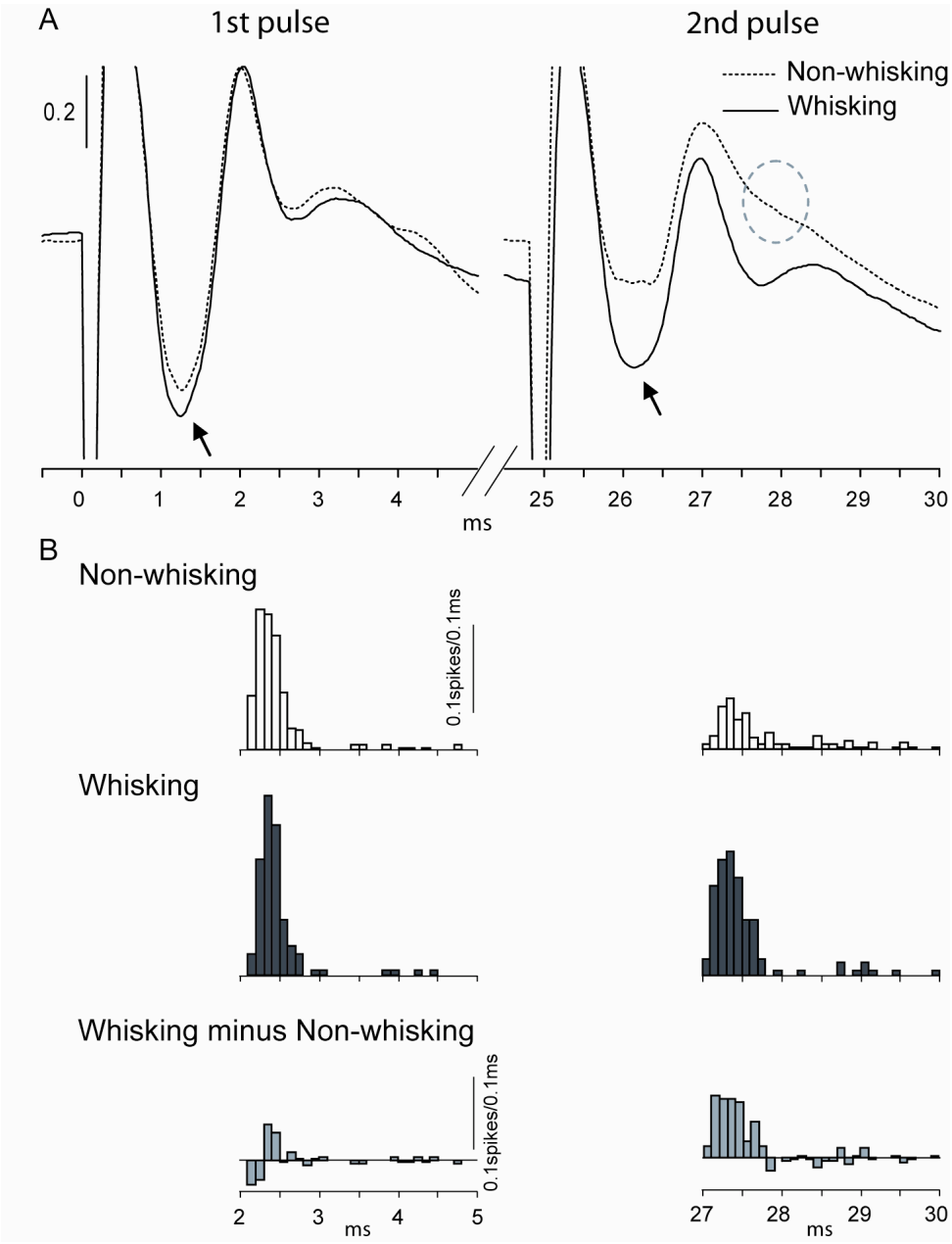


Figure 19. Example of thalamic barreloid responses to paired pulse medial lemniscal stimulation. (A) During whisking, evoked LFP (A) and MUA (B) are less reduced to 25ms paired pulse stimulation compared to those during non-whisking. Arrows indicate evoked LFPs. Note that the late response to 2nd stimulation is hardly noticeable during non-whisking (dotted gray circle). (B) PSTHs of simultaneously recorded MUA (bin=0.1ms) with LFP (above). The bottom PSTH was generated by subtracting MUA during whisking from non-whisking. LFP traces (A) and PSTHs (B) are temporally aligned.

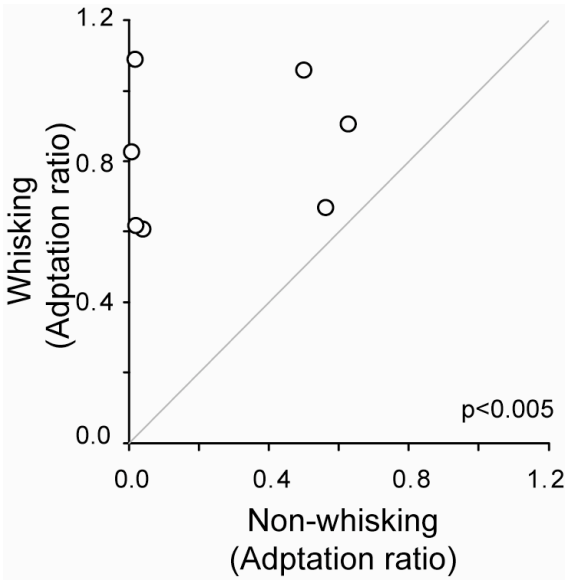


Figure 20. Adaptation of thalamic barreloid responses to paired pulse stimulation of the medial lemniscus during whisking and non-whisking periods. Adaptation ratios were calculated by dividing the response to the second pulse by the response to the first pulse. Adaptation ratios close to 1 indicate that both pulses evoked similar responses. Adaptation ratios less than 1 indicate a reduction in the response to the second pulse. Gray line indicates the unity line. *P*-value is based on a Wilcoxon signed rank test.

dotted circle in Fig. 19A). In response to the second pulse of the pair, ML stimulation evoked 4 times more spikes during whisking than during non-whisking (0.45±0.08 vs 0.11±0.07 spikes/stimulus, $p<0.005$). We calculated an adaptation ratio by dividing the MUA spike count evoked by the 2nd pulse by the spike count for the 1st pulse. On average, adaptation ratios were significantly larger during whisking than non-whisking (Fig. 20, non-whisking 0.25±0.15, whisking 0.82±0.10, $p<0.005$). These data suggest that VPm neurons respond better to repetitive stimuli during whisking, the active state of vMCx.

The data shown in Figures 1-8 were obtained from animals that underwent either WF or ML stimulation. This experimental design leaves open the possibility that the opposite effects of whisking that we observed in VPm are due to behavioral or other differences between the two

groups of animals. To eliminate this possibility, we tested one rat in which we implanted *both* WF and ML stimulating wires. We recorded thalamic responses to WF and ML stimulation during the same recording session, alternating the stimuli in pseudorandom blocks. In this animal, both effects of whisking were observed depending on whether the stimulus was delivered to the whisker follicle or whether it was delivered to the medial lemniscus, by-passing the brainstem. Specifically, during whisking, WF stimulation evoked significantly smaller thalamic responses (Fig. 21A, whisking 0.052±0.058, non-whisking 0.090±0.060, $p<0.05$), whereas ML stimulation elicited significantly larger responses during whisking in the same recording session (Fig. 21B, whisking 0.964±0.067, non-whisking 0.815±0.146, $p<0.005$). Thus, differences in VPm responses in whisking vs. non-whisking conditions appear to depend critically on the involvement of brainstem components of the trigeminothalamic system.

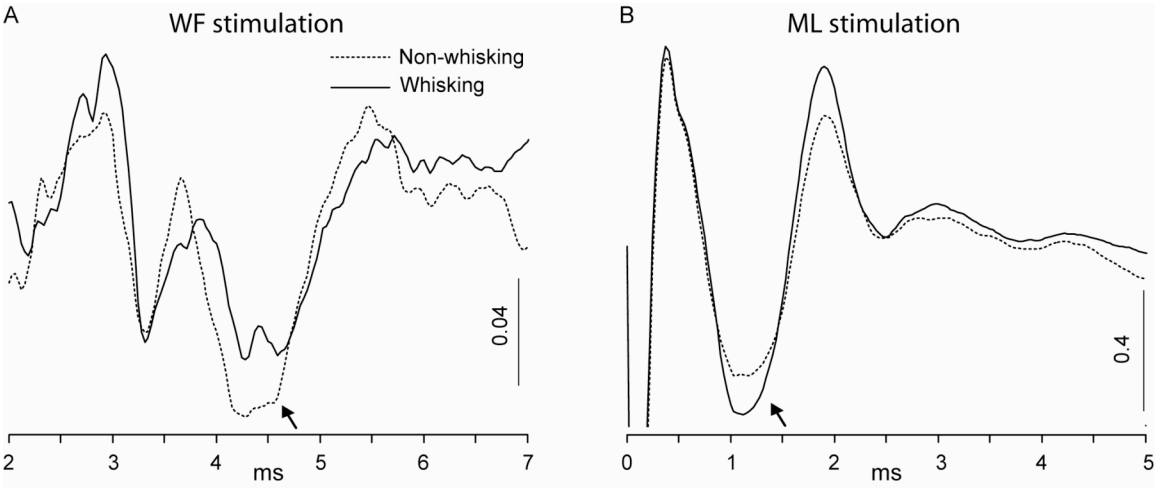


Figure 21. Thalamic barreloid LFPs evoked by (A) whisker follicle stimulation and (B) medial lemniscus stimulation during non-whisking (dotted line) vs. whisking (solid line) conditions. Arrows indicates evoked VPm LFPs to WF and ML stimulation. Both plots show data from the same animal in the same recording session.

Inactivation of SpVi

Motor-related corticofugal signals could alter activity prior to its arrival in VPm by direct effects on PrV and/or indirectly via SpVi. Importantly, SpVi provides inhibitory inputs to PrV, and activity in SpVi can be strongly gated by behavioral state/arousal (see Discussion). Therefore in three rats, we inactivated SpVi. A cannula was implanted into the caudal portion of SpVi ipsilateral to the site of WF stimulation and contralateral to the recordings in VPm (see Methods). We targeted the caudal aspect of SpVi because the majority of neurons there are inter-nuclear projection cells (i.e., SpVi to PrV; Furuta et al. 2006). In order to avoid excessive damage to SpVi, we attempted to position the cannula just dorsal to the subnucleus (Fig 22C). Local anesthetic bupivacaine, a sodium channel blocker, was injected into the brainstem in order to inactivate neuronal firing in the vicinity of the cannula tip. For each animal, we recorded VPm responses to WF stimulation before inactivating SpVi. We then slowly infused bupivacaine using a syringe pump. Five to ten minutes after termination of drug infusion, we collected another set of data.

Inactivation of SpVi reversed the effects of whisking on WF-evoked thalamic responses. Thus, during SpVi inactivation, WF stimulation elicited larger thalamic responses, results similar to those obtained during ML stimulation but opposite to WF stimulation under control (SpVi intact) conditions. We show one such example in Figure 22 (A and B). Consistent with previous data, WF stimulation evoked smaller thalamic VPm response during whisking compared to non-whisking (0.25±0.10 vs 0.33±0.10, $p<0.0005$). After bupivacaine infusion into SpVi, however, identical WF stimuli evoked a larger thalamic response during whisking (whisking 0.36±0.10; non-whisking 0.29±0.10, $p<0.0005$). Similar results were observed in a second similarly studied rat. A third animal displayed WF-evoked VPm responses that were

