EFFECTS OF ADAPTATION ON RESPONSES IN A SOMATOSENSORY THALAMOCORTICAL CIRCUIT

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Submitted to the Graduate Faculty of

Center for Neuroscience in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

University of Pittsburgh

2005

UNIVERSITY OF PITTSBURGH

Center for Neuroscience

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October 29, 2005

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In the mammalian brain, thalamocortical circuits perform the initial stage of processing before information is sent to higher levels of the cerebral cortex. Substantial changes in receptive field properties are produced in the thalamocortical response transformation. In the whisker-to-barrel thalamocortical pathway, the response magnitude of barrel excitatory cells is sensitive to the velocity of whisker deflections, whereas in the thalamus, velocity is only encoded by firing synchrony. The behavior of this circuit can be captured in a model which contains a window of opportunity for thalamic firing synchrony to engage intra-barrel recurrent excitation before being 'damped' by slightly delayed, but strong, local feedforward inhibition. Some remaining aspects of the model that require investigation are: (1) how does adaptation with ongoing and repetitive sensory stimulation affect processing in this circuit and (2) what are the rules governing intra-barrel interactions. By examining sensory processing in thalamic barreloids and cortical barrels, before and after adaptation with repetitive high-frequency whisker stimulation, I have determined that adaptation modifies the operations of the thalamocortical circuit without fundamentally changing it. In the non-adapted state, higher velocities produce larger responses in barrel cells than lower velocities. Similarly, in the adapted barrel, putative excitatory and inhibitory neurons can respond with temporal

fidelity to high-frequency whisker deflections if they are of sufficient velocity. Additionally, before and after adaptation, relative to putative excitatory cells, inhibitory cells produce larger responses and are more broadly-tuned for stimulus parameters (e.g., the angle of whisker deflection). In barrel excitatory cells, adaptation is angularly-nonspecific; that is, response suppression is not specific to the angle of the adapting stimulus. The angular tuning of barrel excitatory cells is sharpened and the original angular preference is maintained. This is consistent with intra-barrel interactions being angularly-nonspecific. The maintenance of the original angular preference also suggests that the same thalamocortical inputs determine angular tuning before and after adaptation. In summary, the present findings suggest that adaptation narrows the window of opportunity for synchronous thalamic inputs to engage recurrent excitation so that it can withstand strong, local inhibition. These results from the whisker-to-barrel thalamocortical response transformation are likely to have parallels in other systems.

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ACKNOWLEDGEMENTS

There are many people to whom I am grateful for their support throughout my time in graduate school. The person who helped me the most, is of course my mentor, Dan Simons. About 4 years ago, I gave him a call one morning and I told him that I had to ask him a question, to which his answer would affect the future of my life. He told me to come down to his office. I wanted to join his laboratory, but I knew that he was already training four graduate students and I was skeptical as to whether there was room for another. But when I went to see him, he told me that he could make room for me in the lab. Consequently, during the last 4 years, I have learned a lot from him. The most important lesson that I received from his example, is that one can run an excellent lab and still be genuinely concerned about the future of the people who are working for you. Dan and the rest of my thesis committee (Allen Humphrey, Pete Land, Karl Kandler, Nathan Urban, and Asaf Keller) have provided me with a great education.

In addition to Dan, the rest of the Simons lab has been wonderful to work with as well. I'd like to thank Simona Temereanca, Brad Minnery, Mish Shoykhet, Anissa Meyers, Jed Hartings, Randy Bruno, Anjey Su, Ernest E. Kwegyir-Afful, and George Fraser for all their help. Jed and I worked together on the project comparing thalamic and cortical responses to repetitive whisker deflections. Randy and I did several experiments together to learn about the barrel's angular tuning domains. Anjey helped with several of the surgical preparations. George kindly let me compare his trigeminal ganglion data to my thalamic and cortical data. Last, but not least from the lab, I would like to thank SooHyun Lee and Harold Kyriazi. SooHyun is a very talented and kind person who helped create the lab's pleasant atmosphere and helped me

work through ideas. Harold provided a lot of technical and intellectual assistance. He also reminded me, every now and then, that there were other things in the world, besides rat whiskers.

Outside of lab, there are of course, my wonderful family and friends. I'd like to thank Mads Larsen, Savanh Chanthaphavong, Marci Chew, Eve Wider, and Sriram Vennetti for their friendships. I could not have done this without my friends or my family. My parents, sister, brother-in-law, and nephew have supported me whole-heartedly, both financially and emotionally. Thank you.

1.0 INTRODUCTION

A major function of the brain is to process informative sensory stimuli. The discovery of neurons with remarkably complex selectivity has produced insights as to how sensory systems operate. Such efforts were pioneered by Kuffler and Barlow who elucidated the center-surround sub-structure within the receptive fields of retinal ganglion cells (Kuffler, 1953; Barlow, 1953). Subsequently, the receptive field characteristics of neurons in many other parts of the brain have been revealed. However, a major deficit in our understanding of sensory processing as expressed by Vernon Mountcastle (1998) is: "We have learned a great deal about the functional properties of individual cortical neurons and of their synaptic interactions, much less of operations executed by groups of those linked in processing chains". As Mountcastle states, we lack insights into why neurons are embedded in a network, or *processing chain*, as opposed to existing in isolation.

Mountcastle did initiate the process of understanding neural circuits with his discovery of the *cortical column* as a fundamental processing unit. He observed that all cells encountered in a vertical electrode penetration of cat primary somatosensory cortex share the same receptive field location upon the body. Since that classic study, many other examples of columnar organization have been demonstrated both electrophysiologically and anatomically for cortical areas, such as rat primary somatosensory cortex (Woolsey, 1967). Using evoked potentials, Woolsey demonstrated a functional relationship between each whisker on the rat's face and specific cytoarchitectonically-defined regions of layer 4 that have been named "barrels" (Woolsey,

1967). Later it was shown that stimulation of a single whisker elicits a larger response than other whiskers from a column of cells throughout all layers of barrel cortex.

Realization of the existence of cortical columns lays the foundation for asking more sophisticated questions of neural processing in defined neural pathways, such as the thalamocortical circuit. Questions that arise include: 1) how do the interplay of thalamic and intracortical inputs determine receptive field properties of cortical neurons, and 2) how does recent stimulus history modify the thalamocortical response transformation. In the rat whisker-to-barrel pathway, answers to the first question have been provided by previous investigations with punctate sensory stimulation (e.g. single, isolated whisker deflections). However, rats derive tactile information about a surface with ongoing and repetitive whisker deflections. The purpose of my work is to examine how adaptation - the presentation of repetitive and ongoing sensory stimulation - modifies the non-adapted thalamocortical response transformation. Adaptation is operationally defined here as the short-term effects of ongoing and repetitive sensory stimulation on neuronal responses. Ongoing stimulation may change the dynamics of a neural circuit due to the accumulation of synaptic depression and/or local inhibition, both of which last for around a few hundred milliseconds. These short-term processes differ from long-term ones such as those due to learning and memory. The model system being used here to examine effects of adaptation and thalamocortical interactions is the lemniscal *barreloid-to-barrel* pathway of the rat whisker system.

1.1 PSYCHOPHYSICAL MOTIVATIONS FOR EXAMINING EFFECTS OF ADAPTATION IN NEURAL CIRCUITS

Adaptation with ongoing sensory stimulation must have significant effects on neural processing. In addition to simply fatiguing sensory neurons that have responded to the adapting stimulus, evidence from psychophysical studies of vision, audition, and somatosensation, indicates that adaptation can facilitate discrimination. For example, in humans, discrimination of features of finger pad tactile stimulation (e.g., frequency or intensity) is enhanced following presentation of prior stimuli of similar characteristics (Goble and Hollins, 1993; Goble and Hollins, 1994). Similar studies in vision (e.g., Dragoi et al., 2002) and audition (e.g., Getzmann, 2004) have provided conceptually identical results. These findings from psychophysics suggest the utility of examining how adaptation influences neural processing in a defined circuit. The whisker-to-barrel thalamocortical circuit provides an excellent model for further characterizing the role of adaptation in sensory systems. Its properties in the non-adapted state are well-characterized (e.g., see Pinto et al., 2003). In addition, the model provides some potential generalizability by sharing properties with the thalamocortical response of cat primary visual cortex (Miller et al. 2001) which also appears to utilize thalamic firing synchrony.

1.2 WHY TARGET LAYER 4 FOR STUDYING THE EFFECTS OF ADAPTATION?

Because of the homogeneity of its constituent cells, layer 4 of sensory neocortex is an excellent target for studying cortical columns and the effect of adaptation on circuit

computations. Layer 4 cells, both inhibitory and excitatory, appear to receive thalamic input (White and Rock, 1981; Benshalom and White, 1986), and intracortical input from other cells in layers 4 and 6 (McGuire et al., 1994; Ahmed et al, 1994; Zhang et al., 1997). Furthermore, layer 4 cells appear to influence receptive field properties throughout a column of cortex. In layer 4 of barrel cortex, the preferred whisker (PW) for a barrel is specified by its thalamic input from the corresponding *barreloid*. Simons (1978) has shown that the PW remains constant throughout the depth of cortex, both above and below the barrel. Similarly, in cat primary visual cortex, Hubel and Wiesel (1962) revealed the existence of *ocular dominance* columns, namely that a given column, extending across cortical layers, is driven more by one eye than the other (Hubel and Wiesel, 1962). They also showed that ocular dominance (OD) was established in layer 4 by thalamic afferents from eye-specific layers of the lateral geniculate nucleus (Hubel and Wiesel, 1969).

The appeal of layer 4 is also due to it being where dramatic receptive field changes occur. In cat primary visual cortex, layer 4 is where orientation selectivity emerges (Hubel and Wiesel, 1958). In the whisker-to-barrel pathway, the layer 4 barrel is the first site where increasing the velocity of whisker deflections consistently results in increased firing by individual excitatory cells, while amplitude has a negligible effect (Pinto et al., 2000). In contrast, the thalamic neuronal population represents greater velocity with more initial firing synchrony (Pinto et al., 2000; Temereanca et al., 2002), but individual thalamocortical (TC) cells may either increase or decrease their overall response magnitude as velocity is increased (Pinto et al., 2000). TC cells also differ from excitatory barrel cells in that they are also amplitude-sensitive if response magnitude is determined for the entire duration of their responses (Pinto et al., 2000).