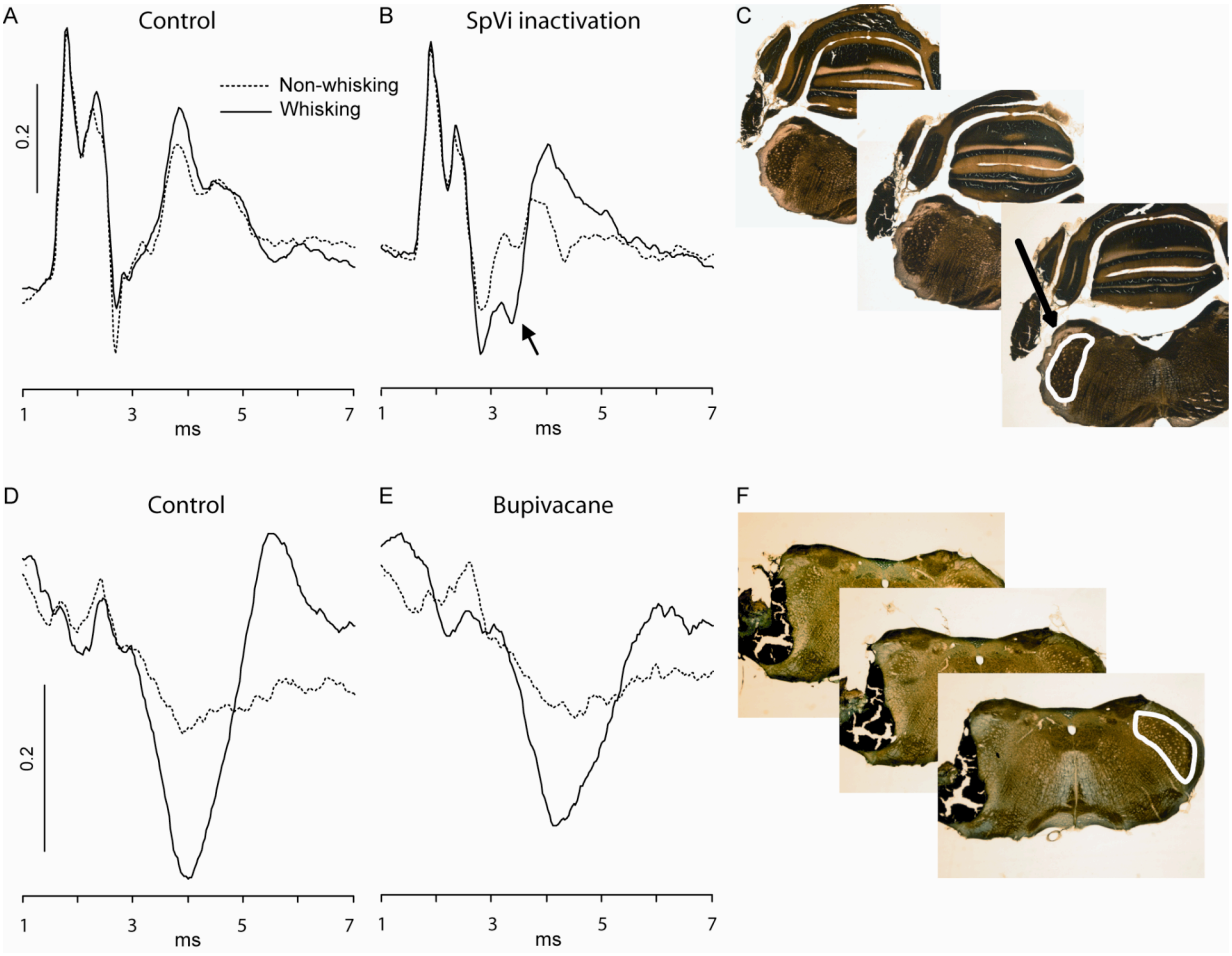


Figure 22. WF stimulation evokes larger thalamic VPm responses during whisking when SpVi is inactivated. (A) Whisking reduces the amplitude of the first negative component in the VPm elicited by WF stimulation. (B) Inactivation of SpVi by bupivacane infusion reverses the impact of whisking on the first negative component in the VPm. (C) Photomicrographs of sections through spinal trigeminal nucleus in the brainstem. Arrow indicates histological localization of an implanted cannula. White line indicates SpVi. Horizontal sections (70 µm) were reacted for cytochrome oxidase and counter-stained with thionin. (D-E) Damage to the SpVi results in a larger thalamic VPm response to WF stimulation,in the absence of bupivacane infusion (D) and during drug infusion (E). (F) Photomicrographs of sections through spinal trigeminal nucleus in the brainstem show damage to the SpVi by the implanted cannula. Datasets in A-B and D-E were collected from two different animals.

larger during whisking even before bupivacaine infusion (Fig. 22D, whisking 0.28, non-whisking 0.10, $p<0.005$). The effect remained upon subsequent drug infusion (Fig. 22E, whisking 0.22, non-whisking 0.09, $p<0.005$). Histological analysis revealed extensive unintended damage in

SpVi due to the presence of the cannula itself, which was found to be located deep in SpVi in the third experimental animal (Fig. 22F). Results suggest that whisking-related suppression observed in VPm following WF stimulation occurs in the brainstem and that, by extension, facilitation effects observed with ML stimulation, when the brainstem is by-passed, reflect corticothalamic feedback.

3.4 DISCUSSION

We investigated sensory transmission in the thalamic VPm nucleus during whisking and no-whisking states. We found that sensory responses during voluntary whisker movements, when motor cortex is likely to be active, are reduced relative to responses that occur during periods of wakeful quiescence. Enhancement of thalamic activity during whisking can be observed, however, when signal processing in sub-thalamic centers is either by-passed or experimentally altered. Findings suggest that afferent somatosensory activity is simultaneously suppressed and facilitated by different corticofugal systems that together may function to enhance the saliency of particular sensory signals by preserving them within a background of global suppression.

LFP as neuronal response

We reported local field potential as a measure of evoked thalamic population responses to WF or ML stimulation. LFPs refer to the low frequency component of the recorded activity within a volume of brain tissue, reflecting postsynaptic potentials in a population of neurons (Purpura 1959; Leung, 1990). Comparison of movement predictions from LFP with those from spikes recorded in motor cortex showed that LFPs contain as much information as contained in spiking

activity (Mehring et al., 2003; Bokil et al., 2006). In thalamic VPm, simultaneously recorded LFP and MUA shows nearly coincident onset, suggesting that LFP signal is dominated by postsynaptic activity (Temereanca and Simons 2003). Furthermore, LFPs showed a strong positive correlation between the magnitude of the initial negative peak and the strength of the stimulus (i.e. velocity/acceleration of whisker deflection) (Temereanca and Simons 2003). The present study also demonstrated that stimulus evoked spikes are aligned to the initial negative peak of LFPs (see Figure 15). Together, it suggests that the early negative peak of LFP represent initial, afferent excitation of barreloid neurons.

Methodological considerations

We applied electrical stimuli to single whisker follicles in order to generate controlled peripheral sensory stimulation in freely behaving rats. Sensory stimulation in other studies has been evoked by electrical stimulation of the infraorbital nerve or whisker pad to evoke sensory responses during movement (Fanselow and Nicolelis 1999; Castro-Alamancos 2004). WF stimulation has several advantages over these other methods. First, it is more specific in that it allows the stimulation of a single whisker. Second, because only brief, low intensity currents are needed, WF stimulation may evoke afferent signals that more closely mimic those during natural object contact. Our stimulation parameters (average 0.6mA (0.2-0.8mA) with duration of 0.04-0.05ms) are substantially lower in amplitude and/or briefer in duration than those used in other studies (Fanselow and Nicolelis 1999,e.g., infraorbital nerve stimulation, 7mA with 0.1 ms; Castro-Alamancos 2004, whisker pad stimulation, 0.2-0.8mA with 1ms), possibly avoiding unintended activation of smaller diameter fibers that might not normally be engaged during exploratory whisking. WF evoked reliable responses only in the corresponding thalamic barreloid, and not in

neighboring barreloids (Fig. 14A). The response profile evoked by WF stimulation is quite similar to responses evoked by whisker deflections recorded in lightly sedated animals.

Our ML stimulation evoked thalamic responses limited to 4-5 adjacent barreloids, despite the dense packing of fibers in ML tract (see Fig. 14B). The current that we applied (60μA average, 40-80μA range) was smaller than the currents used in WF stimulation experiments. This difference was because the electric shock was directly applied to presynaptic fibers. Our ML stimulation parameters consistently elicited two negative peaks in the LFP trace; an early and a late component. These two components had latencies of 0.59±0.05ms and 1.92±0.10, respectively. Considering the latency of the early component, the early component likely represents the thalamic response directly evoked by electrical stimulation of presynaptic fibers. Three possible scenarios can explain the late component. First, the late component may reflect thalamic responses to firing of PrV neurons which are antidromically activated by back-propagating action potentials elicited by the ML electric shock. To test this possibility, in a control experiment we pharmacologically inactivated PrV using tetrodotoxin (TTX), a Na^+ channel blocker. Inactivation of PrV neurons would abolish VPm responses evoked by antidromic spikes in PrV. We found that ML stimulation still induced the late component after PrV inactivation of the PrV; thus, antidromically reflected PrV firing is not responsible for the late component (Fig. 23). A second possibility is that the early thalamic response evoked by ML stimulation excites neurons in L6, including corticothalamic cells. In this scenario, corticothalamic feedback would provide a second, longer-latency source of thalamic activation. However, there are at least two synapses in this thalamo-cortico-thalamic circuit with conduction times of at least 3.5 ms (thalamocortical - 2ms plus corticothalamic - 1.5ms; Kyriazi and Simons, 1993; Kelly et al., 2001); the time difference between early and late components (1.3 ms) is

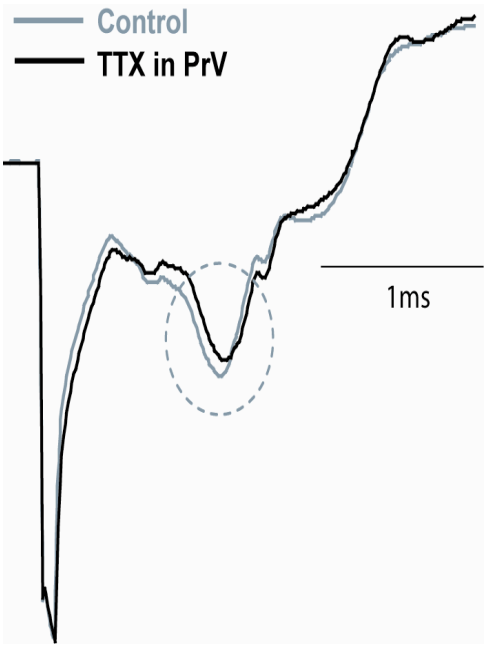


Figure 23. Thalamic VPm response to ML stimulation during PrV inactivation. TTX application to PrV did not change thalamic LFP waveform to ML stimulation. The late (gray dotted circle) components are intact during PrV inactivation. Recording was obtained from a anesthetized rat.

therefore too small to be evoked via cortical feedback. Lastly, direct electrical stimulation of presynaptic fibers likely causes strong depolarization and robust spiking of VPm neurons. The second peak may thus reflect synchronous discharge of VPm neurons with the timing of the second peak determined by the neurons' absolute refractory period of ~1.0-1.5 ms.

We identified whisker movements based on both EMG recordings and videographic monitoring. We recorded EMGs from the mystacial pad ipsilateral to the VPm recording side in order to avoid interference with the whisker follicle stimulating wire (located in the contralateral side of the face). Rats typically move whiskers bilaterally during exploratory whisking, although it is not always the case that both sides are synchronized (Towal and Hartmann, 2006). We did not attempt to correlate VPm activity with detailed whisking kinematics. EMG recordings from the ipsilaternal side and video tape analysis were used simply to define whisking and non-whisking periods.

What is the pattern of activity in the vMCx during whisker motor commands? Activity in vMCx is tonically elevated during whisking. vMCx activity is also often correlated with changes in whisking kinetics (Carvell et al., 1996, Friedman et al., 2005). Carvell et al. additionally reported that individual neurons in infragranular layers of vMCx do not discharge rhythmically in a one-to-one fashion with the periodicity of whisking movements. A series of studies by Keller et al. suggests that vMCx regulates whisking via a serotonergic CPG in the brainstem (Hattox et al., 2003; Cramer and Keller, 2006; Cramer et al., 2007). Other evidence, however, suggests that neuronal activity, measured as field potentials in vMCx, can be matched with each whisking cycle (Ahrens and Kleinfeld, 2004), though it is not clear whether such activity reflects motor commands or sensory (or other) feedback. Electrical stimulation of vMCx can evoke rhythmic whisking (Berg and Kleinfeld, 2003; Brecht et al., 2004; Donoghue and Wise 1982; Haiss and Schwarz 2005), but it is unclear how well such activation mimics the natural state. Seizure activity induced in vMCx activity by pharmacological disinhibition is also capable of driving whisker movement on a cycle-by-cycle basis (Castro-Alamancos, 2006). Whether vMCx exerts direct control over whisker movement or indirect control via serotonergic CPG in the brainstem remains somewhat controversial. It is clear, however, that on-going neural activity in the vMCx is related to voluntary whisker movement.

Circuits for thalamic/cortical sensorimotor integration

Our experiments employing ML stimulation suggest that thalamic activity is facilitated via the cerebral cortex. How might cortical activity generated in vMCx affect activity in VPm, the primary sensory nucleus for tactile sensation? Elevated neuronal discharge in the vMCx during whisking may be transmitted to thalamic VPm via corticothalamic neurons in L6 of barrel

cortex. In Chapter 2, we provided evidence that, in lightly sedated rats, pharmacological facilitation of vMCx activity enhances neuronal responses in topographically aligned infragranular layer in S1 and in thalamic barreloid neurons. The effects could be mediated by direct descending projections from S1, inasmuch as antidromically identified CT cells there display increased activity and enhanced whisker-evoked response with vMCx facilitation.

Two groups of neurons in the vMCx contribute to motor-to-sensory projections: callosal and corticofugal neurons (Hoffer and Alloway, 2000; Veinante and Deschenes, 2003). The main axons of callosal neurons project to contralateral motor cortex but their collaterals project ipsilaterally to infragranular layers of barrel cortex. These collateral projections from vMCx provide a major input to Layer 6 of barrel cortex, the site of origin of corticothalamic projections. Together, this excitatory circuit (vMCx to L6 of S1 to thalamic VPm) provides a means for vMCx to influence thalamic VPm.

Corticofugal neurons, another group of projecting neurons from vMCx to sensory regions, project to subcortical areas including thalamus, striatum and brainstem (Veinante and Deschenes, 2003). In light of such sensorimotor interconnections (reviewed in Kleinfeld et al., 1999), we can not rule out other possible pathways that may enable the vMCx to modulate activity of thalamic VPm. One such candidate is thalamic POm, the paralemniscal thalamic nucleus. POm which itself has reciprocal connections with vMCx may play a role as a mediator between vMCx and thalamic VPm. Despite a lack of direct projections from POm to VPm, POm may modulate VPm activity via the thalamic reticular nucleus (nRT). In this scenario, facilitation effects would probably require circuit interactions that produce polysynaptic disinhibition of VPm neurons.

Excitatory corticofugal effects on thalamic VPm neurons during whisking

Direct stimulation to presynaptic axon fibers in the ML results in a larger response in thalamic VPm during whisking. This suggests that the effect of cortical activity on thalamic VPm neurons is one of facilitation during whisking. ML stimulation by-passes whisking-related subthalamic modulation of tactile transmission by avoiding brainstem trigeminal circuitry. In addition to direct effects of afferent fibers, ML stimulation can affect thalamic VPm activity via CT neurons and via neurons in the thalamic reticular nucleus (nRT), the major inhibitory source to VPm (Spacek and Lieberman, 1974; Barbaresi et al., 1986; Harris and Hendrickson, 1987). Inhibitory neurons in nRT do not receive direct subthalamic sensory input. Instead, the collaterals of TC and CT axons are the major driving sources to nRT (Scheibel and Scheibel, 1966; Bourassa et al., 1995). Thus, elevated activity in vMCx during whisking can provide both excitation and inhibition to thalamic VPm neurons via CT-VPm and CT-nRT-VPm projections, respectively. Previous studies have shown that facilitation of L6 neurons in a barrel-related column provides net excitation to the topographically aligned barreloid and slight suppression to non-aligned barreloid (Temereanca and Simons, 2004). Along the same lines, studies in our laboratory show that pharmacological inactivation of vMCx in freely behaving rats significantly reduces spontaneous and whisker-evoked neuronal discharges of VPm neurons, demonstrating that vMCx is capable of modulating the excitability of thalamic and/or brainstem neurons in the lemniscal system (Prigg, Carvell and Simons, unpublished data).

Larger VPm responses to ML stimulation may also be due to different degrees of activity-dependent depression of trigeminothalamic synapses in whisking and non-whisking periods. The level of spontaneous discharge of presynaptic neurons is known to modulate synaptic depression: high spontaneous discharge rates induce stronger synaptic depression,

whereas low tonic firing produces less synaptic depression (Castro-Alamancos and Oldford, 2002; Castro-Alamancos, 2004a and b). During alertness/aroused states, for example, high spontaneous thalamic activity dampens thalamocortical synapses, resulting in diminished sensory evoked responses in barrel cortex (Fanselow and Nicolelis, 1999; Swadlow and Gusev 2001; Castro-Alamancos and Oldford, 2002; Castro-Alamancos, 2004a and b). Similarly, different levels of spontaneous firing of PrV neurons between whisking and non-whisking conditions can cause different magnitudes of thalamic responses depending on brain/behavior states (Castro-Alamancos, 2002).

We used paired-pulse ML stimulation as one approach for controlling potential effects of trigeminothalamic synaptic depression. The two pulses likely activated most, though perhaps not all, of the fibers terminating in the recorded thalamic barreloid. With the 25-ms interval used the second pulse thus occurred when trigeminothalamic synapses were near-maximally or at least substantially depressed (Yuan et al., 1985, 1986; Fanselow and Nicolelis, 1999; Castro-Alamancos, 2002). Thus, any differences in the evoked thalamic response to the second pulse likely reflect effects other than trigeminothalamic synaptic depression or other activity-related mechanisms in PrV. With this manipulation, the relative increase in VPm responses during whisking vs non-whisking was even larger for the second pulse. We attribute this increase to recurrent corticothalamic activity evoked by the first pulse. Excitatory effects of CT synapses mediated by metabotropic glutamate receptors (McCormick and von Krosigk, 1992; Eaton and Salt, 1996; Goldshani et al., 1998), are facilitating (Turner and Salt, 1998; Castro-Alamancos and Calcagnotto, 1999; Von Krosigk et al., 1999), not depressing as in the case of trigeminothalamic synapses, and relatively long-lasting (Hirata et al., 2006). Evidence from both *in vitro* and *in vivo* studies suggests that CT feedback may be particularly effective in influencing thalamic activity

during high frequency inputs (Yuan et al., 1985, 1986; Fanselow and Nicolelis, 1999; Castro-Alamancos and Calcagnotto 2001). Suppression of S1 cortex attenuates VPm responses to tactile or medial lemniscal stimulation (Yuan et al., 1985, 1986) in anesthetized and in awake animals. Interestingly, this effect was most pronounced using stimulus frequencies of 10-30Hz.

SpVi may modulate the background activity of PrV neurons. First, SpVi is a major source of input to PrV along with the sensory neurons of the trigeminal nerve (Clarke and Bowsher 1962; Hayashi 1980; Jacquin at al., 1990; Jacquin et al., 1990, 1993; Frututa at al., 2006). Second, SpVi receives cholinergic projections from the pedunculopontine tegmental region (PPTg) (Timofeeva et al., 2005). PPTg, a part of brainstem reticular formation, is involved with regulating brain activity levels during arousal states (Aston-Jones et al., 1991; Buzaki et al., 1988). Thus, unlike inputs from the trigeminal ganglion, which are solely dependent on sensory stimulation, the modulatory pathway of PPTg-SpVi-PrV can affect PrV activity in a stimulus-independent fashion. One such stimulus-independent effect would be engagement of the circuit by descending projects to PPTg and/or SpVi from vMCx. We reasoned that inactivation of SpVi would produce a condition wherein the spontaneous discharges of PrV neurons would be largely equivalent during whisking and non-whisking conditions. In support of this, when SpVi was inactivated, thalamic responses to WF stimulation were larger during whisking, the converse of what we observed in functionally intact animals. This result suggests that the larger ML-evoked thalamic responses observed during whisking are not due to diminished depression of trigeminothalamic synapses that would occur if spontaneous PrV activity is reduced during whisking. Future studies involving recordings in PrV are needed to address this issue more directly.

Whisker-movement related suppression of sensory responses in the brainstem

Two lines of evidence presented here suggest that whisker movement-related suppression of sensory responses occurs at the level of the brainstem trigeminal complex. First, whisker movement-related suppression of VPm responses was not observed during ML stimulation, when afferent stimuli bypassed the whisker periphery and the brainstem trigeminal complex. Second, when SpVi was inactivated, thalamic responses were not suppressed during whisking, rather evoked thalamic responses were larger.

A recent study showed that during active wrist movements in primates cutaneous inputs are presynaptically inhibited at the level of the spinal cord (Seki et al., 2003). The timing of the attenuation suggests that descending motor commands generate the inhibition, rather than movement-induced sensory inputs from the periphery. Similarly, in cats, sensory transmission in the cuneate nucleus is suppressed during limb movements (Ghez and Lenzi, 1971; Ghez and Pisa, 1972; Coulter, 1974). In the whisker-to-barrel system, the brainstem reticular formation may contribute to suppression of PrV neurons via its effects on inhibitory neurons in SpVi. Projections from vMCx to the brainstem reticular formation are extensive and widespread (Miyashita et al., 1994; Hattox et all., 2002), providing a means for whisker-related elevated firing in motor cortex to engage inhibition within PrV, albeit indirectly by a polysynaptic cholinergic pathway. Taking into account that voluntary, exploratory whisking is a highly motivated state, strong cholinergic modulation of SpVi may result in enhanced inhibition to PrV neurons.

Movement-related suppression and facilitation in the whisker-to-barrel pathway

We interpret the present experiments to indicate that during voluntary whisker movement, activity in the whisker-to-barrel pathway is simultaneously enhanced and suppressed by descending, corticofugal signals that produce converse effects at different levels of the neuraxis. We propose that, at the level of the brainstem, second-order neurons in PrV are inhibited during whisking in a fashion at least qualitatively similar to that described as "gating" in second order somatosensory neurons of cats and monkeys (Ghez and Lenzi, 1971; Ghez and Pisa, 1972; Coulter, 1974; Chapman et al., 1988; Seki et al., 2003). Reduced transmission through brainstem circuits may disproportionately suppress relatively weak and unintended or potentially confusing sensory signals produced by movement of peripheral tissues. For fore- and hind-limbs, such signals might arise during incidental contact of the skin surface with nearby objects. In the case of whiskers, the potentially distracting signals could arise from movement of whiskers that the animal is not intentionally using for exploration or object palpation. Also, trigeminal ganglion cells are active during whisking in air, though at rates approximately an order of magnitude smaller than those associated with direct object contact (Leiser and Moxon, 2007). Future studies are needed to examine more directly whisking-related effects in PrV and whether corticofugally mediated suppression is global or topographically organized as in the case of the S1-CT system.

Facilitating CT effects during whisking were revealed in our experiments under three conditions in which potential brainstem influences were either by-passed (ML stimulation), neutralized (paired-pulse ML stimulation) or eliminated (SpVi inactivation). In the normal condition whisker-evoked VPm activity is smaller during whisking, and this likely reflects strong inhibition in sub-thalamic stations. Net suppression within VPm must, however, be viewed in light of the findings here and in Chapter 2 that motor cortex-mediated S1 corticothalamic

influences are both net excitatory and topographic. As will be addressed more fully in Chapter 5, context-dependent facilitation of activity in subsets of VPm could increase their saliency within thalamocortical circuits even if the activity is reduced relative to non-whisking states, provided that surrounding subsets of VPm neurons are suppressed even more.

4.0 MODULATION OF LAYER 4 ACTIVITY BY PRIMARY MOTOR CORTEX

4.1 INTRODUCTION

In chapter 2, we showed that enhancement of activity within vMCx increases neuronal responses in infragranular layers of barrel cortex including those of corticothalamic neurons, many of which are normally silent. At the same time, the spontaneous activities and whisker-evoked responses of neurons in thalamic VPm barreloids are increased. In chapter 3, we further examined how vMCx influence activity in thalamic VPm nucleus in a freely behaving rat. Consistent with the data from acute experiments, we found enhancement of thalamic activity to sensory stimulation during whisking, when sensory processing bypassed the brainstem trigeminal complex. Whisker-evoked thalamic activity, however, is reduced during whisking under conditions where brainstem circuitry is functioning normally. Together, our findings suggest that during voluntary movement, sensory activity within the lemniscal system is globally diminished, perhaps at brainstem levels. At the same time that activity within specific thalamocortical neuronal populations is facilitated.

Here, we investigate how motor cortex facilitation affects responses in layer IV barrels, another major circuit component of the cortico-thalamo-cortical loop in lightly sedated rats. Because different neuronal types in L4 barrel cortex (i.e. excitatory and inhibitory neurons) show different intrinsic response properties and thalamocortical connectivity, we first examined how

vMCx activation differently influence regular-spike units (RSUs), presumably excitatory neurons, and fast-spike units (FSUs), presumably inhibitory neurons. Second, we asked how the spatial resolution of L4 neurons is modulated by vMCx activation. During active whisking, it is often the case that neighboring whiskers make contact an object in sequence. To mimic a natural behavior situation, we applied paired whisker deflections with a short interval to assess suppressive effects of adjacent whisker deflection.

4.2 METHODS

Surgical procedure, electrophysiological recordings, whisker stimulation, data analysis and histology are same as described in chapter2. L4 corresponds to recording depths of 700–950 μm (Kyriazi et al. 1998).

Whisker stimulation

In order to assess the effect of vMCx activation on inhibitory receptive fields of barrel neurons, a condition-test protocol was used. One stimulator was attached to the PW and another to its caudally adjacent whisker (AW). The ramp-and-hold deflection described in chapter 2 was delivered. The caudal AW was chosen because, among all four adjacent whiskers, it elicits the strongest suppression of evoked responses to PW deflection (Brumberg et al., 1996). Inhibitory receptive fields of barrel neurons were examined by deflecting the PW and the AW in paired combination (Simons and Carvell, 1989; Kyriazi et al., 1996; Shoykhet et al., 2005). The suppressive effect of the first deflection on the neuron's response to the second deflection was taken as a measure of the former's contribution to the cell's inhibitory receptive field. The PW

and the AW were deflected sequentially 30 ms apart. This interval was chosen, because it is within the period of strong condition-test suppression observed previously (Simons and Carvell, 1989; Kyriazi et al., 1996; Shoykhet et al., 2005). The first-moved whisker is termed the conditioning whisker, and the second one is called the test whisker. The conditioning stimulus was delivered to the conditioning whisker for all 8 directions, while the test stimulus was delivered in the test whisker's preferred direction. In addition, the condition-test paradigm included 10-test stimuli delivered alone wherein the AW or the PW were deflected without the conditioning stimulus. Stimuli in which the AW and PW each served both roles as conditioning or test whisker were randomly interleaved and delivered 10 times for each condition at 1.5 s intervals.

Data Analysis

Suppression of whisker responses during the condition-test protocol was evaluated using the condition-test ratio. Condition-test ratio for each cell was calculated as the average response to the test stimulus when it is preceded by the conditioning stimulus divided by the average response evoked by the test stimulus presented alone.

Data were analyzed using Microsoft Excel/Visual Basic and Matlab (The MathWorks, Natick, MA). Means ± standard deviations are given throughout the text. Results are displayed as means ± standard errors.

4.3 RESULTS

Effect of vMCx activation on neurons in L4 of S1

We recorded 21 RSUs and 19 FSUs from L4 barrels while facilitating activity in corresponding areas in vMCx by BMI microiontophoresis. The effect of vMCx activation on neurons in L4 barrel cortex is cell-type dependent. The majority of FSUs (12 of 19, 64%) became significantly more responsive to PW deflection during BMI application in vMCx. FSUs showed an average 30% increase in their ON responses (Control vs. BMI, ON 2.16 ± 0.51 vs. 2.77 ± 0.63 spikes/stimulus, $p = 0.0007$, paired t-test). OFF responses and spontaneous firing rate were also significantly enhanced during vMCx activation (Control vs. BMI, OFF 1.80 ± 0.40 vs. 2.14 ± 0.35 spikes/stimulus, $p = 0.01$; Spontaneous activity 12.80 ± 6.90 vs. 16.36 ± 7.81Hz, $p = 0.02$, paired t-test). Similar results were obtained for the stimulus-evoked responses after subtracting each unit's spontaneous activity. No differences were observed during control and vMCx activation for FSU OFF/ON and angular tuning ratios (control vs. vMCx activation: angular tuning 0.77 ± 0.04 vs. 0.78 ± 0.04, $p = 0.82$; OFF/ON ratio 0.88 ± 0.15 vs. 0.83 ± 0.14, $p = 0.15$). Note that L4 FSUs are normally poorly tuned for deflection angle and also that OFF and ON responses are normally equivalent in size.

vMCx stimulation had a smaller and more variable effect on RSUs than FSUs. On average, the ON and OFF responses and spontaneous activities of RSUs during vMCx activation are similar to those during the control condition (control vs. vMCx activation: ON 0.64±0.23 vs. 0.70±0.26 spikes/stimulus, $p=0.37$; OFF 0.47±0.20 vs. 0.50±0.24, $p=0.48$; Spontaneous 0.28±0.18 vs. 0.30±0.18 Hz, $p=0.67$, paired t-tests). Cell by cell analyses showed, however, that 24% of RSUs (5 of 21) became significantly more responsive. Two RSUs showed significantly less robust responses, as evident in Figure 24A. Angular tuning and OFF/ON ratios of RSUs did not differ during the vMCx activation condition relative to the control condition (control vs.