Another advantage of studying barrel cortex is that the receptive field properties of inhibitory neurons have been distinguished from excitatory neurons by their different extracellular waveforms. In somatosensory cortex, Mountcastle et al. (1969) suggested that cells with 'thin spikes' may be inhibitory neurons. Simons identified *fast-spike* units (FSUs) in barrel cortex and showed that they have receptive field properties that differ from those of *regular-spike* units (RSUs). FSUs are more responsive, having large multi-whisker receptive fields and broader tuning for the direction of whisker deflections (Simons, 1978). Subsequent intracellular studies indicated that a class of cortical neurons with short-duration action potentials had an aspinous or sparsely spinous non-pyramidal morphology (McCormick et al., 1985; Connors and Kriegstein, 1986), a characterisitic of inhibitory neurons. RS waveforms, on the other hand, may be assigned an excitatory status, albeit cautiously. 'Regular spikes' are discharged by excitatory spiny cells, which comprise 90% of the cortical population (Beaulieu, 1993), but the RS waveform is also displayed by a sparse subpopulation of GABAergic neurons (Kawaguchi and Kubota, 1993; Gibson et al., 1999). Fortunately, this subpopulation of GABAergic neurons is sparser in layer 4 than other cortical layers (Gibson et al., 2003). The utility of the RSU-FSU dichotomy can be seen in a recent study of whisker barrel cortex where the two cell types' receptive field properties correlated with their receiving different thalamic inputs (Bruno et al., 2003). Studies of the visual system are also beginning to make use of the RSU-FSU distinction. For example, Contreras and Palmers (2003) have demonstrated that FSUs are more sensitive to stimulus contrast than RSUs, suggesting utility in distinguishing these two groups of neurons in other systems as well.

The homogeneity of a barrel and the complex stimulus selectivity of its constituent excitatory and inhibitory neurons make it an ideal circuit in which to examine the proposal made

by Barlow (1961) that adaptation *decorrelates* the activity of cortical neurons. By decorrelation, he means that after adaptation, the responses of two neurons will be more independent, whereas prior to adaptation their responses will be more correlated. The motivation behind his hypothesis is that the brain may use decorrelation to remove spatial and temporal redundancies characteristic of natural stimuli. As of yet, no study has directly examined Barlow's hypothesis by simultaneously recording from multiple cortical neurons and examining their response correlations before and after adaptation. However, a study of the moth antennal lobe indicates that the odor-evoked responses of simultaneously recorded neurons are decorrelated during the first few hundred milliseconds after stimulus presentation (Daly et al., 2004). By recording from individual neurons and then comparing their responses in the analysis as though they were simultaneously recorded, Muller et al. (1999) demonstrated that in primary visual cortex, adaptation to a particular orientation made complex cells with different orientation preferences more sensitive to changes near that orientation, and that their orientation tuning curves became less similar. Alternatively stated, they were decorrelated by adaptation. Perhaps, if they had done the same analysis with similarly-tuned cells, they would have observed that correlations are retained after adaptation. An alternative to global decorrelation has been proposed to account for the fact that neural circuits are inherently noisy. Correlation among neurons has been proposed as a means to overcome response variability (Wainwright, 1999). A direct demonstration of decorrelation or the retention of correlations after adaptation with simultaneous recordings is therefore necessary and will be done here. If activity is decorrelated by adaptation in layer 4, then Barlow's hypothesis will be supported, but simultaneous recording from thalamic neurons will be necessary before one can that state that decorrelation originates in the cortex.

1.3 THE NON-ADAPTED THALAMOCORTICAL RESPONSE TRANSFORMATION

The role of thalamocortical input

We are prepared to examine how adaptation affects the barreloid-to-barrel thalamocortical response transformation, because much is already known about this transformation in the non-adapted state. Additionally, it will be made apparent how adaptation will reveal further insight into certain aspects of the thalamocortical circuit that is utilized in the non-adapted state.

Despite the fact that only 5 to 20% of the excitatory synapses onto layer 4 neurons are thalamocortical (White, 1989), thalamic input from VPM (ventral posterior medial nucleus) plays a major role in determining the response characteristics of barrel cells. In slices of barrel cortex, individual TC (thalamocortical) axons were found to drive spiny layer 4 neurons more strongly than intracortical (IC) axons (Gil et al., 1999). The larger effect of the TC axon was attributed to a greater number of release sites and a higher release probability. Consistent with the *in vitro* data, other studies in barrel cortex and primary visual cortex have found that response properties of layer 4 cells can be accounted for by TC input. Simultaneous recording of layer 4 barrel cells and somatotopically-aligned thalamic barreloid cells has shown that many barrel cell properties are inherited from TC input (Bruno and Simons, 2002). Barrel RSUs are more likely to be connected to a particular TCU if the two cells have similar principal and adjacent whisker (PW and AW) responses. More frequent TCU connections to FSUs that have larger caudal AW responses can account for the previous demonstration that the response of a barrel RSU to its PW is reduced more if preceded by stimulation of a caudal AW, rather than other AWs (Brumberg et al., 1996).

Additional support for the prominent role of TC input in the production of barrel neuron receptive fields comes from experiments that I did with Randy Bruno. We simultaneously recorded individual TCUs and multiple spike-triggered local field potentials throughout the barrel. We also determined angular tuning for stimulus-evoked multi-unit activity at the same sites in the barrel where the LFPs were collected. Using a previous characterization of the different components of the TCU spike-triggered LFP (Swadlow and Gusev, 2000), we were able to show that a TCU strongly influences a barrel sub-region if the angular tuning of both sites are similar (Bruno et al., 2003).

Studies of cat primary visual cortex also indicate that TC input is responsible for many layer 4 cell properties. Cross-correlation analyses of simultaneously recorded thalamic and cortical simple cells with overlapping receptive fields revealed connections only when ON and OFF sub-regions were in correspondence for both cells (Alonso et al., 2001). Additionally, the organization of TC inputs appears to be sufficient for generating orientation selectivity in cortical cells (Chung and Ferster, 1998). This was determined by silencing most of the cortex with an electrical shock and then whole-cell recording the visually-evoked EPSPs of cortical cells, presumably due to thalamic inputs received by cortical cells.

There is strong evidence that the thalamus plays a key role in determining the receptive field properties of layer 4 cells, but several findings cannot be accounted for by TC input alone. Barrel cells are sensitive to the velocity of a whisker movement, but relatively unaffected by its amplitude. By contrast, thalamic cells are sensitive to both velocity and amplitude (Pinto et al., 2000). The magnitude of TC neuronal firing increases with velocity during the first 2-7 msec of their response, but they also fire more for larger amplitudes throughout their entire response duration (Pinto et al., 2000). Local cortical processing, strong feedforward inhibition and

recurrent excitation, appear to be required to relate barrel RSU activity to TC input (Pinto et al., 2002). Intracortical processing is also needed to account for a *push-pull* model of cat primary visual cortex simple cells (Ferster, 1988; Hirsch et al., 1998). The presentation of a bright spot in an off-subregion of a simple cell evokes hyperpolarization. Thus, the construction of a simple cell receptive field cannot be due to TC input alone, which is purely excitatory.

A 'damping' circuit: a model of the layer 4 barrel

The velocity-sensitivity of RSUs was first predicted in a *damping* model of the barrel (Pinto et al., 1996). In order to create velocity sensitive RSUs, the model requires three major components: 1) the amount of thalamic firing synchrony should increase as the velocity of a whisker deflection is raised, 2) feedforward inhibition is stronger than feedforward excitation and 3) recurrent excitation is activated in a non-linear manner by thalamic input (e.g. a sigmoidal activation function). The strong feedforward inhibition creates a *window of opportunity* for the population of excitatory cells to be engaged by synchronous thalamic firing. If the network of excitatory cells is sufficiently driven before a slightly delayed wave of inhibition arrives, then the recurrent excitation is able to overcome the *damping* effect of local inhibition. The non-linear nature of the recurrent excitation refers to the threshold level of activation needed to overcome damping by feedforward inhibition. Ultimately, barrel excitation recruits feedback inhibition to shut itself down.

Several requirements of the model have been verified experimentally. The response magnitudes of thalamocortical neurons do increase with velocity during the first 2-7 msec of their response (Pinto et al., 2000; Temereanca and Simons, 2003). FSUs are more easily driven

by whisker stimulation than RSUs; they are able to respond to higher temporal frequencies (Simons, 1978) and smaller amplitudes of whisker movement (Swadlow, 1989). The ease with which FSUs can be driven is consistent with their TCU connections, which are stronger and more frequent than those between TCUs and RSUs (Bruno, 2003).

Evidence for sub-networks within a barrel

One aspect of the model that has not been specified is whether the barrel is a homogenous processing unit. Anatomical features strongly suggest that a single barrel contains sub-networks. Cytochrome-oxidase staining of barrels has revealed patterns of low or high reactivity that overlap with other synaptic markers, such as $GABA_A$ receptors (Land et al., 1995). The light CO regions stain heavily for myelin and appear to be conduits for neuronal communication (Land and Erickson, 2005). It has also been shown that while the axon of a single thalamocortical neuron can span the entire horizontal extent of a barrel, individual arbors may terminate most densely within a barrel sub-region of ~200 μm (Jensen and Killackey, 1987; Arnold et al., 2001). Additional evidence for barrel partitioning is provided by the dendritic trees of spiny stellate neurons which also span ~200 μm horizontally (Lubke et al., 2000; Simons and Woolsey, 1984). While the 200 μm spread of TC axonal and stellate dendritic arbors is substantially less than the size of a barrel, the angular tuning domains we have characterized are no wider than ~100 μm. Thus, if the barrel does contain sub-networks that correspond to angular tuning domains, then additional anatomical correlates (e.g. synapse specificity) must be present.

1.4 THE ADAPTED THALAMOCORTICAL RESPONSE TRANSFORMATION

Effects of adaptation on thalamic barreloid neurons

The repetitive manner, in which rats actively sweep their whiskers across a surface can at a first-order approximation, be simulated by repetitively deflecting the whiskers adapting stimuli of different frequencies. The whisking frequencies used by a rat range from ~1 to 8 Hz in air and can reach as high as 40 Hz during object contact (Carvell and Simons, 1995; Harvey et al., 2001). The 1-to-40 Hz frequency range has been used to examine how thalamic neurons in the ventral posterior medial nucleus (VPM) respond to adapting whisker deflections. In lightly-sedated rats, VPM neurons display only a ~20% reduction in response magnitude to even 40 Hz whisker deflections (Hartings et al., 2003). The ability of VPM neurons to respond well to high-frequency stimuli has been shown to be dependent on arousal. VPM responses are suppressed at even 2 Hz in deeply anesthetized rats (e.g. pentobarbital: Diamond et al., 1992; Chung et al., 2002). Overall, these past studies suggest that the responses of thalamic neurons change minimally in response to repetitive whisker deflections.

However, results of several studies indicate that adaptation can modulate the thalamocortical synapse. Chung et al. (2002) demonstrated that the amount of TC synaptic depression evoked by repetitive whisker deflections is correlated with the magnitude of response reduction in cortical neurons. Also, in cat primary visual cortex, TC synaptic depression appears to be able to account for the cortical phenomenon of cross-orientation suppression in which

responses to an optimally-oriented grating are suppressed by superposition with an orthogonal grating (Freeman et al., 2002).