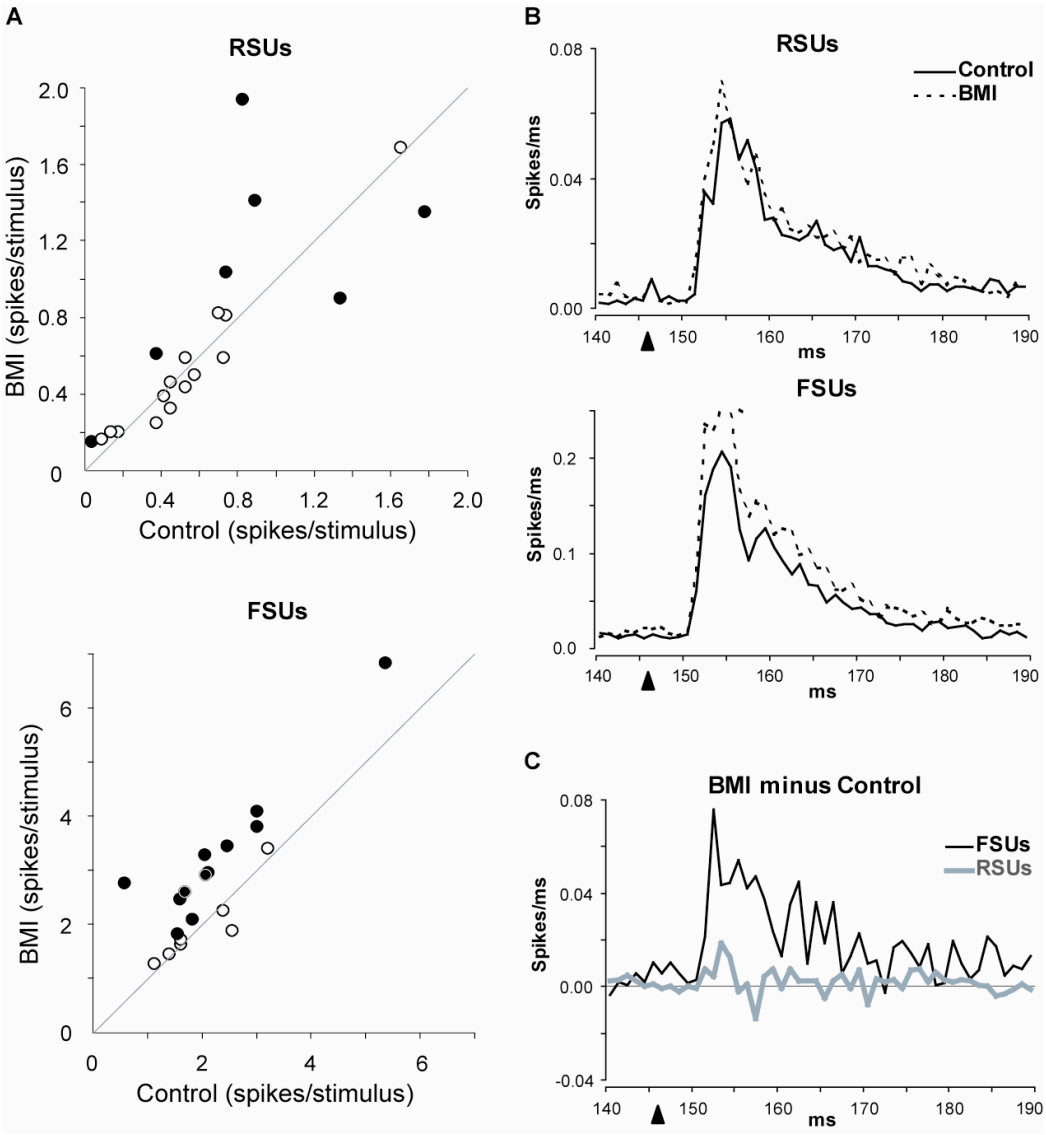


Figure 24. Effects of vMCx activation on layer 4 barrel neurons. (A) vMCx activation increased excitability of L4 FSUs in barrel cortex. Scatter plots compare ON responses for L4 neurons in control condition (x-axis) and BMI condition (y-axis). Gray lines indicate unity. Individual neurons which show a significant difference are indicated as a closed circle. (B) Overlaid population PSTHs compares ON responses during control condition (solid lines) and BMI application to vMCx (dotted line). (C) PSTH shows the difference in response magnitude during BMI and control conditions for RSUs and FSUs. The PSTHs were generated by subtracting ON responses during the control condition from the ON responses during BMI application. This subtraction was performed for each neuron and the mean was computed.

vMCx activation: angular tuning 0.49 ± 0.08 vs. 0.50 ± 0.09, p = 0.57; OFF/ON ratio 0.60 ± 0.21 vs. 0.64 ± 0.21, p = 0.46).

We constructed population PSTHS to examine the effects of vMCx activation on the temporal profile of responses of neurons in barrel cortex (Fig. 24B). Application of BMI to vMCx leads to an elevated firing of FSUs throughout the ON response. The effect is strongest during the first 10ms of the ON response. Response latency, however, was not changed. Population firing was affected only slightly in RSUs, primarily during the earliest part of the response. PSTHs in Figure 24C compares effects in two types of neurons, illustrating the overall increase in FSU but not RSU firing.

Inhibition in the barrel circuit

The increased excitability of FSUs during BMI application to vMCx suggests that inhibition in barrel circuits is enhanced during vMCx activation. We therefore hypothesized that inhibitory receptive fields of L4 neurons may be enhanced during vMCx activation. To examine this, we deflected the PW and an immediately adjacent whisker (AW) in a condition-test protocol used previously to assess suppressive effects induced by AW deflection on responses to PW deflection (see Simons and Carvell 1989). The AW was deflected first followed 30 ms later by deflection of the PW; previous studies have shown this to be a time of maximal AW-evoked suppression. Data were quantified by computing a condition-test ratio in which the PW response following AW deflection was divided by the PW-alone response; a smaller value indicates more AW-evoked response suppression.

An example is presented in Figure 25A. The top PSTH shows responses of an RSU to PW-alone deflection. The bottom PSTH displays response of the same neuron to caudal AW

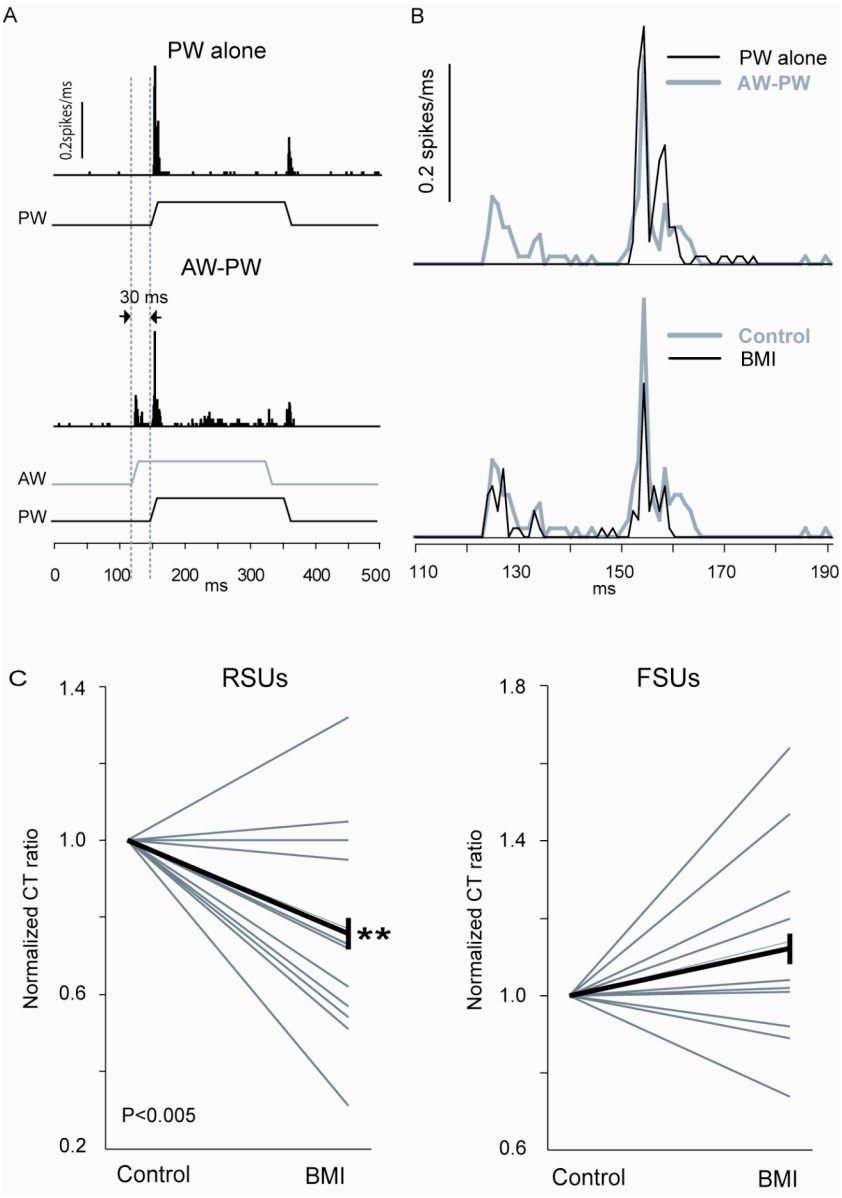


Figure 25. Activation of vMCx suppresses inhibitory receptive fields in L4 of S1. (A-B) Representative PSTHs of a barrel RSU in the condition-test protocol during control condition and vMCx activation. (A) Responses to PW deflection only (PW alone, top) and responses to PW-deflection preceded by AW-deflection (condition-test) with a 30-ms inter-stimulus interval (AW-PW, bottom) from the same neuron. Stimulus waveforms are indicated below the PSTHs. Dotted lines show onsets of each whisker deflection. (B) PSTHs of ON responses to condition-test and 'PW alone' from the same neuron at an expanded time scale (top).The bottom PSTHs compare ON responses to condition-test during the control condition and BMI application in vMCx. (C) AW-evoked suppression of RSUs is greater during vMCx activation. Condition-test (CT) ratio was calculated as ON response to PW stimulation preceded by AW (AW-PW) to ON response to PW deflection only. Condition test (CT) ratios after BMI application in vMCx were normalized to the CT ratio during control condition for each L4 neuron. Thick solid lines represent mean CT ratio (+/- with standard errors). ** *P* value < 0.005, paired t-test.

deflection followed 30 ms later by PW deflection. The overlaid PSTH (Fig. 25B top) demonstrates that the ON response is suppressed by 20% (PW only, 1.40 spikes/stimulus; AW-PW 1.11). During vMCx activation, preceding AW deflection further suppressed the ON response (0.54 spikes/stimulus). For twelve RSUs examined, preceding AW deflections resulted in a 24% greater response suppression when BMI was applied to vMCx (Figure 25C, normalized CT ratio 0.76±0.14, paired t-test, p=0.009). For eleven FSUs, there was a small difference at trend level in CT ratios between control and BMI conditions (Fig. 25C, normalized CT ratio 1.12±0.13, paired t-test, p=0.1).

4.4 DISCUSSION

Our data suggest that neurons in vMCx can enhance the activity of corticothalamic neurons in S1 (Chapter 2). We found that this enhancement can be transmitted to thalamic VPm, which provides the primary topographic input to L4 in S1. Here we asked how the input from motor cortex to cortico-thalamo-cortical circuit affects responses in layer 4, the major recipient zone for VPm input. We recorded RSUs, and FSUs in layer 4, barrel cortex while manipulating activity of vMCx using microiontophoresis of BMI. We found that both spontaneous and stimulus evoked activities in fast-spike units in layer 4 barrels was significantly increased. These layer 4 neurons were presumably a subset of inhibitory barrel neurons. The effect of vMCx activation on regular-spike units, presumed excitatory cells, was more variable, but on average activity of RSUs during vMCx activation is similar to those during control conditions. The FSU data suggest that functional inhibition in the barrel circuit is enhanced when neuronal activity in vMCx is tonically elevated. We further examined this issue by estimating the inhibitory

receptive fields of L4 barrel neurons using paired whisker deflections. vMCx activation enhanced the suppressive effects of adjacent whisker stimulation on subsequent deflections of the principal whisker. Such effects were observed in RSUs and to a much less extent in FSUs. The present study implies that the spatial focus of L4 RSUs is enhanced during tactile discrimination by active whisking.

How does facilitated activity in vMCx enhance responsiveness of FSUs but not RSUs in barrels? Two different, but not mutually exclusive, pathways may contribute to this effect: 1) direct projection from L6 to L4 neurons (vMCx-L6-L4) and 2) indirect projection from L6 to L4 via thalamic VPm (vMCx-L6-VPm-L4). Supporting the first direct projection from L6 to L4 neurons, anatomic studies have demonstrated that axon collaterals from L6 corticothalamic neurons project to L4. These neurons preferentially synapse onto dendritic shafts of aspiny multipolar neurons, i.e. inhibitory cells (White and Keller, 1987; Keller and White 1989). Enhanced activity of L6 neurons due to vMCx facilitation may strengthen inhibition in L4 circuit because of this biased projection from L6 to L4 inhibitory neurons. vMCx may also contact other S1 (excitatory or inhibitory) neurons that could indirectly or directly activate barrel FSUs. Selective vMCx-corticocortical engagement of barrel FSUs is likely to result in a decreased level of RSU activity. However, RSU responses were relatively unaffected. Thus, this intracortical pathway may not be solely responsible for the increased responsiveness of FSUs.