Modifying response properties of cortical barrel neurons with adaptation

Unlike thalamic neurons, cortical neurons show large response reductions for repetitive whisker deflections (Chung et al., 2002; Garabedian et al., 2003). In addition to thalamocortical synaptic depression, the underlying causes of response reduction in the cortex are likely to be strong, local inhibition mediated by FSUs (see Kyriazi et al., 1996) and the depression of synapses among barrel excitatory cells (see Egger et al., 1999; Petersen, 2002). The response reductions of cortical neurons are accompanied by changes in their receptive field properties. For example, Armstrong-James and George (1988) demonstrated that the receptive fields of forepaw cortical neurons become focused spatially with repetitive tactile stimulation. Additionally, Castro-Alamancos demonstrated that electrical stimulation of the brainstem reticular formation suppressed adjacent whisker responses more than principal whisker responses, thereby spatially focusing receptive fields (Castro-Alamancos, 2002) Numerous studies of the other major sensory systems (visual: Dragoi et al., 2002, auditory: Ulanovsky et al., 2002, and olfactory: Wilson, 2000) indicate that adaptation-induced changes in neuronal responses are stimulus-specific, or that response suppression is specific to the characteristics of the adapting stimulus, and that facilitation can be displayed for novel stimuli.

It remains to be determined whether adaptation in the whisker-to-barrel pathway evokes stimulus-specific changes in thalamic barreloid and cortical barrel neuron responses (e.g., for angular tuning). Angularly-specific suppression would be expected from the depression of

angularly-tuned thalamic inputs. However, broadly-tuned intra-barrel inhibition mediated by FSUs should produce angularly-nonspecific suppression. The depression of connections between excitatory cells could produce either specific or nonspecific effects depending on whether or not connections exist between cells with similar or dissimilar angular tuning. It is not known whether excitatory connections only link RSUs with similar receptive field properties (e.g., tuning for the angle of whisker deflections). At the other extreme, RSUs may be interconnected in a completely angularly-nonspecific fashion. The last possibility is that there is a small bias for connections between similarly-tuned neurons, though most connections are angularly-nonspecific. These hypothetical intra-barrel circuits can be distinguished by determining whether adaptation is angularly-specific. If response suppression is greatest when adapting and test angles are the same, then this would provide support for there being angularly-specific connections within a barrel. If intra-barrel interactions link neurons with the same angular preference, this suggests that the purpose of cortical recurrent excitation is to enhance stimulus selectivity. Alternatively, the dominance of angularly-nonspecific intra-barrel interactions (synaptic depression and broadly-tuned inhibition) would indicate that stimulus selectivity is provided by the thalamic input and the purpose of recurrent excitations is to modulate the gain of barrel neuron responses.

1.5 GOAL

These experiments will utilize knowledge gained from the described *in vivo* and *in vitro* studies to investigate how the neural processing of whisker deflections is influenced by recent stimulus history. The goal is to understand how adaptation modifies the non-adapted thalamocortical response transformation and its major components of 1) thalamic firing synchrony, 2) strong feed-forward inhibition, and 3) intra-barrel recurrent excitation.

2.0 ADAPTATION IN THALAMIC BARRELOID AND CORTICAL BARREL NEURONS TO PERIODIC WHISKER DEFLECTIONS VARYING IN FREQUENCY AND VELOCITY

2.1 INTRODUCTION

Using their whiskers, rats can perform high-resolution tactile discriminations (Guic-Robles et al., 1989; Carvell and Simons, 1990; Brecht et al., 1997). Rodents actively whisk their vibrissae back and forth across palpated objects (Welker, 1964), creating rapidly changing patterns of neural activity that, by analogy with active touch in other mammalian tactile systems, enhance sensory discrimination (Lederman and Klatzky, 1987; Hollins et al., 2002). In the brain, individual whiskers are represented by anatomically distinct collections of neurons at each of the central processing stations within the main pathway underlying discriminative touch. These neuronal aggregations are called barrelettes in the principal sensory nucleus of the brain stem (Jacquin et al., 1988), barreloids in the ventral posterior medial (VPM) thalamus (Van der Loos, 1976), and barrels in layer IV of the primary somatosensory cortex (Simons et al., 1989; Welker and Woolsey, 1974). The whisker/barrel system appears well-suited for conveying and processing sensory information rapidly and reliably. Circuits in the brain stem (Minnery and Simons, 2003) and thalamus (Deschenes et al., 2003) faithfully transmit information about temporally precise firing patterns in primary afferent neurons that innervate the whiskers (Jones

et al., 2004), and thalamocortical circuitry is preferentially sensitive to the timing of thalamic spikes (Pinto et al., 2003).

Extracellular recordings of barrel neurons have identified two cell types based on spike waveform. Regular-spike units (RSUs) are thought to be excitatory (spiny stellate or pyramidal neurons), whereas fast-spike units (FSUs) correspond to the largest population of inhibitory neurons (see Bruno and Simons, 2002). Response properties of RSUs and FSUs differ from each other and from those of thalamocortical units (TCUs) in thalamic barreloids (Simons and Carvell, 1989). For example, RSUs have the lowest spontaneous and stimulus-evoked firing rates, FSUs the highest with TCU values intermediate. The highly responsive nature of FSUs is thought to reflect their intrinsic membrane properties (Rudy et al., 1999) and their receipt of highly convergent, strong thalamocortical synaptic input (Bruno and Simons, 2002; Swadlow and Gusev, 2002). Strong thalamic connections directly onto highly responsive inhibitory barrel neurons renders barrel circuitry highly sensitive to the synchronous arrival times of impulses—on the millisecond scale—from populations of barreloid neurons (Pinto et al., 2003). One consequence is that barrel neurons are preferentially sensitive to deflection velocity but not amplitude; the former affecting thalamic firing synchrony and the latter affecting total thalamic response magnitude but not timing (Pinto et al., 2000).

Responses in thalamocortical circuits are strongly affected by adaptation produced by repetitive sensory stimulation (Yuan et al., 1986; Fanselow and Nicolelis, 1999). Periodic stimuli have been employed to examine in vivo effects of central inhibition and synaptic depression, both of which act to reduce responsiveness to repetitive stimuli in a time-dependent fashion (Hellweg et al., 1977; Chung et al., 2002). Behavioral arousal and exploration is associated with pronounced adaptation in thalamocortical circuits (Castro-Alamancos, 2004), raising the

possibility that the vibrissa system is optimized by adaptation to process information at near-whisking frequencies, which occur at ~8 Hz. Consequently, a number of recent studies have focused on the encoding of relatively low-frequency periodic whisker deflections (Moore et al., 1999; Ahissar et al., 2000; Garabedian et al., 2003). Yet to be determined is whether the responses of cortical neurons, including their ability to faithfully reflect the temporal signature of the afferent signal, are affected by the velocity of the periodic whisker deflections.

Recently, we used periodic whisker deflections in the range of 1–40 Hz as a probe for studying thalamic circuitry in the whisker-to-barrel pathway (Hartings et al., 2003). Whisker deflections consisted of high-velocity pulses or lower-velocity sinusoids. Results indicate that TCUs faithfully transmit the high-frequency temporal structure originating from afferent sensory input. Here we examine the consequences of this faithful transmission for neural processing in the thalamocortical circuit. We find that adaptation is considerably greater among barrel than barreloid neurons. Results indicate that RSUs and FSUs retain their distinctive response properties during repetitive whisker stimulation and that as a population barrel neurons encode information about repetitive whisker deflections in a temporally faithful fashion. Moreover, differences in thalamic and cortical responses to pulsatile versus sinusoidal stimuli suggest that barrel circuitry retains its sensitivity to thalamic population firing synchrony in the adapted state.

2.2 METHODS

2.2.1 Animals and surgical preparation

Surgical preparation and maintenance of the rats during electrophysiological recording were identical to those described previously (Hartings et al., 2003). Twenty Sprague-Dawley adult female albino rats (200-300 g) were obtained from a commercial supplier. All surgical preparation was performed under halothane anesthesia. A silastic catheter was inserted into the right jugular vein and led out from the nape of the neck for later drug delivery. A short length (~ 40mm) of polyethylene tubing was inserted into the trachea for later artificial respiration, and the left femoral artery was cannulated using an angiocath catheter in order to measure blood pressure. After exposing the skull, small stainless steel screws were placed over the left occipital and frontal cortex for EEG recordings, and a ground screw was placed over the right frontal cortex. Dental acrylic was used to attach a steel post to the skull. The post, which was used to hold the animal's head without pressure points during the rest of the experiment, permitted unimpeded access to the facial vibrissae. In cortical experiments, the bone overlying the right barrel cortex was thinned and a small (~1 mm^2) craniectomy was made. For thalamic experiments, a craniectomy was made at stereotaxic coordinates overlying VPM (2.0-4.5 mm posterior, 1.5-4.0 mm lateral to bregma). The dura was incised to prevent the brain from dimpling and thus suffering compression damage due to electrode insertion. Lastly, an acrylic dam was constructed around the skull opening and filled with saline.

Body temperature was maintained at 37°C by a servo-controlled heating blanket (Harvard Apparatus, Holliston, MA). For neural recordings, halothane was discontinued and the rat was maintained in a lightly narcotized, sedated state by intravenous infusion of fentanyl (Sublimaze,

~10 μg · kg–^{1} · hr–^{1}; Janssen Biochimica, Berse, Belgium). To prevent spontaneous movement of facial muscles, which would prevent use of our electromechanical stimulators (below), neuromuscular blockade was induced with pancuronium bromide (1.6 mg · kg^{-1} · hr^{-1}), and the animal respired (90-100 breaths/min) using a positive-pressure ventilator. A computer continuously monitored the rat's electroencephalogram, 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 normal physiological ranges; this occurred rarely.

2.2.2 Recordings

Data were obtained from cortical barrels and thalamic barreloids in the ventral posterior medial nucleus (VPm) using high impedance (5-10 MΩ) stainless steel microelectrodes (Frederick Haer, Brunswick, ME) or beveled glass micropipettes. Glass microelectrodes were made from double-barreled capillary tubes; one barrel, for unit recordings, was filled with 3M NaCl and the other, for marking penetration sites, with 10% horseradish peroxidase (Simons and Land, 1987). Whiskers on the contralateral mystacial pad were stimulated manually during electrode advancement. Extracellularly recorded single units were identified by spike amplitude and waveform criteria using an amplitude discriminator and a digital oscilloscope with a triggered delay. When multiple units were present, only the one having the largest amplitude was discriminated. Spike times were digitized at 10 kHz for subsequent analyses (see below). In the cortex, we distinguished two types of neurons based on spike waveform, regular spiking units (RSUs) and fast spiking units (FSUs) (Bruno and Simons, 2002; Kyriazi et al., 1996). These are thought to represent the discharges of excitatory and inhibitory barrel neurons, respectively. In this study, we compared the response properties of RSUs and FSUs with identically studied

thalamocortical units (TCUs) from Hartings et al. (2003). Hereafter, the terms barreloid and thalamic neurons are used interchangeably along with TCU.

2.2.3 Histology and recording locations

At the termination of an experiment, the rat was deeply anesthetized with sodium pentobarbital and perfused transcardially for cytochrome oxidase (CO) histochemistry. The cortex was cut tangentially and the thalamus was sectioned coronally. Alternate tissue sections were reacted for CO or HRP (Simons and Land, 1987), and all sections were counterstained with thionine. Using microdrive readings, signs of tissue disruption, HRP spots, and/or electrolytic lesions made with metal microelectrodes, recording sites were localized with respect to individual barrels; data are presented only for units recorded in CO-rich barrel centers. Because of the complex geometry of thalamic barreloids, no attempt was made to identify thalamic recording sites with respect to individual barreloids, but all recording sites were confirmed as being located within the ventral posterior medial thalamic nucleus.