An alternative explanation involves an indirect pathway involving the corticothalamic loop and thalamocortical (TC) barreloid neurons (vMCx-L6-VPm-L4). TC neurons send convergent and divergent monosynaptic inputs onto both barrel RSUs and FSUs (White, 1978; Agmon and Connors 1991; Swadlow and Gustev 2000, Bruno and Simons 2002; Bruno and Sakmann 2006). RSUs and FSUs, however, respond differently to TC input because of their

intrinsic response properties and different thalamocortical connectivity (McCormick et al., 1985; Kawaguchi and Kubota 1993, Angulo et al., 1999; Bruno and Simons 2002; Bruno and Sakmann 2006). FSUs are strongly driven by TC inputs and thus more likely than RSUs to increase their firing as a result of enhanced TCU activity. Thus L6-mediated, vMCx-produced increases in TC firing could preferentially increase FSU vs RSU activity, as observed. Spontaneous firing rates of FSU but not RSUs increased. Heightened levels of tonic intrabarrel inhibition could counteract the increased stimulus-evoked firing of TCUs, especially for RSUs, most of which are normally only weakly responsive to TCU inputs. During vMCx activation, RSUs may receive increased feedforward excitation from TC inputs and, at the same time, enhanced inhibition from nearby FSUs. Thus, in response to discrete deflections of a whisker, RSU responses might be largely unchanged.

vMCx-faciltation effects on RSUs were evident using the paired whisker-deflection paradigm. AW-evoked response suppression in barrel neurons is thought to be mediated largely by intrabarrel FSUs which are strongly activated by adjacent whiskers as well as by the PW. FSUs are in turn strongly engaged by TC neurons (see above), most of which have excitatory multi-whisker receptive fields (Simons and Carvell, 1989; Bruno and Simons, 2002; Bruno and Sakmann 2006). Thus, AW responses of TC neurons may have been enhanced during vMCx facilitation, leading in turn to a disproportionate increase in FSU vs. RSU firing, as proposed above. This could account for the greater AW-evoked response suppression observed during BMI application in vMCx. Additional studies are needed to establish that AW responses are indeed enhanced by vMCx facilitation as shown for PW responses in Chapter 2.

The robust effects of vMCx facilitation observed with the paired whisker deflection paradigm suggest that corticothalamic effects – and their enhancement by motor cortex

activation – might be better probed using repetitive whisker deflections that can engage the thalamocortical-corticothalamic-thalamocortical loop in a temporally appropriate fashion. Indeed, as yet unpublished data from our laboratory indicates that CT effects can be observed in VPm neurons beginning on the second cycle of a train of whisker deflections. Repetitive deflections of whiskers occur during whisking, and thus the motor cortex might be particularly effective in regulating sensory processing in thalamocortical circuits during active touch.

5.0 CONCLUSIONS AND FUTURE DIRECTIONS

Waking up CT neurons: afferent inputs from other cortical areas

The functional role of CT neurons in somatosensory processing remains unknown, despite numerous proposals regarding the functional significance of these neurons. The role of CT neurons has remained elusive because of their notoriously weak responsiveness under standard experimental conditions. Here we provided evidence that elevated neuronal activity in vMCx, by pharmacological manipulation in lightly sedated rats and during whisker movements in freely behaving rats, can facilitate CT neurons in barrel cortex. The increased excitability of CT neurons in turn enhances responsiveness of thalamic VPm in a topographic manner.

Neurons in infragranular layers of barrel cortex receive afferent inputs from other cortical areas in addition to vMCx, e.g., S2 (Fabri and Burton 1991; Zhang and Deschenes, 1998). This anatomical observation raises the possibility that projections from other cortical areas may also provide facilitatatory inputs to CT neurons. If true, this would raise an important question: to what degree do inputs from vMCx and other cortical afferents overlap and do they equally affect all CT neurons? Depending on which afferents recruit CT neurons, CT neurons might be functionally subdivided. This information could potentially provide new and important insights into the specificity of cortical modulation of sensory processing in thalamocortical circuits.

Why mixed messages? Bottom-up suppression vs. top-down facilitation

Our findings and those of others indicate that tactile signals from the sensory periphery (bottom-up) are attenuated in the brainstem. During the very same period, however, cortical activity associated with whisker motor commands (top-down) enhances sensory transmission in the thalamus. The output of these two opposing influences is net suppression of thalamic VPm response to tactile stimulation. If the net output is suppression, what is the role of cortical excitatory modulation of thalamic neurons? Whisker motor commands from vMCx may influence thalamic sensory transmission according to specific stimulus features. Our preliminary data suggests that cortical activity related to whisker movements may affect thalamic firing synchrony (Fig 26). The degree of enhanced firing synchrony during vMCx activation appears to be related to the similarity of angular tuning among thalamic neurons; the more similar angular tuning, the higher firing synchrony of the neurons during vMCx activation. Stimulus-selective enhancement of thalamic firing synchrony would enhance the saliency of the thalamic signal for cortical processing, because circuitry within layer 4 barrels is preferentially activated by synchronously firing thalamic neurons (Pinto et al., 2000, 2003; Bruno and Sakmann, 2006).

Whisker motor commands from the vMCx may influence thalamic activity in a context-dependent manner. In Chapter 2, we found that the effects of pharmacologically elevated activity in the vMCx are topographically specific. This modulation consists of an enhancement of neuronal activity in topographically aligned infragranular layers in S1 and in similarly aligned thalamic barreloids. Considering that a rat can move its whiskers independently to some degree (Sachdev et al., 2002) and that whisking occurs in relation to coordinated head movements, specific activation patterns emerging from the whisker/face motor map may facilitate specific thalamocortical populations dynamically during active touch.

Gating in the brainstem trigeminal complex

Trigeminal ganglion neurons fire when whiskers simply move through the air without making contact with an object (Leiser and Moxon, 2007). This observation suggests that whisking itself, in the absence of physical contact, could induce neuronal discharges along the ascending lemniscal pathway (O'connor et al., 2002). One role of sensory suppression in PrV of the brainstem trigeminal complex thus may be to diminish firing levels unrelated to object contact, selectively allowing transmission to VPm thalamus and layer 4 cortical barrels of information about the details of contacted objects. Firing of trigeminal ganglion neurons during whisking may nevertheless provide important non-contact related, sensory information to higher brain centers. For example, trigeminal ganglion neurons may encode information related to the location of individual whiskers in space (Szwed et al., 2003, 2005). Such information may be carried in a different pathway, such as the paralemniscal pathway (Yu et al., 2006).

The present data suggest that the SpVi plays a key role in sensory suppression along the lemniscal pathway by providing inhibition to the PrV. The SpVi also has excitatory projections to the PrV (Jacquin et al., 1990b). These excitatory inputs from SpVi to PrV form the multi-whisker receptive fields of PrV neurons (Timofeeva et al., 2004; Kwegyir-Afful, 2005). How can SpVi provide inhibition to PrV during whisking, if SpVi neurons have both excitatory and inhibitory projections to PrV? One possible scenario is that excitatory and inhibitory neurons in SpVi are independently modulated by different sources. An excitatory channel from SpVi to PrV may relay needed information about the detailed deflections of groups of nearby whiskers. On the other hand, an inhibitory channel may transmit neuromodulatory effects from reticular formation in brainstem, in particular, PPTg. Independent SpVi modulation of excitation and inhibition to PrV due to different and separated input sources to SpVi, i.e. sensory and

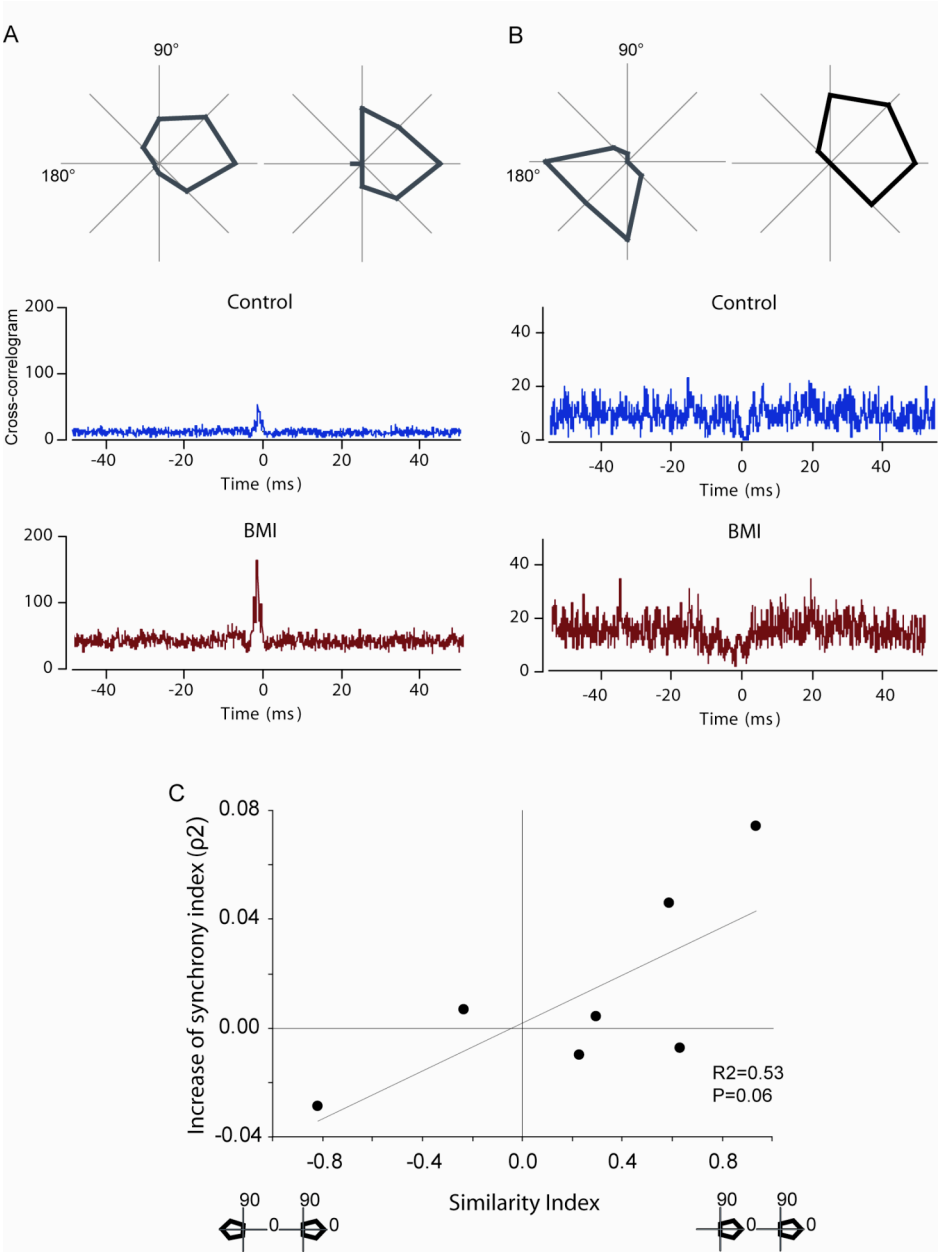


Figure 26. Effects of vMCx activation on thalamic firing synchrony are dependent on angular tuning. A) Example of two neurons simultaneously recorded from a single barreloid. Polar plots show similar angular tuning of two neurons (similarity index of two polar plots: 0.9). Raw cross-correlograms were computed based on simultaneously recorded spike trains of two thalamic neurons during noise stimulation. Synchrony index (ρ^2) increased from 0.03 to 0.1 during BMI application to corresponding vMCx. B) Another example. Polar plots show opposite angular tuning of two thalamic neurons from a barreloid (similarity index: -0.9). Synchrony index (ρ^2) is decreased from -0.05 to -0.08 during BMI application to corresponding vMCx. C) Difference in firing synchrony of two thalamic neurons between control and BMI conditions are plotted as a function of similarity of the two neurons' angular tuning. Similarity index=1: identical angular tuning, -1: opposite angular tuning. Frozen white noise stimulation was used.

neuromodulation, may allow SpVi to be a center for "gating". As a first step to test this hypothesis, it is necessary to examine whether cholinergic neurons from PPTg preferentially contact inhibitory neurons in SpVi. In addition, it is important to determine the source of SpVi projecting cholinergic neurons in PPTg. Considering the anatomical evidence that vMCx projects to PPTg (Hattox et al., 2002), it will be interesting to know whether vMCx directly activates neurons in PPTg. Finally, recordings of VPm-projecting PrV neurons in behaving animials are needed to establish more directly that whisking is associated with a net decrease in PrV spiking.

Lemniscal vs. paralemniscal pathways (VPm vs. POm)

In the present studies, we provided evidence that vMCx can modulate thalamic sensory transmission in the ascending lemniscal system via corticothalamic neurons in the primary somatosensory cortex. As noted in Chapter 1, POm, thought to be a "higher-order" thalamic nucleus, is a component of a parallel ascending, paralemniscal pathway. POm also receives CT inputs from primary somatosensory barrel cortex. Unlike CT-VPm neurons which are located almost exclusively in layer 6, CT-POm neurons arise also from L5 (Hoogland et al., 1991; Bourassa et al., 1995; Veinante et al., 2000). As discussed in Chapter 1, morphological and physiological properties of CT projections differ according to their laminar distribution and the destination of their axon terminals (reviewed in Deschenes et al., 1998; Guillery and Sherman, 2002; Jones, 1998). Thus, CT-mediated vMCx modulation of POm neurons may affect POm neurons differently than CT-VPm neurons affect transmission in the lemniscal system. It has been proposed, for example, that POm circuit may carry sensory as well as motor information of whisker movements (Sharp and Evans, 1982). In this regard, it is important to understand how

vMCx differently and similarly modulates POm via CT projections compared to VPm. Unlike VPm, POm is directly interconnected with vMCx (Hoffer and Alloway, 2001; Hoffer et al., 2003). Nearly no information is available how these two areas influence each other (see Yu et al., 2006). How the direct and indirect influence of vMCx interplay within POm will provide a better understanding of sensory processing in the paralemniscal pathway. Another pathway in which POm may be gated by vMCx is via its action on inhibitory neurons of zona incerta (ZI) (see Trageser and Keller, 2004). The ZI, subthalamic nucleus, provide GABAergic inputs to POm neurons (Power et al., 1999; Bartho et al., 2002), and received input from vMCx (Porter and White, 1983). Understanding such differences and similarities corticothalamic systems involving VPm vs POm will likely provide important insights into corticothalamic function and its regulation of sensorimotor integration.