2.2.4 Whisker stimulation protocols

For each unit, we first used hand-held probes to identify the whisker, hereafter denoted the principal whisker (PW), evoking the strongest or most reliable response. The PW was trimmed to 12-15 mm in length and a multi-angle piezoelectric stimulator was advanced over the terminal 2-5 mm of the cut end of the whisker (Simons 1983). The following stimulation protocols were used:

Standard protocol. The whisker was deflected 1 mm with onset and offset velocities of ~125 mm/sec and a plateau duration of 200 msec. Stimuli were delivered randomly in 8 angular directions (spanning 360^{o} in 45 ^{o}. increments), and each randomized battery was repeated 20 times. This protocol enabled quantitative identification of each unit's maximally effective or 'best' deflection angle.

Periodic pulsatile stimulation. The PW was deflected repetitively using 10 msec long deflections of 700. μm; rise and fall-times were 5 msec, corresponding to average movement velocities of ~140 mm/sec (Fig.1A). The smaller (<1 mm) amplitude of pulsatile deflections was necessitated by limitations in the stimulators, and a need to keep individual pulses brief in duration. These rapid pulses evoke only a single response, rather than distinct responses to stimulus onset and offset. Identical pulses were delivered at 1, 2, and 4 Hz for 4 sec, 8 and 12 Hz for 2 sec and 16, 20, 30, and 40 Hz for 1 sec. The upper limit of 40 Hz was chosen because its period of 25 msec corresponds to the time of maximal cortical response suppression evoked by a prior whisker deflection (Simons, 1985). Twenty trials of each frequency were randomly interleaved, and stimuli were applied in each cell's preferred direction and in the caudal direction. Data analyses indicated that all measures of adaptation were equivalent for the two sets, and therefore we only report findings from preferred-direction stimuli.

Periodic sinusoidal stimulation. The PW was also deflected periodically using sinusoidal movements. Deflections were 1 mm in peak-to-peak amplitude and began with the resting vibrissa position corresponding to the trough of the sine wave; maximal amplitude deflection thus occurred after one-half cycle of the stimulus, with the return to rest position completing one full cycle (Fig. 1B). Sinusoidal stimuli were delivered at the same frequencies and train durations as the pulsatile stimuli. Sinusoidal deflections were applied in each unit's preferred direction and

in the caudal direction. Because deflection amplitude was constant at 1.0 mm, average movement velocity increased with the frequency of the sinusoidal deflections, allowing us to examine the interaction between adaptation and velocity-sensitivity. Average onset velocity was measured as the peak distance divided by the time of the deflection from its start deflection to its termination at peak amplitude. Average onset velocities of the 1, 2, 4, 8, 10, 12, 16, 20, 30, and 40 Hz sinusoids were 0.23, 0.46, 0.92, 1.94, 3.88, 7.75, 15.5, 31, 62, and 124 mm/sec, respectively.

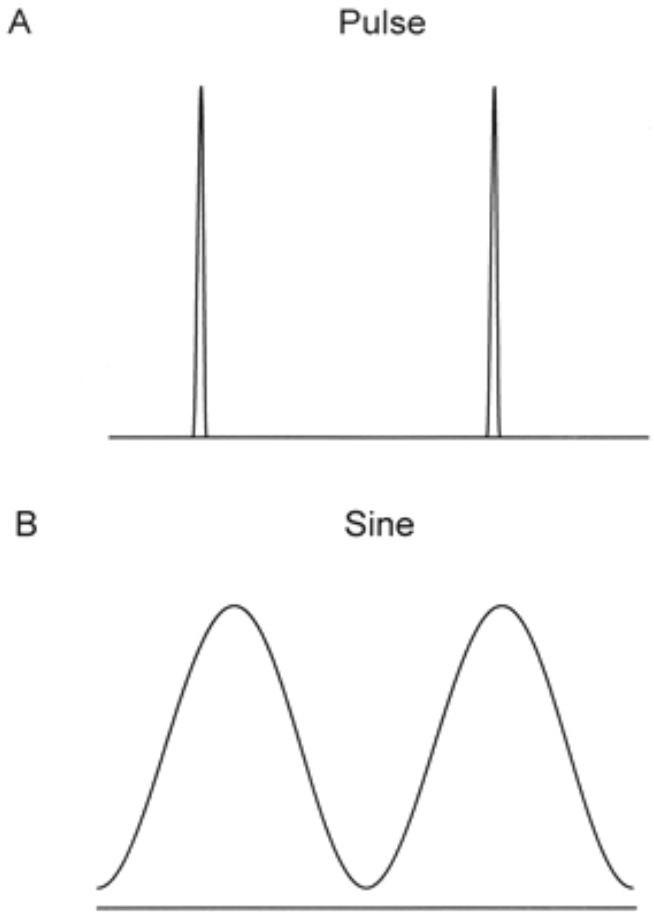


Figure 1. The 2 different types of periodic whisker deflections. A. Pulses. **B.** Sines. Pulsatile deflections had a fixed duration of 10 ms with peak amplitude of 0.7 mm and average onset velocity of 140 mm/s. Sinusoid duration and onset velocity varied with frequency; peak amplitude was 1.0 mm. Waveforms not drawn to scale.

For all stimuli, waveforms were filtered using a 4th-order bessel filter in order to remove high-frequency signal components near the resonance frequency of the stimulator. This filtering prevented unwanted ringing of the stimulator at the termination of a deflection (see Simons, 1983). The behavior of the stimulator for the pulsatile and sinusoidal deflections used here was calibrated off-line using a sensitive photodiode circuit. The stimulator faithfully reproduced the intended waveforms except that for the highest velocity movements (e.g., pulses), the termination of the pulse was associated with two cycles of mechanical ringing at 200 Hz having a maximum peak amplitude of 35 μm (compared to the 700 μm deflection peak). These small deflections occurred immediately upon stimulus offset, a period during which thalamic and cortical neurons are refractory to even large amplitude deflections (Kyriazi et al., 1996).

2.2.5 Data analysis

Sequential spike times were recorded at a resolution of 100 μsec using either a DEC LS11 computer or, in later experiments, a PC equipped with a fast A/D converter (National Instruments, Austin, TX). The computers also controlled the whisker stimuli. Spike data from multiple stimulus presentations were first accumulated in 1 msec bins, and responses were quantified by calculating spike counts during specific time windows. For periodic stimuli peri-stimulus time histograms (PSTHs) were constructed by accumulating responses during the entire length of the trial, including pre- and post-stimulus activities. Data were examined also by constructing cycle-time histograms (CTHs) for each frequency's first cycle and *steady-state*. The steady-state periods for each cell type and stimulus were determined by examining population cycle-by-cycle spike counts and identifying the first cycle at which changes in firing rate appeared similar to those obtained with the 1 Hz (pulse or sinusoid) stimulus; 1 Hz was

chosen as the standard of comparison, because there is virtually no adaptation at this stimulus frequency. The method is illustrated in Figure 2 which shows cycle-by-cycle spike counts at selected frequencies of pulsatile stimulation for RSUs. Interestingly, it appears that, for all cell types, steady-state occurs at progressively earlier times in the stimulus train as stimulus frequency increases. We did not analyze this in detail, however.

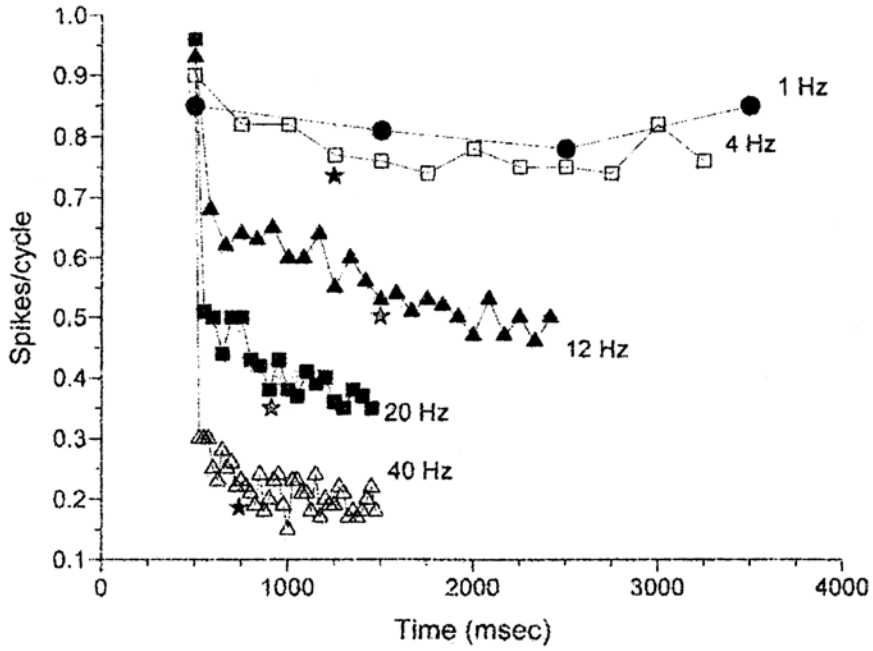


Figure 2. Designation of steady-state onsets. Average cycle spike counts are shown for multiple frequencies of pulsatile stimulation in the regular-spike barrel unit (RSU) population. Stars: steady-state onsets as determined above.

The effects of repetitive pulsatile or sinusoidal whisker stimulation were assessed using an *adaptation index (AI)*. A unit's response in spikes/deflection was quantified by dividing the mean response to all steady-state stimulus cycles by the mean response to the first stimulus cycle. A value of <1.0 indicates that the unit decreased its firing upon repetitive stimulation. We also examined responses on a cycle-by-cycle basis. For pulsatile deflections, which evoked transient responses with distinct onsets and offsets, unit activity was measured during a 25 msec period. The time of response onsets for sinusoidal deflections, especially those at low frequencies and thus low velocities, was difficult to determine accurately; therefore spike counts were taken for the entire stimulus cycle. Because of the sparseness of firing of many of the cells with high frequency stimuli, response durations were computed for TCU, RSU and FSU populations rather than individual units. For steady-state responses, the beginning and ending times were defined as the first and last bins in population CTHs having a spike count that exceeded mean spontaneous activity by 2 SDs; the difference was taken as the response duration. Such analyses were not performed for sinusoidal population responses because their low velocities produced such gradual increases in firing that well-defined response onsets could not be determined using the same statistical procedure.

The degree to which neurons fired at a given phase of the stimulus cycle (i.e. phase-locking) was quantified using a measure of vector strength (VS) (Goldberg and Brown 1969):

$$VS = \sqrt{\sum (\cos\theta)^2 + \sum (\sin\theta)^2}\,/n$$

$$\theta = 2\pi(t/T)$$

n is the total number of evoked spikes, t is the time between cycle onset and an evoked spike, and T is the period of the stimulus frequency. Measures of VS were calculated separately

for CTHs constructed for first-cycle and steady-state responses. We found that the VS measure is influenced complexly by a number of interacting factors, including period length, level of spontaneous (inter-deflection) activity, response shape, and response duration relative to the stimulus period. As suggested by Eggermont (2002), these complications can be overcome by dividing the VS for the steady-state response by that of the first cycle so as to derive a normalized measure of VS. This procedure also accounts for the overall firing rates of different units. In addition, we normalized the CTHs to a common length corresponding to a full 360 deg of the stimulus cycle. The normalized VS thus provides a quantification of the extent to which phase-locking during the stimulus cycle changes in the adapted vs. non-adapted state. A value of 1 indicates the maintenance of phase-locking in the adapted state, and 0 signifies a complete loss of phase-locking.

We also quantified the degree to which neurons fired periodically at the stimulus frequency or integer multiples thereof (i.e., entrainment). Autocorrelograms were constructed from individual spike trains accumulated over all trials of a given frequency and then analyzed using a discrete Fourier transform (DFT). This analysis was performed on a unit-by-unit basis and on autocorrelograms constructed from all spike trains for a given population. In order to account for different firing rates among individual units or between populations (e.g., RSUs vs FSUs), the autocorrelogram was normalized to its maximum bin prior to the frequency analysis.

Comparisons among TCUs, RSUs and FSUs were conducted using ANOVAs followed by post-hoc pair-wise comparisons. For the ANOVAs data were compiled across all frequencies for a given cell type in order to minimize the number of statistical comparisons. Inspection of frequency dependent measures (e.g., Fig. 4) revealed that, for virtually all measures, relationships were consistent across frequencies.

2.3 RESULTS

Responses to pulsatile periodic stimuli were examined in 29 TCUs, 44 RSUs, and 18 FSUs. Responses to sinusoidal deflections were examined in 27 TCUs, 32 RSUs, and 12 FSUs. Approximately, two-thirds of each population was studied with both stimulus sets. Below figure 3 shows population PSTHs illustrating the characteristic responses of the three cell populations to pulsatile stimuli. At low frequencies, each cell type fired relatively uniformly throughout the stimulus train, but with higher-frequency deflections responses were largest for the first deflection in a series. As quantified below, TCUs and FSUs fire more stimulus-evoked and spontaneous spikes than RSUs. FSUs and RSUs are more similar to each other and different from TCUs, however, in that both show greater response decrements to the second and subsequent stimulus cycles. The extent to which responses decreased after the first cycle was quantified using an *adaptation index*, wherein the average response evoked by steady-state stimulus cycles is divided by the mean response to the first deflection in the train.

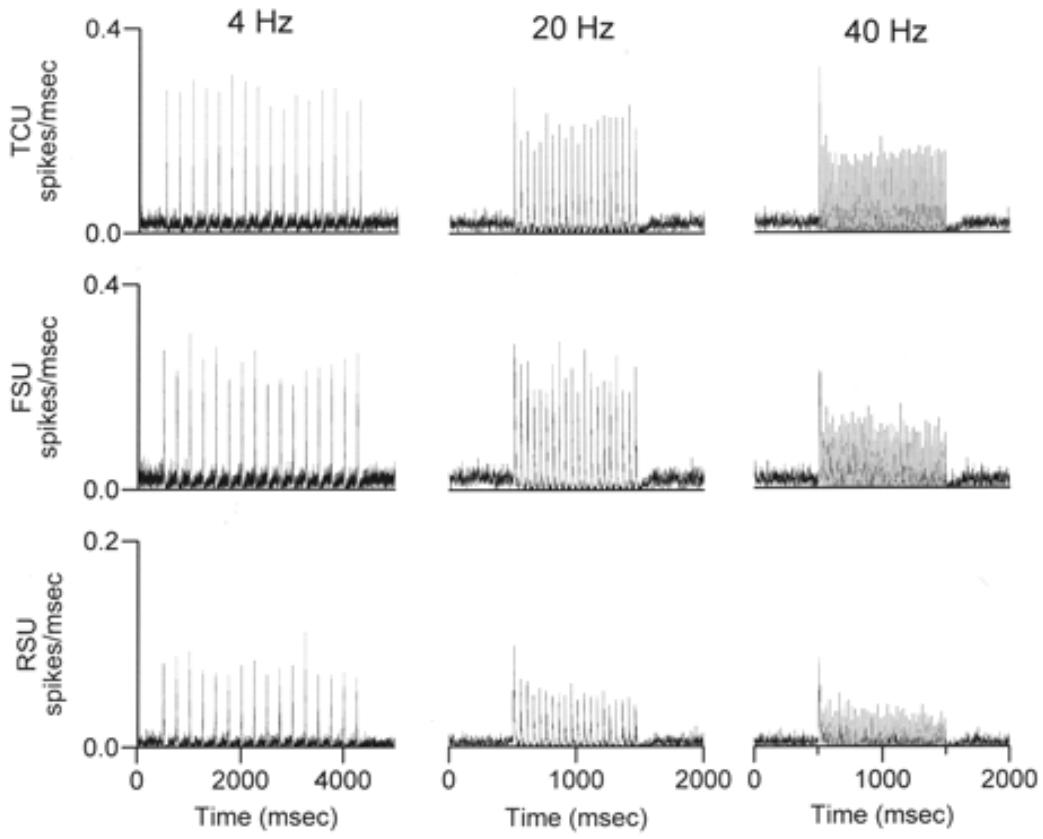


Figure 3. Population peristimulus time histographs (PSTHs) of 29 thalamocortical units (TCUs), 18 fast-spike barrel units (FSUs), and 44 RSUs for periodic stimulation with identical pulses delivered at frequencies of 4, 20, and 40 Hz. Note that with 20 and 40 Hz, response peaks are smaller after the 1st cycle.

Figure 4B below shows frequency-dependent adaptation for the three studied populations. At frequencies where adaptation occurred, adaptation is greater in FSUs and RSUs than in TCUs. For example, at the highest frequency tested (40 Hz) TCU responses decreased by 33% (e.g. adaptation index = 0.67), whereas the responses of RSUs and FSU were 77 and 72% smaller, respectively. For each cell population, we computed a mean adaptation index across all frequencies tested. An ANOVA indicated that the amount of adaptation differed among the three cell populations ($p < 0.001$). Both RSUs and FSUs adapted more than TCUs (Student's unpaired t-tests, p values < 0.001), but FSUs adapted slightly less than RSUs ($p = 0.02$). Within each studied population, individual units varied in terms of their adaptation and initial response magnitude. We therefore examined whether the amount of adaptation displayed by an individual cell is related to its response during the first stimulus cycle, i.e., in the non-adapted state. A correlation coefficient was computed by comparing, for the 20 Hz stimulus train, the magnitude (spikes/stimulus) of each unit's first-cycle response with its calculated adaptation index. For all three cell populations adaptation was independent of non-adapted response magnitude.

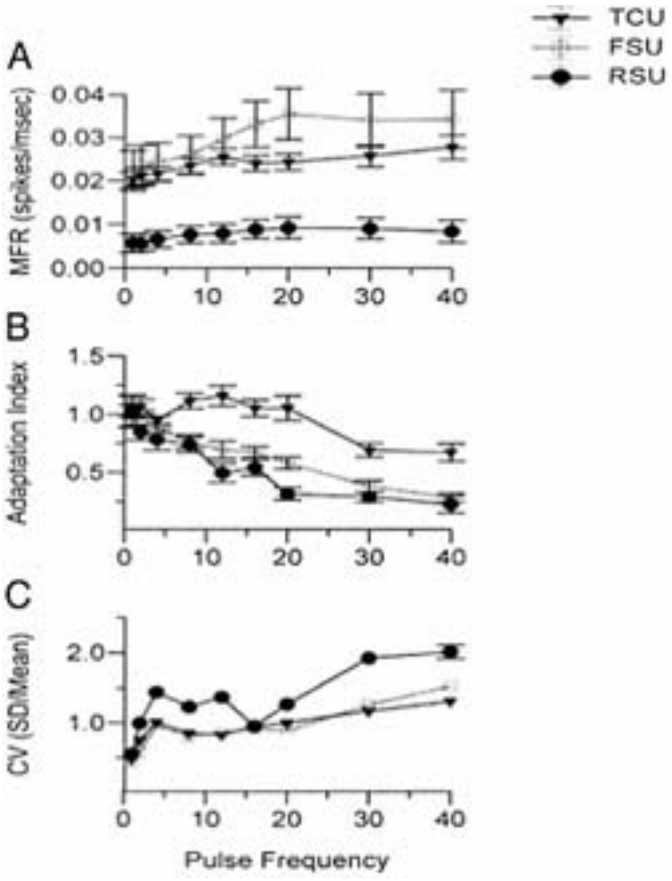


Figure 4. Adaptation characteristics for pulsatile stimuli. **A**. steady-state mean firing rate (MFR) as a function of the pulsatile stimulation frequency. The MFRs of all 3 cell types change minimally with increases of frequency. **B**. pulse adaptation indices calculated by taking the steady-state response and dividing that by the 1st-cycle response. A value of 1.0 indicates no response decrement, and a value of 0 indicates the absence of a steady-state response. Both FSUs and RSUs adapt more than TCUs especially at frequencies of 8 Hz. **C**. trial-by-trial coefficients of variation (CV = SD/mean) for steady-state spike counts. The CVs of FSUs and TCUs increase minimally and equivalently as frequency is increased, whereas RSUs become noticeably more variable. Error bars = ±1 SE.

RSUs adapted slightly more than FSUs, and inspection of trial raster-plots suggested that RSU adapted responses also varied more widely from trial-to-trial and from cycle-to-cycle. For each unit we examined trial-to-trial variability by computing a coefficient of variation (standard deviation/mean) for steady-state responses (Fig. 4C above). Variability substantially increased in a frequency-dependent fashion for RSUs, but CV's remained relatively constant in FSUs, which in turn were equivalent to those of TCUs. Similar results were observed when CV's were calculated across individual cycles within a train. Again, RSU responses varied most, with FSU and TCU responses being similar to each other. In all three populations cycle-by-cycle variability was roughly equivalent to trial-by-trial variability.

2.3.1 Adaptation and response timing

High frequency pulsatile stimulation substantially reduced response magnitudes of cortical neurons without greatly degrading the temporal fidelity, or phase-locking, of their responses. This is illustrated qualitatively by the cycle-time histograms (CTHs) in Figure 5. With 4 Hz trains, TCUs and FSUs are similar in the rapid rise of their responses to both the first and subsequent, steady-state stimulus cycles; the RSU response develops more slowly. Within each cell type the time course of the response is similar for the first and steady-state cycles. At 20 and 40 Hz, TCU responses are largely similar to those at 4 Hz, except that the peak response for steady-state cycles occurs 1-3 msec later than the peak response for the first cycle; this right-ward shift causes a reduction in total response magnitude. For RSUs and FSUs at 20 and 40 Hz, the time to peak increases by ~5 msec for the steady-state response, and firing rates are reduced throughout. Responses are briefer as well as smaller (Fig. 6A; also see Fig. 5), however, yielding

a CTH that, though scaled down in size, has a distinct, time-locked peak. We quantified the temporal fidelity of responses using a measure of vector strength (Figure 6B below; see Methods). Vector strengths differed across cells types (ANOVA: $p < .001$). Both TCUs and FSUs were equally phase-locked, and both were better than RSUs (Student's unpaired T-tests: TCUs vs. FSUs, $p = 0.61$; TCUs vs RSUs, $p < .001$; TCUs vs FSUs, $p < .001$).