Implications for active touch

Accumulating evidence indicates that centrally generated motor commands regulate sensory transmission in a top-town fashion during voluntary movement (Mignard and Malpeli, 1991; Nelson, 1996; Chapman et al., 1998; Fanselow and Nicolelis, 1999; Hupe at al., 1998; Roelfsema et al., 1998; Castro-Alamancos, 2004a; Krupa et al., 2004; Hentschke et al., 2006; Sommer and Wurtz, 2006). In the somatosensory system, 'motor-related gating' is generally viewed as consisting of suppression of tactile-evoked activity (Chapin and Woodward, 1982a,b; Shin and Chapin 1990b,c; Chapman, 1994). Whisking-related suppression has been reported in thalamic barreloids and in somatosensory barrel cortex (Fanselow and Nicolelis, 1999; Krupa et al., 2004; Hentschke et al., 2006). Diminished neural responsiveness may be associated with stronger receptive field focus and response tuning (Alloway et al., 1989; Kyriazi et al., 1996).

These findings discussed here appear to be at variance with our data indicating vMCx facilitation of sensory activity via corticothalamic circuitry. Focal BMI application in layer 6 of barrel cortex facilitates sensory-evoked responses in corresponding thalamic barreloids (Temereanca and Simons, 2004), and in behaving animals, inactivation of S1 diminishes the responsiveness of VPm neurons to periodic electrical stimuli delivered to the medial lemniscus (Yuan et al., 1986; see also Krupa et al., 1999; but see Diamond et al., 1992). Corticobulbar projections are known to engage inhibition within the gracile and cuneate nuclei, as well as within the brainstem trigeminal system. This circuitry could mediate potent cortically-regulated inhibition within the principal sensory nucleus, which is the whisker relay to VPm (Timofeeva et al., 2005; Furuta et al., 2006).

Together, the present findings raise the possibility that during voluntary movement activity within the lemniscal system is globally diminished, perhaps at early, brainstem levels (see Temofeeva et al., 2003) at the same time that activity within specific thalamocortical neuronal populations is facilitated. Though activity levels are reduced system-wide, activity within some local circuits may be subject to less net suppression. This decrease in suppression may occur on a moment-to-moment basis in a context-dependent manner. Thus, during voluntary whisker movement, sensory transmission in thalamocortical circuits may be modulated according to specific activation patterns distributed across the motor map.

BIBLIOGRAPHY

Agmon A, Connors BW. Thalamocortical responses of mouse somatosensory (barrel) cortex in vitro. *Neuroscience*. 41(2-3):365-79, 1991.

Ahissar E, Kleinfeld D. Closed-loop neuronal computations: focus on vibrissa somatosensation in rat. *Cereb Cortex* 13(1):53-62, 2003.

Ahissar E, Sosnik R, Bagdasarian K, Haidarliu S. Temporal frequency of whisker movement. II. Laminar organization of cortical representations. *J Neurophysiol* 86(1):354-67, 2001

Ahrens KF, Kleinfeld D. Current flow in vibrissa motor cortex can phase-lock with exploratory rhythmic whisking in rat. *J Neurophysiol* 92(3):1700-7, 2004.

Akers RM, Killackey HP. Organization of corticocortical connections in the parietal cortex of the rat. *J Comp Neurol* 181(3):513-37,1978.

Alitto HJ, Usrey WM. Corticothalamic feedback and sensory processing. *Curr Opin Neurobiol* 13(4):440-5, 2003.

Alloway KD, Rosenthal P, Burton H. Quantitative measurements of receptive field changes during antagonism of GABAergic transmission in primary somatosensory cortex of cats. *Exp Brain Res* 78(3):514-32, 1989.

Alloway KD, Zhang M, Chakrabarti S. Septal columns in rodent barrel cortex: functional circuits for modulating whisking behavior. *J Comp Neurol* 480(3):299-309, 2004.

Angulo MC, Staiger JF, Rossier J, Audinat E. Developmental synaptic changes increase the range of integrative capabilities of an identified excitatory neocortical connection. *J Neurosci* 19(5):1566-76, 1999.

Aston-Jones G, Chiang C, Alexinsky T. Discharge of noradrenergic locus coeruleus neurons in behaving rats and monkeys suggests a role in vigilance. *Prog Brain Res* 88:501-20, 1991.

Baker FH, Malpeli JG. Effects of cryogenic blockade of visual cortex on the responses of lateral geniculate neurons in the monkey. *Exp Brain Res* 29(3-4):433-44, 1977.

Barbaresi P, Spreafico R, Frassoni C, Rustioni A. GABAergic neurons are present in the dorsal column nuclei but not in the ventroposterior complex of rats. *Brain Res* 382(2):305-26, 1986.

Beierlein M, Connors BW. Short-term dynamics of thalamocortical and intracortical synapses onto layer 6 neurons in neocortex. *J Neurophysiol* 88(4):1924-32, 2002.

Belford GR, Killackey HP. Vibrissae representation in subcortical trigeminal centers of the neonatal rat. *J Comp Neurol* 183(2):305-21, 1979.

Beloozerova IN, Sirota MG, Swadlow HA. Activity of different classes of neurons of the motor cortex during locomotion. *J Neurosci* 23(3):1087-97, 2003.

Berg RW, Kleinfeld D. Rhythmic whisking by rat: retraction as well as protraction of the vibrissae is under active muscular control. *J Neurophysiol* 89(1):104-17, 2003.

Bernardo KL, Woolsey TA. Axonal trajectories between mouse somatosensory thalamus and cortex. *J Comp Neurol*. 258(4):542-64 1987.

Berntson GG, Shafi R and Sarter M, Specific contributions of the basal forebrain corticopetal cholinergic system to electroencephalographic activity and sleep/waking behaviour. *Eur J Neurosci* 16. pp. 2453–2461, 2002.

Blakemore SJ, Wolpert DM, Frith CD. Central cancellation of self-produced tickle sensation. *Nat Neurosci* 1(7):635-40, 1998.

Blakemore SJ, Wolpert DM, Frith CD.The cerebellum contributes to somatosensory cortical activity during self-produced tactile stimulation. *Neuroimage* 10(4):448-59, 1999

Bourassa J, Deschenes M. Corticothalamic projections from the primary visual cortex in rats: a single fiber study using biocytin as an anterograde tracer. *Neuroscience* 66(2):253-63, 1995.

Bourassa J, Pinault D, Deschenes M. Corticothalamic projections from the cortical barrel field to the somatosensory thalamus in rats: a single-fibre study using biocytin as an anterograde tracer. *Eur J Neurosci* 7(1):19-30, 1995.

Brecht M, Schneider M, Sakmann B, Margrie TW. Whisker movements evoked by stimulation of single pyramidal cells in rat motor cortex. *Nature* 427(6976):704-10, 2004a.

Brecht M, Krauss A, Muhammad S, Sinai-Esfahani L, Bellanca S, Margrie TW. Organization of rat vibrissa motor cortex and adjacent areas according to cytoarchitectonics, microstimulation, and intracellular stimulation of identified cells. *J Comp Neurol* 479(4):360-73, 2004b.

Bruce LL, McHaffie JG, Stein BE. The organization of trigeminotectal and trigeminothalamic neurons in rodents: a double-labeling study with fluorescent dyes. *J Comp Neurol* 262(3):315-30, 1987.

Brumberg JC, Pinto DJ, Simons DJ. Spatial gradients and inhibitory summation in the rat whisker barrel system. *J Neurophysiol* 76(1):130-40, 1996.

Bruno RM, Sakmann B. Cortex is driven by weak but synchronously active thalamocortical synapses. *Science* 312(5780):1622-7, 2006.

Bruno RM, Simons DJ. Feedforward mechanisms of excitatory and inhibitory cortical receptive fields. *J Neurosci* 22(24):10966-75, 2002.

Buzsaki G, Bickford RG, Armstrong DM, Ponomareff G, Chen KS, Ruiz R, Thal LJ and Gage FH, Electric activity in the neocortex of freely moving young and aged rats. *Neuroscience* 26 pp. 735–744, 1988.

Carvell GE, Miller SA, Simons DJ. The relationship of vibrissal motor cortex unit activity to whisking in the awake rat. *Somatosens Mot Res* 13(2):115-27, 1996.

Carvell GE, Simons DJ, Lichtenstein SH, Bryant P. Electromyographic activity of mystacial pad musculature during whisking behavior in the rat. *Somatosens Mot Res* 8(2):159-64, 1991.

Carvell GE, Simons DJ. Biometric analyses of vibrissal tactile discrimination in the rat. *J Neurosci.* 10(8):2638-48, 1990.

Carvell GE, Simons DJ. Thalamic and corticocortical connections of the second somatic sensory area of the mouse. *J Comp Neurol* 265(3):409-27, 1987.

Castro-Alamancos MA, Calcagnotto ME. High-pass filtering of corticothalamic activity by neuromodulators released in the thalamus during arousal: in vitro and in vivo. *J Neurophysiol* 85(4):1489-97, 2001.

Castro-Alamancos MA, Calcagnotto ME. Presynaptic long-term potentiation in corticothalamic synapses. *J Neurosci* 19(20):9090-7, 1999.

Castro-Alamancos MA, Connors BW. Spatiotemporal properties of short-term plasticity sensorimotor thalamocortical pathways of the rat. *J Neurosci* 16(8):2767-79, 1996

Castro-Alamancos MA, Oldford E. Cortical sensory suppression during arousal is due to the activity-dependent depression of thalamocortical synapses. *J Physiol* 541(Pt 1):319-31, 2002.

Castro-Alamancos MA. Absence of rapid sensory adaptation in neocortex during information processing states. *Neuron* 41(3):455-64, 2004a.

Castro-Alamancos MA. Dynamics of sensory thalamocortical synaptic networks during information processing states. *Prog Neurobiol* 74(4):213-47, 2004b.

Castro-Alamancos MA. Properties of primary sensory (lemniscal) synapses in the ventrobasal thalamus and the relay of high-frequency sensory inputs. *J Neurophysiol* 87(2):946-53, 2002

Castro-Alamancos MA. Vibrissa myoclonus (rhythmic retractions) driven by resonance of excitatory networks in motor cortex. *J Neurophysiol* 96(4):1691-8, 2006.

Catsman-Berrevoets CE, Kuypers HG. Differential laminar distribution of corticothalamic neurons projecting to the VL and the center median. An HRP study in the cynomolgus monkey. *Brain Res* 154(2):359-65, 1978.

Chakrabarti S, Alloway KD. Differential origin of projections from SI barrel cortex to the whisker representations in SII and MI. *J Comp Neurol* 498(5):624-36, 2006.

Chapin JK, Sadeq M, Guise JL. Corticocortical connections within the primary somatosensory cortex of the rat. *J Comp Neurol* 263(3):326-46, 1987.

Chapin JK, Woodward DJ. Somatic sensory transmission to the cortex during movement: gating of single cell responses to touch. *Exp Neurol* 78(3):654-69, 1982a.

Chapin JK, Woodward DJ. Somatic sensory transmission to the cortex during movement: phasic modulation over the locomotor step cycle. *Exp Neurol* 78(3):670-84, 1982b.

Chapman CE, Jiang W, Lamarre Y. Modulation of lemniscal input during conditioned arm movements in the monkey. *Exp Brain Res* 72(2):316-34, 1988.

Chapman CE. Active versus passive touch: factors influencing the transmission of somatosensory signals to primary somatosensory cortex. Can *J Physiol Pharmacol* 72(5):558-70, 1994.

Chmielowska J, Carvell GE, Simons DJ. Spatial organization of thalamocortical and corticothalamic projection systems in the rat SmI barrel cortex. *J Comp Neurol* 285(3):325-38, 1989.

Clarke WB, Bowsher D. Terminal distribution of primary afferent trigeminal fibers in the rat. *Exp Neurol* 6:372-83, 1962.

Coulter JD. Sensory transmission through lemniscal pathway during voluntary movement in the cat. *J Neurophysiol* 37(5):831-45, 1974.

Cramer NP, Keller A. Cortical control of a whisking central pattern generator. *J Neurophysiol* 96(1):209-17, 2006.

Cramer NP, Li Y, Keller A.The whisking rhythm generator: a novel mammalian network for the generation of movement. *J Neurophysiol* 97(3):2148-58, 2007.

Cullen KE, Sensory signals during active versus passive movement. *Curr Opin Neurobiol* 14(6):698-706, 2004.

Deschenes M, Veinante P, Zhang ZW. The organization of corticothalamic projections: reciprocity versus parity. *Brain Res Brain Res Rev* 28(3):286-308, 1998.

Diamond ME, Armstrong-James M, Budway MJ, Ebner FF. Somatic sensory responses in the rostral sector of the posterior group (POm) and in the ventral posterior medial nucleus (VPM) of the rat thalamus: dependence on the barrel field cortex. *J Comp Neurol* 319(1):66-84, 1992.

Diamond ME, Somatosensory thalamus of the rat. In Cerebral Cortex: The Barrel Cortex of the Rat. Edited by Jones EG, Diamond IT. New York and London: Plenum Press 189-220, 1995.

Donoghue JP, Parham C. Afferent connections of the lateral agranular field of the rat motor cortex. *J Comp Neurol* 217(4):390-404, 1983.

Donoghue JP, Wise SP. The motor cortex of the rat: cytoarchitecture and microstimulation mapping. *J Comp Neurol* 212(1):76-88. 1982.

Durham D, Woolsey TA. Effects of neonatal whisker lesions on mouse central trigeminal pathways. *J Comp Neurol* 223(3):424-47, 1984.

Eaton SA, Salt TE. Role of N-methyl-D-aspartate and metabotropic glutamate receptors in corticothalamic excitatory postsynaptic potentials in vivo. *Neuroscience* 73(1):1-5, 1996.

Ergenzinger ER, Glasier MM, Hahm JO, Pons TP. Cortically induced thalamic plasticity in the primate somatosensory system. *Nat Neurosci* 1(3):226-9, 1998.

Erisir A, Van Horn SC, Sherman SM. Relative numbers of cortical and brainstem inputs to the lateral geniculate nucleus. *Proc Natl Acad Sci U S A* 94(4):1517-20, 1997.

Fabri M, Burton H. Ipsilateral cortical connections of primary somatic sensory cortex in rats. *J Comp Neurol* 311(3):405-24, 1991.

Fanselow EE, Nicolelis MA. Behavioral modulation of tactile responses in the rat somatosensory system. *J Neurosci* 19(17):7603-16, 1999.