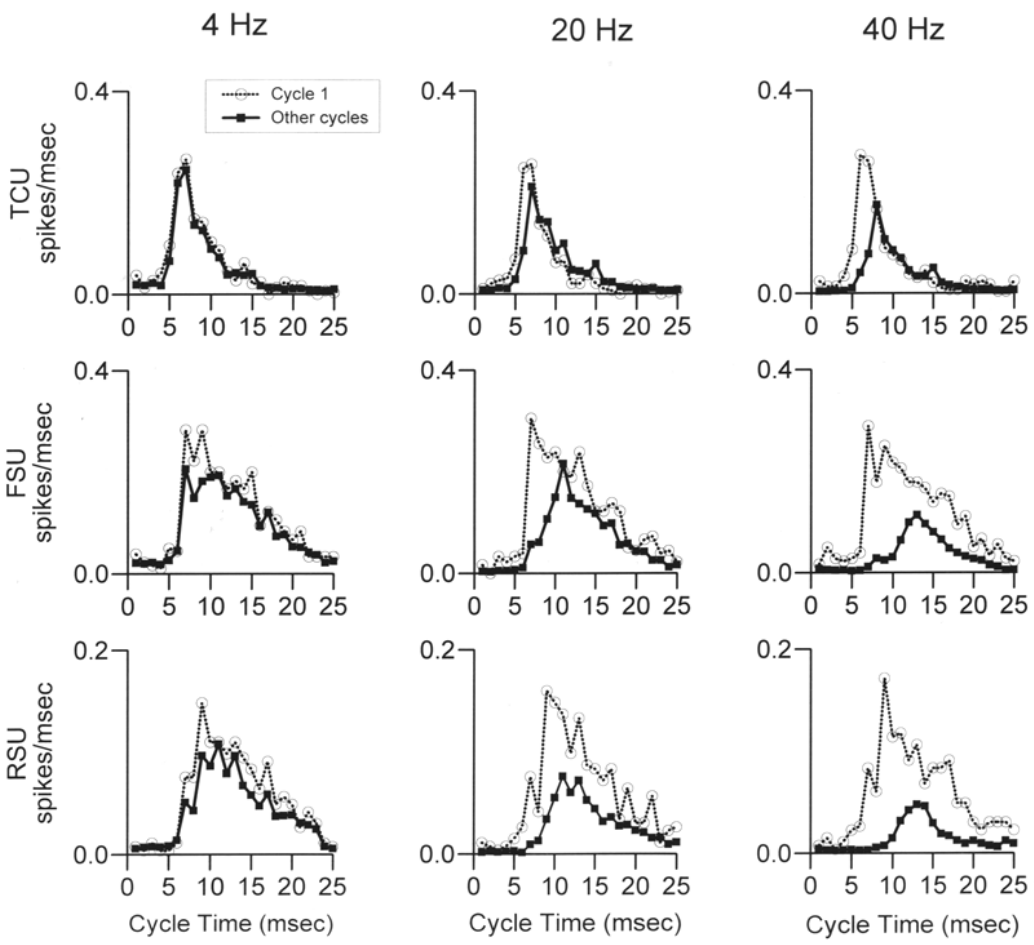


Figure 5. Pulse cycle-time histograms (CTHs). Responses to the 1st cycle and all other cycles are depicted. Note rightward phase shift of responses to the 20- and 40-Hz stimuli.

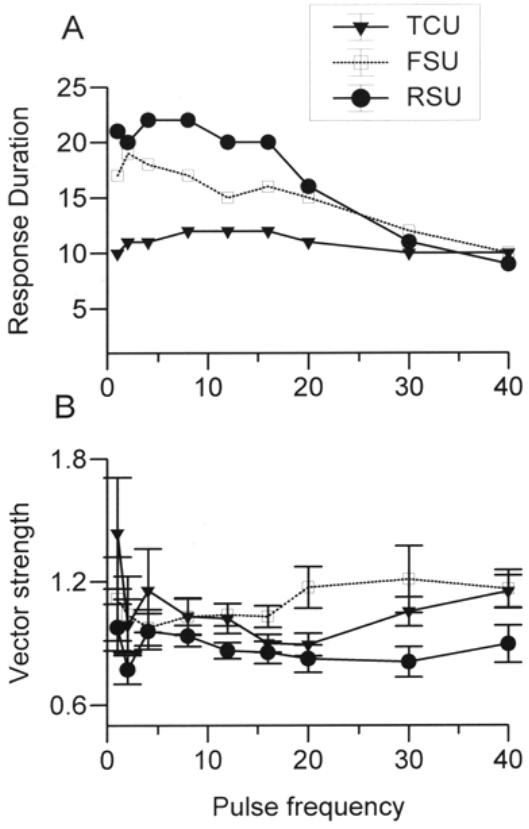


Figure 6. Temporal fidelity for pulsatile stimulation. A. Response durations of steady-state pulsatile population responses. The TCU response duration remains constant across frequencies, whereas for FSUs and RSUs responses become briefer with increases of stimulus frequency (see text). **B**. Normalized vector strengths for individual cells calculated by dividing the steady-state VS by that of the 1st cycle.

Analyses of steady-state CTHs suggest that rapidly repeating stimuli induce cortical neurons to fire periodically, albeit especially sparsely in the case of RSUs. Figure 7A below shows an autocorrelogram of spike trains evoked by 40 Hz stimulation in an RSU; this unit was among the best entrained cells, and the data provide a good example of the numerical measures we used. The neuron fired preferentially at intervals of 25 msecs or integer multiples thereof. Figure 7B shows a discrete Fourier transform (DFT) of the autocorrelogram and illustrates the periodicity of spiking at 40 with a secondary peak at 80 Hz (the first harmonic). To quantify this, we divided the power at 40 Hz by the (total) power summed from 5-100 Hz; the value for this unit is 0.14. Approximately 20% of RSUs had values > 0.14. RSUs displayed lower mean values (.057+0.010) than FSUs (.154 +.020) and TCUs (.160 + .010); variances relative to the means were larger for cortical than thalamic neurons. Thus both quantitative measures, vector strength and autocorrelogram frequency domain, indicate that RSU firing displays the least temporal fidelity for pulsatile whisker deflections. We performed a similar analysis on population autocorrelograms constructed by accumulating spikes across all units and trials. In all three cell types population-level values at 40 Hz were larger than mean values of individual units (compare Fig. 7C and D), and, interestingly, RSUs (.21) were virtually identical to FSUs (.22) and TCUs (.20).

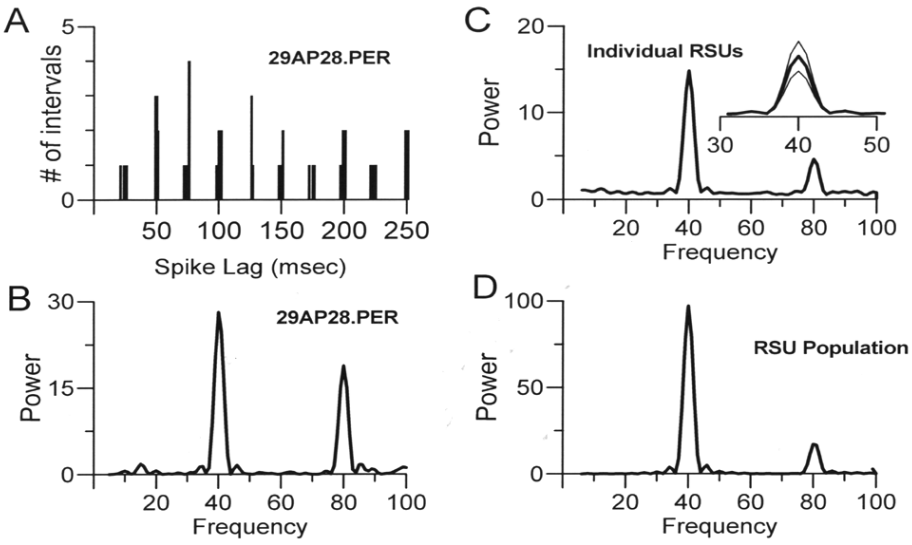


Figure 7. A well-entrained RSU. A. Autocorrelogram for the response of a well-entrained RSU to the 40 Hz pulsatile stimulus. The depicted cell is typical of the RSU population in terms of its adaptation index and CV. **B.** Power spectrum of the autocorrelogram in A. Note the peak at 40 Hz. **C.** Power spectrum for autocorrelogram averaged across all individual RSU responses at 40 Hz. Inset depicts spectrum from 30 to 50 Hz, the black line, mean; the grey lines, $\pm$ 1 S.E.M. **D.** Power spectrum for autocorrelogram of 40 Hz responses summed across the population of RSUs.

We also examined whether firing rates at steady-state increased with higher stimulus frequencies (Fig. 4A above). For all three cell populations, mean firing rates (MFR) increased only ~50% from 1 to 12 Hz, despite the 12-fold increase in stimulus frequency. MFRs remained relatively constant from 12 to 40 Hz, presumably because the suppressive effects of interdeflection intervals <70 msecs counterbalance, and then dominate, the excitatory effects of more frequent whisker deflections. Thus, despite adaptation-induced decreases in responsiveness, populations of thalamic and cortical neurons appear to represent stimulus frequencies up to 40 Hz by the temporal pattern of their firing not by their overall number of spikes.

2.3.2 Responses to sinusoidal deflections

In all three cell populations, adaptation to sinusoidal movements increased with higher frequencies (Fig. 8). ANOVA and post-hoc analyses revealed that, as in the case of pulsatile deflections, TCUs adapted less than RSUs and FSUs (Fig. 9B) which were equivalent to each other. Effects of deflection velocity are clearly evident in the population CTHs constructed for the first stimulus cycle, i.e., the non-adapted state (Fig. 10). With higher frequency stimuli and thus higher velocity deflections, population responses in the non-adapted state have shorter latency, faster rise-times, greater peak magnitudes and higher mean firing rates. In the adapted state TCU response latencies are longer, and differences in latency between non-adapted and adapted states are greater for lower frequency sinusoids. Reflecting their thalamic inputs, FSUs and RSUs show similar frequency-dependent latency shifts. In all three populations the number of spikes per cycle in the adapted state increases at higher stimulus frequencies, because the

velocity of the stimulus increases as well. Effects are modest, however; overall mean firing rates increase only modestly (~25 %) with stimulus frequencies from 1-12 Hz and then asymptote (Fig.9A). Response variability as quantified by CVs was virtually identical for FSUs and TCUs, with RSU firing being least variable (ANOVA, $p < .001$) perhaps because many cells fired either a maximum of 1 or no spikes per cycle. Mean firing rates were similar for sinusoidal and pulsatile stimuli (compare Figs. 4A and 9A) in spite of the clearly less distinct responses evoked by sinusoidal movements. This reflects the fact that responses to the latter are considerably more temporally dispersed. Autocorrelograms based on unit responses to 40 Hz stimuli were examined using DFTs in a fashion similar to those for pulsatile stimulation. From 5-100 Hz, the proportions of power at 40 Hz were $.035 \pm .010$ for RSUs, $.094 \pm .022$ for FSUs and $.149 \pm .014$ for TCUs. As with pulsatile deflections, population level values were larger in all three cell types and equivalent to each other: TCUs (.21), RSUs (.22) and FSUs (.22).