Fee MS, Mitra PP, Kleinfeld D. Central versus peripheral determinants of patterned spike activity in rat vibrissa cortex during whisking. *J Neurophysiol* 78(2):1144-9, 1997.

Friedman WA, Jones LM, Cramer NP, Kwegyir-Afful EE, Zeigler HP, Keller A. Anticipatory activity of motor cortex in relation to rhythmic whisking. *J Neurophysiol* 95(2):1274-7, 2006.

Furuta T, Nakamura K, Deschenes M. Angular tuning bias of vibrissa-responsive cells in the paralemniscal pathway. *J Neurosci* 26(41):10548-57, 2006.

Gao P, Bermejo R, Zeigler HP. Whisker deafferentation and rodent whisking patterns: behavioral evidence for a central pattern generator. *J Neurosci* 21(14):5374-80, 2001

Gao P, Hattox AM, Jones LM, Keller A, Zeigler HP. Whisker motor cortex ablation and whisker movement patterns. *Somatosens Mot Res* 20(3-4):191-8, 2003.

Ghazanfar AA, Krupa DJ, Nicolelis MA. Role of cortical feedback in the receptive field structure and nonlinear response properties of somatosensory thalamic neurons. *Exp Brain Res* 141(1):88-100, 2001.

Ghez C, Lenzi GL. Modulation of afferent transmission in the lemniscal system during voluntary movement in cat. *Brain Res* 24(3):542, 1970.

Ghez C, Lenzi GL. Modulation of sensory transmission in cat lemniscal system during voluntary movement. *Pflugers Arch* 323(3):273-8, 1971.

Ghez C, Pisa M. Inhibition of afferent transmission in cuneate nucleus during voluntary movement in the cat. *Brain Res* 40(1):145-55, 1972.

Ghosh S, Murray GM, Turman AB, Rowe MJ. Corticothalamic influences on transmission of tactile information in the ventroposterolateral thalamus of the cat: effect of reversible inactivation of somatosensory cortical areas I and II. *Exp Brain Res* 100(2):276-86, 1994.

Gibson JM, Welker WI. Quantitative studies of stimulus coding in first-order vibrissa afferents of rats. 1. Receptive field properties and threshold distributions. *Somatosens Res* 1(1):51-67, 1983a.

Gibson JM, Welker WI. Quantitative studies of stimulus coding in first-order vibrissa afferents of rats. 2. Adaptation and coding of stimulus parameters. *Somatosens Res* 1(2):95-117, 1983b.

Gilbert CD, Kelly JP. The projections of cells in different layers of the cat's visual cortex. *J Comp Neurol* 163(1):81-105, 1975.

Gioanni Y, Lamarche M. A reappraisal of rat motor cortex organization by intracortical microstimulation. *Brain Res* 344(1):49-61, 1985.

Golshani P, Warren RA, Jones EG. Progression of change in NMDA, non-NMDA, and metabotropic glutamate receptor function at the developing corticothalamic synapse. *J Neurophysiol* 80(1):143-54, 1998.

Grinevich V, Brecht M, Osten P. Monosynaptic pathway from rat vibrissa motor cortex to facial motor neurons revealed by lentivirus-based axonal tracing. *J Neurosci* 25(36):8250-8, 2005.

Guillery RW, Sherman SM. The thalamus as a monitor of motor outputs. *Philos Trans R Soc Lond B Biol Sci* 357(1428):1809-21, 2002.

Haiss F, Schwarz C. Spatial segregation of different modes of movement control in the whisker representation of rat primary motor cortex. *J Neurosci* 25(6):1579-87, 2005.

Harris RM, Hendrickson AE. Local circuit neurons in the rat ventrobasal thalamus--a GABA immunocytochemical study. *Neuroscience* 21(1):229-36, 1987.

Hattox A, Li Y, Keller A. Serotonin regulates rhythmic whisking. *Neuron* 39(2):343-52, 2003.

Hattox AM, Priest CA, Keller A. Functional circuitry involved in the regulation of whisker movements. *J Comp Neurol* 442(3):266-76, 2002.

Hayashi H. Distributions of vibrissae afferent fiber collaterals in the trigeminal nuclei as revealed by intra-axonal injection of horseradish peroxidase. *Brain Res* 183(2):442-6, 1980.

He J. Modulatory effects of regional cortical activation on the onset responses of the cat medial geniculate neurons. *J Neurophysiol* 77(2):896-908, 1997.

Hentschke H, Haiss F, Schwarz C. Central signals rapidly switch tactile processing in rat barrel cortex during whisker movements. *Cereb Cortex* 16(8):1142-56, 2006.

Hirata A, Aguilar J, Castro-Alamancos MA. Noradrenergic activation amplifies bottom-up and top-down signal-to-noise ratios in sensory thalamus. *J Neurosci* 26(16):4426-36, 2006.

Hoffer ZS, Alloway KD. Organization of corticostriatal projections from the vibrissal representations in the primary motor and somatosensory cortical areas of rodents. *J Comp Neurol* 439(1):87-103, 2001.

Hoffer ZS, Hoover JE, Alloway KD. Sensorimotor corticocortical projections from rat barrel cortex have an anisotropic organization that facilitates integration of inputs from whiskers in the same row. *J Comp Neurol* 466(4):525-44, 2003.

Hoogland PV, Wouterlood FG, Welker E, Van der Loos H. Ultrastructure of giant and small thalamic terminals of cortical origin: a study of the projections from the barrel cortex in mice using Phaseolus vulgaris leuco-agglutinin (PHA-L). *Exp Brain Res* 87(1):159-72, 1991.

Hupe JM, James AC, Payne BR, Lomber SG, Girard P, Bullier J. Cortical feedback improves discrimination between figure and background by V1, V2 and V3 neurons. *Nature* 394(6695):784-7, 1998.

Izraeli R, Porter LL. Vibrissal motor cortex in the rat: connections with the barrel field. *Exp Brain Res* 104(1):41-54, 1995.

Jacquin MF, Chiaia NL, Haring JH, Rhoades RW. Intersubnuclear connections within the rat trigeminal brainstem complex. *Somatosens Mot Res* 7(4):399-420, 1990b.

Jacquin MF, Golden J, Panneton WM. Structure and function of barrel 'precursor' cells in trigeminal nucleus principalis. Brain Res 471(2):309-14, 1988.

Jacquin MF, McCasland JS, Henderson TA, Rhoades RW, Woolsey TA. 2-DG uptake patterns related to single vibrissae during exploratory behaviors in the hamster trigeminal system. *J Comp Neurol* 332(1):38-58, 1993.

Jacquin MF, Renehan WE, Rhoades RW, Panneton WM. Morphology and topography of identified primary afferents in trigeminal subnuclei principalis and oralis. *J Neurophysiol* 70(5):1911-36, 1993.

Jacquin MF, Wiegand MR, Renehan WE. Structure-function relationships in rat brain stem subnucleus interpolaris. VIII. Cortical inputs. *J Neurophysiol* 64(1):3-27, 1990a.

Jones EG, Powell TP. An electron microscopic study of the mode of termination of cortico-thalamic fibres within the sensory relay nuclei of the thalamus. *Proc R Soc Lond B Biol Sci* 172(27):173-85, 1969.

Kawaguchi Y, Kubota Y. Correlation of physiological subgroupings of nonpyramidal cells with parvalbumin- and calbindinD28k-immunoreactive neurons in layer V of rat frontal cortex. *J Neurophysiol* 70(1):387-96, 1993.

Keller A, White EL. Triads: a synaptic network component in the cerebral cortex. *Brain Res* 496(1-2):105-12, 1989.

Kelly MK, Carvell GE, Hartings JA, Simons DJ. Axonal conduction properties of antidromically identified neurons in rat barrel cortex. *Somatosens Mot Res* 18(3):202-10, 2001.

Kleinfeld D, Ahissar E, Diamond ME. Active sensation: insights from the rodent vibrissa sensorimotor system. *Curr Opin Neurobiol* 16(4):435-44, 2006.

Kleinfeld D, Berg RW, O'Connor SM. Anatomical loops and their electrical dynamics in relation to whisking by rat. *Somatosens Mot Res* 16(2):69-88, 1999.

Koralek KA, Olavarria J, Killackey HP. Areal and laminar organization of corticocortical projections in the rat somatosensory cortex. *J Comp Neurol* 299(2):133-50, 1990.

Krupa DJ, Ghazanfar AA, Nicolelis MA. Immediate thalamic sensory plasticity depends on corticothalamic feedback. *Proc Natl Acad Sci U S A* 96(14):8200-5, 1999.

Krupa DJ, Wiest MC, Shuler MG, Laubach M, Nicolelis MA. Layer-specific somatosensory cortical activation during active tactile discrimination. *Science* 304(5679):1989-92, 2004.

Kwegyir-Afful EE, Bruno RM, Simons DJ, Keller A. The role of thalamic inputs in surround receptive fields of barrel neurons. *J Neurosci* 25(25):5926-34, 2005.

Kyriazi HT, Carvell GE, Brumberg JC, Simons DJ. Quantitative effects of GABA and bicuculline methiodide on receptive field properties of neurons in real and simulated whisker barrels. *J Neurophysiol* 75(2):547-60, 1996.

Kyriazi H, Carvell GE, Brumberg JC, Simons DJ. Laminar differences in bicuculline methiodide's effects on cortical neurons in the rat whisker/barrel system. *Somatosens Mot Res* 15(2):146-56, 1998.

Kyriazi HT, Simons DJ. Thalamocortical response transformations in simulated whisker barrels. *J Neurosci* 13(4):1601-15, 1993.

Land PW, Buffer SA Jr, Yaskosky JD. Barreloids in adult rat thalamus: three-dimensional architecture and relationship to somatosensory cortical barrels. *J Comp Neurol* 355(4):573-88, 1995.

Landry P, Dykes RW. Identification of two populations of corticothalamic neurons in cat primary somatosensory cortex. *Exp Brain Res* 60(2):289-98, 1985.

Leiser SC, Moxon KA. Responses of trigeminal ganglion neurons during natural whisking behaviors in the awake rat. *Neuron* 53(1):117-33, 2007.

Li L, Ebner FF. Cortical modulation of spatial and angular tuning maps in the rat thalamus. *J Neurosci* 27(1):167-79, 2007.

Lichtenstein SH, Carvell GE, Simons DJ. Responses of rat trigeminal ganglion neurons to movements of vibrissae in different directions. *Somatosens Mot Res* 7(1):47-65, 1990.

Liu XB, Honda CN, Jones EG Distribution of four types of synapse on physiologically identified relay neurons in the ventral posterior thalamic nucleus of the cat. *J Comp Neurol* 352(1):69-91, 1995.

Lovick TA. The behavioural repertoire of precollicular decerebrate rats. *J Physiol* 226(2):4P-6P, 1972.

Ma PM, Woolsey TA. Cytoarchitectonic correlates of the vibrissae in the medullary trigeminal complex of the mouse. *Brain Res* 306(1-2):374-9, 1984.

Ma PM. The barrelettes--architectonic vibrissal representations in the brainstem trigeminal complex of the mouse. I. Normal structural organization. J Comp Neurol 309(2):161-99, 1991.

McCormick DA, Connors BW, Lighthall JW, Prince DA. Comparative electrophysiology of pyramidal and sparsely spiny stellate neurons of the neocortex. *J Neurophysiol*. 54(4):782-806, 1985.

McCormick DA, von Krosigk M. Corticothalamic activation modulates thalamic firing through glutamate "metabotropic" receptors. *Proc Natl Acad Sci U S A* 89(7):2774-8, 1992.

Mercer A, West DC, Morris OT, Kirchhecker S, Kerkhoff JE, Thomson AM. Excitatory connections made by presynaptic cortico-cortical pyramidal cells in layer 6 of the neocortex. *Cereb Cortex* 15(10):1485-96, 2005.

Mignard M, Malpeli JG. Paths of information flow through visual cortex. *Science* 251(4998):1249-51, 1991.

Minnery BS, Simons DJ. Response properties of whisker-associated trigeminothalamic neurons in rat nucleus principalis. *J Neurophysiol* 89(1):40-56, 2003.

Miyashita E, Keller A, Asanuma H. Input-output organization of the rat vibrissal motor cortex. *Exp Brain Res* 99(2):223-32, 1994.

Mountcastle VB. Introduction. Computation in cortical columns. *Cereb Cortex* 13(1):2-4, 2003

Neafsey EJ, Bold EL, Haas G, Hurley-Gius KM, Quirk G, Sievert CF, Terreberry RR. The organization of the rat motor cortex: a microstimulation mapping study. *Brain Res* 396(1):77-96, 1986.

Nelson RJ. Interactions between motor commands and somatic perception in sensorimotor cortex. *Curr Opin Neurobiol* 6(6):801-10, 1996.

Nguyen QT, Kleinfeld D. Positive feedback in a brainstem tactile sensorimotor loop. *Neuron* 45(3):447-57, 2005.

Ojima H. Terminal morphology and distribution of corticothalamic fibers originating from layers 5 and 6 of cat primary auditory cortex. *Cereb Cortex* 4(6):646-63, 1994.

O'Connor SM, Berg RW, Kleinfeld D. Coherent electrical activity between vibrissa sensory areas of cerebellum and neocortex is enhanced during free whisking.*J Neurophysiol* 87(4):2137-48, 2002.

O'Keefe J, Gaffan D. Response properties of units in the dorsal column nuclei of the freely moving rat: changes as a function of behaviour. *Brain Res* 31(2):374-5, 1971.

Pierret T, Lavallee P, Deschenes M. Parallel streams for the relay of vibrissal information through thalamic barreloids. J Neurosci 20(19):7455-62, 2000.

Pinto DJ, Hartings JA, Brumberg JC, Simons DJ. Cortical damping: analysis of thalamocortical response transformations in rodent barrel *cortex.Cereb* Cortex 13(1):33-44, 2003.

Pinto DJ, Brumberg JC, Simons DJ. Circuit dynamics and coding strategies in rodent somatosensory cortex. *J Neurophysiol* 83(3):1158-66, 2000.