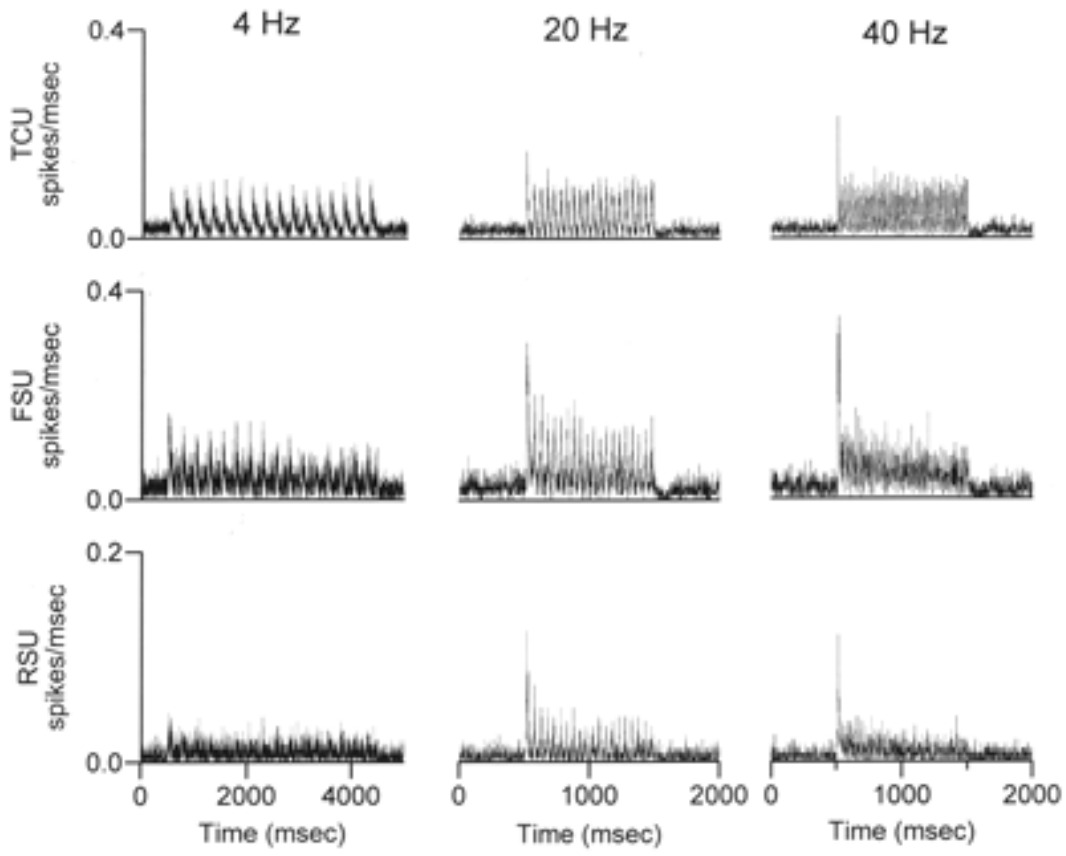


Figure 8. Population PSTHs of 27 TCUs, 13 FSUs, and 31 RSUs for periodic sinusoidal stimulation at frequencies of 4, 20, and 40 Hz. Note the lower firing rates relative to those in Fig. 2.

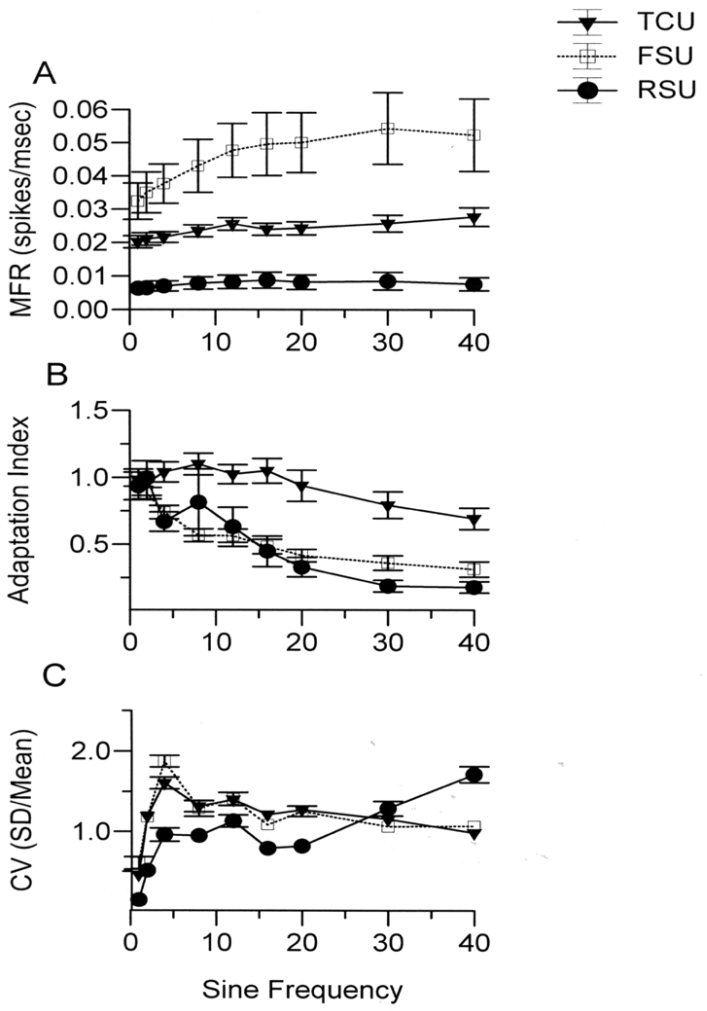


Figure 9. Adaptation characteristics for sinusoidal stimuli. Refer back to legend of Fig.5. **A.** Steady-state mean firing rate (MFR) as a function of the sinusoidal stimulation frequency. **B.** Sinusoid adaptation indices **C.** Trial-by-trial coefficients of variation (CV = S.D./Mean) for steady-state spike counts.

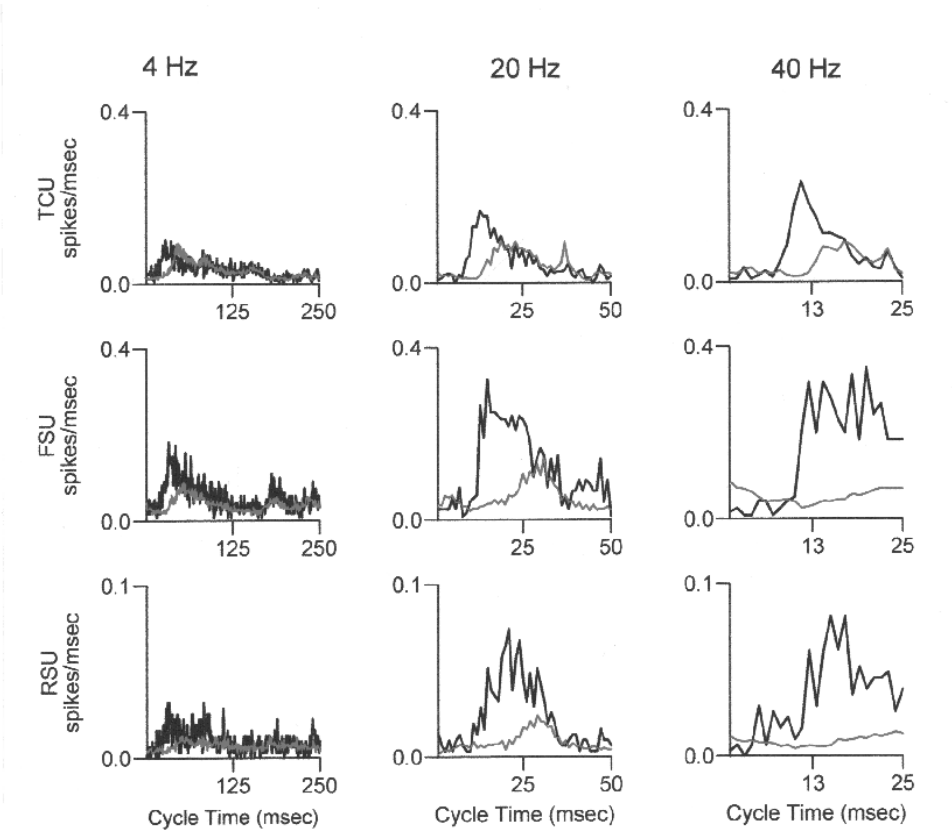


Figure 10. Sinusoidal CTHs of all three cell populations for 4, 20, and 40 Hz stimuli. Both responses to the first cycle (black trace) and steady-state (grey trace) are depicted. Note the time scale differences across columns.

2.3.3 **Frequency- and velocity-dependent effects of adaptation**

For a given stimulus frequency, the velocity of whisker movement was higher for pulsatile than sinusoidal deflections, even at 40 Hz. This difference enables an assessment of possible interactions between adaptation and deflection velocity. For these analyses we collapsed data across frequencies in order to calculate mean values per cell. As illustrated by the bar graph in Figure 11A below, adaptation indices were similar for sinusoidal and pulsatile deflections, except that FSUs adapted slightly more for sines (Student's unpaired t-test, $p = 0.01$). Thus, deflection velocity affects non-adapted (first-cycle) firing rates but not the proportional decrease in steady-state firing. Also, as noted above steady-state mean firing rates reflect stimulus period poorly for both sines and pulses. Ten-fold increases in frequency (4 to 40 Hz) produced maximal increases of ~70% in MFR (Fig. 11B).

In the temporal domain, the timing of spike occurrences remained more faithful to the non-adapted pattern when higher velocity, pulsatile deflections were used. For all cell types adaptation-induced shifts in peak response times (in msecs) were substantially larger for sinusoidal than pulsatile stimuli (Fig. 11C). Phase-locking, as measured by vector strength, was also velocity-dependent but only in cortical, not thalamic neurons (Fig. 11D). Average vector strengths were greater for pulses than sines in RSUs and FSUs (Student's unpaired T-test: RSUs, $p < 0.001$; FSUs, $p = 0.003$), whereas TCU phase-locking was equivalent for both types of stimuli (Student's unpaired t-test: TCU, $p = 0.33$). Similarly, entrainment, as measured by the frequency spectrum of the autocorrelogram, was larger (Student's unpaired T-test: $p << 0.001$) for pulsatile than sinusoidal deflections for RSUs (0.057 ± 0.010 vs. 0.035 ± 0.010) and FSUs

(0.154 ± 0.020 vs 0.094 ± 0.022), whereas the converse was the case for TCUs (0.102 ± 0.056 vs. 0.131 vs. 0.076), perhaps due to the more slowly adapting-like responses which were tonically modulated by the sinusoidal deflection.