Prigg T, Goldreich D, Carvell GE, Simons DJ. Texture discrimination and unit recordings in the rat whisker/barrel system. Physiol Behav 77(4-5):671-5, 2002.

Porter LL, White EL. Afferent and efferent pathways of the vibrissal region of primary motor cortex in the mouse. *J Comp Neurol* 214(3):279-89, 1983.

Przybyszewski AW, Gaska JP, Foote W, Pollen DA. Striate cortex increases contrast gain of macaque LGN neurons. *Vis Neurosci* 17(4):485-94, 2000.

Rauschecker JP. Cortical processing of complex sounds. *Curr Opin Neurobiol* 8(4):516-21, 1998.

Rocco MM, Brumberg JC. The sensorimotor slice. *J Neurosci Methods* 162(1-2):139-47, 2007.

Roelfsema PR, Lamme VA, Spekreijse H. Object-based attention in the primary visual cortex of the macaque monkey. *Nature* 395(6700):376-81, 1998.

Rouiller EM, Welker E. A comparative analysis of the morphology of corticothalamic projections in mammals. *Brain Res Bull* 53(6):727-41, 2000.

Sachdev RN, Berg RW, Champney G, Kleinfeld D, Ebner FF. Unilateral vibrissa contact: changes in amplitude but not timing of rhythmic whisking. *Somatosens Mot Res* 20(2):163-9, 2003.

Sachdev RN, Sato T, Ebner FF. Divergent movement of adjacent whiskers. *J Neurophysiol* 87(3):1440-8, 2002.

Sarter M, Givens B and Bruno JP, The cognitive neuroscience of sustained attention: Where top–down meets bottom–up. *Brain Research and Brain Research Review* 35 pp. 146–160, 2001.

Scheibel ME, Scheibel AB. The organization of the ventral anterior nucleus of the thalamus. A Golgi study. *Brain Res* 1(3):250-68, 1966.

Seki K, Perlmutter SI, Fetz EE. Sensory input to primate spinal cord is presynaptically inhibited during voluntary movement. *Nat Neurosci* 6(12):1309-16. Epub 2003 Nov 16, 2003.

Semba K, Komisaruk BR. Neural substrates of two different rhythmical vibrissal movements in the rat. *Neuroscience* 12(3):761-74, 1984.

Sharp FR, Evans K. Regional (14C) 2-deoxyglucose uptake during vibrissae movements evoked by rat motor cortex stimulation. *J Comp Neurol* 208(3):255-87, 1982.

Sherman SM, Guillery RW. Functional organization of thalamocortical relays. *J Neurophysiol* 76(3):1367-95, 1996.

Shin HC, Chapin JK. Mapping the effects of motor cortex stimulation on somatosensory relay neurons in the rat thalamus: direct responses and afferent modulation. *Brain Res Bull* 24(2):257-65, 1990a.

Shin HC, Chapin JK. Modulation of afferent transmission to single neurons in the ventroposterior thalamus during movement in rats. *Neurosci Lett* 108(1-2):116-20, 1990b.

Shin HC, Chapin JK. Movement induced modulation of afferent transmission to single neurons in the ventroposterior thalamus and somatosensory cortex in rat. *Exp Brain Res* 81(3):515-22, 1990c.

Shoykhet M, Doherty D, Simons DJ. Coding of deflection velocity and amplitude by whisker primary afferent neurons: implications for higher level processing. *Somatosens Mot Res* 17(2):171-80, 2000.

Shoykhet M, Land PW, Simons DJ. Whisker trimming begun at birth or on postnatal day 12 affects excitatory and inhibitory receptive fields of layer IV barrel neurons. *J Neurophysiol* 94(6):3987-95, 2005.

Sikich L, Woolsey TA, Johnson EM Jr. Effect of a uniform partial denervation of the periphery on the peripheral and central vibrissal system in guinea pigs. *J Neurosci* 6(5):1227-40, 1986.

Sillito AM, Jones HE, Gerstein GL, West DC. Feature-linked synchronization of thalamic relay cell firing induced by feedback from the visual cortex. *Nature* 369(6480):479-82, 1994.

Sillito AM, Jones HE. Corticothalamic interactions in the transfer of visual information. Philos *Trans R Soc Lond B Biol Sci* 357(1428):1739-52, 2002.

Simons DJ, Carvell GE. Thalamocortical response transformation in the rat vibrissa/barrel system. *J Neurophysiol* 61(2):311-30,1989.

Simons DJ. Temporal and spatial integration in the rat SI vibrissa cortex. *J Neurophysiol* 54(3):615-35, 1985.

Simons DJ.Response properties of vibrissa units in rat SI somatosensory neocortex. *J Neurophysiol* 41(3):798-820, 1978.

Sirota MG, Swadlow HA, Beloozerova IN. Three channels of corticothalamic communication during locomotion. *J Neurosci* 25(25):5915-25, 2005.

Sommer MA, Wurtz RH. Influence of the thalamus on spatial visual processing in frontal cortex. *Nature*. 444(7117):374-7, 2006.

Spacek J, Lieberman AR. Ultrastructure and three-dimensional organization of synaptic glomeruli in rat somatosensory thalamus. *J Anat* 117(Pt 3):487-516, 1974.

Swadlow HA, Gusev AG. The influence of single VB thalamocortical impulses on barrel columns of rabbit somatosensory cortex. *J Neurophysiol* 83(5):2802-13, 2000.

Swadlow HA, Gusev AG. The impact of 'bursting' thalamic impulses at a neocortical synapse. *Nat Neurosci* 4(4):402-8, 2001.

Swadlow HA, Hicks TP. Somatosensory cortical efferent neurons of the awake rabbit: latencies to activation via supra--and subthreshold receptive fields. *J Neurophysiol* 75(4):1753-9, 1996.

Swadlow HA. Efferent neurons and suspected interneurons in second somatosensory cortex of the awake rabbit: receptive fields and axonal properties. *J Neurophysiol* 66(4):1392-409, 1991.

Swadlow HA. Descending corticofugal neurons in layer 5 of rabbit S1: evidence for potent corticocortical, but not thalamocortical, input. *Exp Brain Res* 130(2):188-94, 2000.

Swadlow HA. Efferent neurons and suspected interneurons in binocular visual cortex of the awake rabbit: receptive fields and binocular properties. *J Neurophysiol* 59(4):1162-87, 1988.

Swadlow HA. Efferent neurons and suspected interneurons in motor cortex of the awake rabbit: axonal properties, sensory receptive fields, and subthreshold synaptic inputs. *J Neurophysiol.* Feb;71(2):437-53, 1994.

Swadlow HA. Efferent neurons and suspected interneurons in S-1 vibrissa cortex of the awake rabbit: receptive fields and axonal properties. *J Neurophysiol* 62(1):288-308, 1989.

Swadlow HA. Efferent neurons and suspected interneurons in S-1 vibrissa cortex of the awake rabbit: receptive fields and axonal properties. *J Neurophysiol* 62(1):288-308, 1989

Szwed M, Bagdasarian K, Ahissar E. Encoding of vibrissal active touch. *Neuron.* Oct 30;40(3):621-30, 2003.

Szwed M, Bagdasarian K, Blumenfeld B, Barak O, Derdikman D, Ahissar E. Responses of trigeminal ganglion neurons to the radial distance of contact during active vibrissal touch. *J Neurophysiol* 95(2):791-802, 2006.

Temereanca S, Simons DJ. Functional topography of corticothalamic feedback enhances thalamic spatial response tuning in the somatosensory whisker/barrel system. *Neuron* 19;41(4):639-51, 2004.

Temereanca S, Simons DJ. Local field potentials and the encoding of whisker deflections by population firing synchrony in thalamic barreloids. *J Neurophysiol* 89(4):2137-45, 2003.

Timofeeva E, Dufresne C, Sik A, Zhang ZW, Deschenes M. Cholinergic modulation of vibrissal receptive fields in trigeminal nuclei. *J Neurosci* 25(40):9135-43, 2005.

Timofeeva E, Lavallee P, Arsenault D, Deschenes M. Synthesis of multiwhisker-receptive fields in subcortical stations of the vibrissa system. *J Neurophysiol* 91(4):1510-5, 2004.

Timofeeva E, Merette C, Emond C, Lavallee P, Deschenes M. A map of angular tuning preference in thalamic barreloids. *J Neurosci* 23(33):10717-23, 2003.

Towal RB, Hartmann MJ. Right-left asymmetries in the whisking behavior of rats anticipate head movements. *J Neurosci* 26(34):8838-46, 2006.

Trageser JC, Keller A. Reducing the uncertainty: gating of peripheral inputs by zona incerta. *J Neurosci* 24(40):8911-5, 2004.

Tsumoto T, Creutzfeldt OD, Legendy CR. Functional organization of the corticofugal system from visual cortex to lateral geniculate nucleus in the cat (with an appendix on geniculo-cortical mono-synaptic connections*). Exp Brain Res* 32(3):345-64, 1978.

Turner JP, Salt TE. Characterization of sensory and corticothalamic excitatory inputs to rat thalamocortical neurones in vitro*. J Physiol* 510 (Pt 3):829-43, 1998.

Usrey WM, Fitzpatrick D. Specificity in the axonal connections of layer VI neurons in tree shrew striate cortex: evidence for distinct granular and supragranular systems. *J Neurosci* 16(3):1203-18, 1996.

van der Loos H. Neuronal circuitry and its development. *Prog Brain Res* 45:259-78, 1976.

Veinante P, Deschenes M. Single- and multi-whisker channels in the ascending projections from the principal trigeminal nucleus in the rat. *J Neurosci* 19(12):5085-95, 1999.

Veinante P, Deschenes M. Single-cell study of motor cortex projections to the barrel field in rats*. J Comp Neurol* 464(1):98-103, 2003.

Veinante P, Lavallee P, Deschenes M. Corticothalamic projections from layer 5 of the vibrissal barrel cortex in the rat. *J Comp Neurol* 424(2):197-204, 2000.

von Krosigk M, Monckton JE, Reiner PB, McCormick DA. Dynamic properties of corticothalamic excitatory postsynaptic potentials and thalamic reticular inhibitory postsynaptic potentials in thalamocortical neurons of the guinea-pig dorsal lateral geniculate nucleus. *Neuroscience* 91(1):7-20, 1999.

Wang W, Jones HE, Andolina IM, Salt TE, Sillito AM. Functional alignment of feedback effects from visual cortex to thalamus. *Nat Neurosci* 9(10):1330-6, 2006.

Webb BS, Tinsley CJ, Barraclough NE, Easton A, Parker A, Derrington AM. Feedback from V1 and inhibition from beyond the classical receptive field modulates the responses of neurons in the primate lateral geniculate nucleus. *Vis Neurosci* 19(5):583-92, 2002.

Welker C. Microelectrode delineation of fine grain somatotopic organization of (SmI) cerebral neocortex in albino rat. *Brain Res* 26(2):259-75, 1971.

Welker WI, Johnson JI Jr, Pubols BH Jr. Some morphological and physiological characteristics of the somatic sensory system in raccoons*. Am Zool* 4:75-94, 1964.

West DC, Mercer A, Kirchhecker S, Morris OT, Thomson AM. Layer 6 cortico-thalamic pyramidal cells preferentially innervate interneurons and generate facilitating EPSPs. *Cereb Cortex* 16(2):200-11, 2006.

White EL. Identified neurons in mouse Sml cortex which are postsynaptic to thalamocortical axon terminals: a combined Golgi-electron microscopic and degeneration study. *J Comp Neurol* 181(3):627-61, 1978.

White EL, DeAmicis RA. Afferent and efferent projections of the region in mouse SmL cortex which contains the posteromedial barrel subfield. *J Comp Neurol* 175(4):455-82, 1977.

White EL, Hersch SM. A quantitative study of thalamocortical and other synapses involving the apical dendrites of corticothalamic projection cells in mouse SmI cortex. *J Neurocytol* 11(1):137-57, 1982.

White EL, Keller A. Intrinsic circuitry involving the local axon collaterals of corticothalamic projection cells in mouse SmI cortex. *J Comp Neurol* 262(1):13-26, 1987.

Williams MN, Zahm DS, Jacquin MF. Differential foci and synaptic organization of the principal and spinal trigeminal projections to the thalamus in the rat. *Eur J Neurosci* 6(3):429-53, 1994.

Woolsey TA, Van der Loos H. The structural organization of layer IV in the somatosensory region (SI) of mouse cerebral cortex. The description of a cortical field composed of discrete cytoarchitectonic units. *Brain Res* 17(2):205-42, 1970.

Wurtz RH, Sommer MA. Identifying corollary discharges for movement in the primate brain. *Prog Brain Res* 144:47-60, 2004.

Yan J, Suga N. Corticofugal amplification of facilitative auditory responses of subcortical combination-sensitive neurons in the mustached bat. *J Neurophysiol* 81(2):817-24, .1999

Yu C, Derdikman D, Haidarliu S, Ahissar E. Parallel thalamic pathways for whisking and touch signals in the rat. *PLoS Biol* 4(5):e124, 2006.

Yuan B, Morrow TJ, Casey KL. Corticofugal influences of S1 cortex on ventrobasal thalamic neurons in the awake rat. *J Neurosci* 6(12):3611-7, 1986.

Yuan B, Morrow TJ, Casey KL. Responsiveness of ventrobasal thalamic neurons after suppression of S1 cortex in the anesthetized rat. *J Neurosci* 5(11):2971-8, 1985.

Zhang Y, Suga N. Modulation of responses and frequency tuning of thalamic and collicular neurons by cortical activation in mustached bats. *J Neurophysiol* 84(1):325-33, 2000.

Zhang ZW, Deschenes M. Intracortical axonal projections of lamina VI cells of the primary somatosensory cortex in the rat: a single-cell labeling study. *J Neurosci* 17(16):6365-79, 1997.

Zhang ZW, Deschenes M. Projections to layer VI of the posteromedial barrel field in the rat: a reappraisal of the role of corticothalamic pathways. *Cereb Cortex* 8(5):428-36, 1998.

Zucker E, Welker WI. Coding of somatic sensory input by vibrissae neurons in the rat's trigeminal ganglion. *Brain Res* 12(1):138-56, 1969.