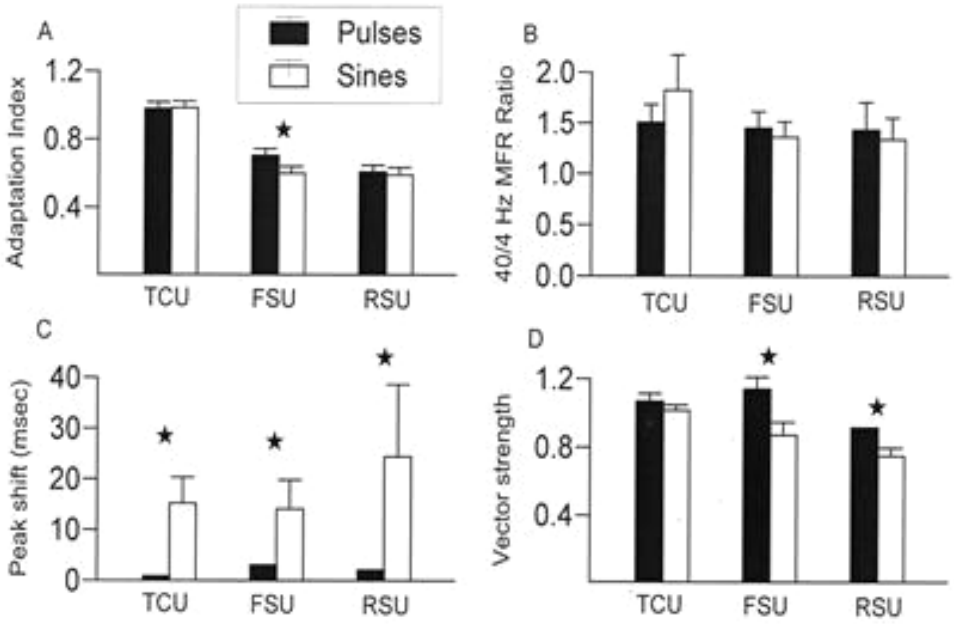


Figure 11. Comparison of responses evoked by pulsatile and sinusoidal stimulation.

A. Adaptation indices derived by averaging responses across all frequencies for both sines and pulses (*, p< 0.01). **B.** Ratio of mean firing rate (MFR) evoked by 40 Hz versus 4 Hz stimuli. MFRs increase only 20-70% for sines and pulses in all cell types despite a 10-fold increase in stimulus frequency. **C.** Response peak latency shifts derived by accumulating responses across all frequencies (*, p<.001). **D.** Vector strengths derived by averaging responses across all frequencies (*, p<.001).

2.4 DISCUSSION

The present study investigated thalamocortical response transformations in the whisker-to-barrel pathway using periodic whisker deflections having different frequencies and velocities. Frequency-dependent reductions in firing were observed in thalamic and cortical neurons for both high (pulse) and low (sinusoid) velocity deflections. Consistent with previous reports (Gottschaldt et al., 1983; Chung et al., 2002), we found that cortical neurons adapt more than their thalamic input neurons and that presumed excitatory (RSU) and inhibitory (FSU) barrel neurons adapt equivalently to each other. Greater adaptation in the cortex may reflect more pronounced depression at thalamocortical (Chung et al. 2002) vs. trigeminothalamic synapses (Castro-Alamancos, 2002), stronger intra-barrel (Goldreich et al., 1999) vs thalamic RT-mediated inhibition (Hartings and Simons, 2000), and/or the presence in barrels of recurrent synaptic connections (e.g., excitatory-to-excitatory) that also depress (Egger et al., 1999; Petersen, 2002). Despite smaller responses in the adapted state, periodic firing of cortical neurons closely reflects that of thalamic barreloid neurons, especially with higher velocity whisker movement. Thus, in an adapted state produced by passive whisker deflection in sedated animals, firing within the barrel is sparse but still temporally faithful to the occurrence of the stimulus and the thalamic input signal. Available evidence suggests that the barreloid-barrel circuit operates similarly during adaptation that accompanies behavioral arousal. During arousal adaptation to repetitive stimuli is less pronounced, because the thalamocortical circuit is already in a suppressed state; nevertheless, periodic stimuli produce a further temporal focusing and magnitude reduction, albeit smaller, of the steady-state response (Fanselow and Nicolelis, 1999; Castro-Alamancos, 2004).

Responses of thalamic and cortical neurons are determined by the frequency of whisker deflections and by their velocity. In both thalamic and cortical neurons, response onsets and peaks occurred at longer latencies for steady-state compared to first-cycle stimuli, and latency shifts were greater for the lower-velocity (sinusoidal) deflections. With both sinusoidal and pulsatile deflections, changes in the times of RSU and FSU response onsets were virtually identical to those of TCUs. Thalamic activity in turn reflects the firing of primary afferent neurons, which occurs at longer latency and with less population synchrony for lower velocity deflections (Shoykhet et al., 2000). Our thalamic data are consistent with findings that relatively high velocity air puffs delivered up to 8 Hz lead to only small increases in VPM response latency (Ahissar et al., 2000).

Adapted TCU vector strengths were equivalent for the sines and pulses despite the more pronounced latency (phase) shifts that accompanied the former. During adaptation, the slopes of TCU population response onsets decreased more with sinusoidal than pulsatile deflections, however. Perhaps as a result, in the cortex the temporal fidelity of the adapted response is velocity-dependent; for both RSUs and FSUs sinusoidal whisker movements evoke more temporally dispersed responses (as indicated by smaller vector strength values) than pulsatile stimuli, and even with the highest-velocity (125 mm/s at 40 Hz) sinusoidal deflections the period of the sinusoidal stimulus is represented less well in cortical firing patterns than is the case for pulsatile deflections (140 mm/s). In studies employing single deflections, barrel circuitry has been found to be sensitive to population firing synchrony within thalamic barreloids; whisker stimuli that lead to steeper slopes in thalamic population PSTHs evoke larger responses in barrel neurons (Kyriazi et al., 1994; Pinto et al., 2000). The present findings therefore suggest that

barrel circuitry remains sensitive to thalamic population firing synchrony in an adapted state produced by repetitive whisker deflection.

Previous investigators have described response decrements of somatosensory cortical neurons due to a preceding stimulus at the same location (e.g. Gardner and Costanzo, 1980; Kyriazi et al., 1994; Garabedian et al., 2003; Whitsel et al., 2003). Though details vary, response suppression increases with shorter inter-stimulus intervals and is mostly absent, or weaker, with intervals >100 msec. Suppressive effects have been attributed primarily to local inhibition and more recently to depression at TC synapses (see below). Recent *in vitro* evidence indicates that TCU-FSU synapses depress more than TCU-RSU synapses (Beierlein et al., 2004). Our adaptation indices show that *in vivo*, however, the combined affects of inhibition and synaptic depression appear to act nearly equivalently on both RSUs and FSUs; if anything, FSUs showed slightly less adaptation with the pulsatile deflections. In cat vibrissa cortex *in vivo*, cortical EPSPs and IPSPs adapt upon periodic electrical stimulation of the thalamus (Hellweg et al., 1977). The similarity of adaptation in FSUs and RSUs is consistent with their receiving inputs from similar populations of barreloid neurons (Bruno and Simons, 2002) and their extensive interconnections with each other (Petersen and Sakmann, 2000). For example, adaptation of RSUs will reduce the amount of recurrent, intra-barrel excitatory input onto FSUs.

Although we found that cortical neurons differed greatly from thalamic neurons in terms of the magnitude of frequency-dependent adaptation, a number of response characteristics are similar at both levels. Across frequencies steady-state mean firing rates remained relatively constant in thalamus and cortex. Up to 8 Hz, mean firing rates increased moderately with increasing frequency of stimulus cycles, but firing rates reached asymptotic levels at ~12 Hz. Indeed, for both pulses and sines a 10-fold increase in stimulus frequency (4 to 40 Hz) yielded at

most only a 70% increase in MFR (Fig. 11B). This finding appears to be at variance with a recent report (Arabzadeh et al., 2004) in which a robust positive relationship was observed between MFR and the frequency of sinusoidal whisker deflection; for example, MFR doubled with increases in frequency from 19-50 Hz. Movement velocities of stimuli used in that study were, however, considerably lower than those of the present study. Interestingly, neurons did not fire periodically even at frequencies comparable to those used here (e.g., ~20-40 Hz) in which substantial phase-locking was observed both at the single cell and population levels. The lack of phase-locking at 20-40 Hz reported by Arabzadeh et al. may be a consequence of the relatively low velocity of the whisker deflections they used; our analyses indicate that higher velocity movements are more likely to evoke temporally focused responses at high stimulus frequencies.

The monotonic, minimal increase in MFR observed in the present study differs from the finding of Garabedian et al. (2003). Though they too observed pronounced frequency-dependent adaptation, mean firing rates peaked with 8 Hz whisker deflections, whereas firing rates in our cortical cells neared asymptotic values but did not decrease with higher frequencies. Thalamic neurons were not examined in the former study, and it is therefore difficult to directly compare the two sets of cortical data. Moreover, Garabedian et al. recorded from more deeply anesthetized rats, and anesthesia likely contributed to the perhaps related finding that adaptation was substantially greater in that study. They also employed longer stimulus trains (2 seconds of adaptation). As suggested by their simulation work, greater response suppression, due perhaps to stronger anesthesia-related inhibition, likely counteracted the effects of more frequent (> 8 Hz) excitatory inputs onto thalamic and/or cortical neurons. Garabedian et al. also reported a pronounced band-pass effect on steady-state response vector strengths, such that the timing of individual responses was most faithfully preserved at 6-10 Hz. Our analyses using normalized

vector strength measures reveal only subtle changes in entrainment across stimulus frequencies, with no evidence for substantial frequency-dependent filtering up to 40 Hz. Thus, at least in lightly narcotized animals, the temporal dynamics of the barreloid-barrel circuit do not appear to appear to be specialized for processing afferent information in the ~8 Hz range.

FSU responses displayed higher firing rates and greater entrainment than RSUs (see also Simons 1978). As in previous studies (e.g. Simons and Carvell, 1989), FSU responses were highly similar to those of TCUs, consistent with their receiving strong synaptic inputs from thalamocortical axons (Bruno and Simons, 2002; Swadlow and Gusev, 2002). Neurons in the thalamic reticular nucleus (RT), which provide virtually the only source of inhibition to VPm neurons (Desilets-Roy et al., 2002), also receive monosynaptic inputs from barreloid neurons and are strongly driven by whisker deflection. Interestingly, steady-state responses of these two populations of inhibitory neurons differ substantially. With whisker deflections identical to those used in the present study, the firing of RT neurons becomes increasingly unmodulated and tonic at higher stimulus frequencies (Hartings et al., 2003). Such uniform steady-state RT firing may help to preserve and even enhance VPm response transients (see Minnery et al., 2003), which are initially generated in primary afferent neurons. Unlike RT cells, cortical FS cells fire phasically (see also Mountcastle et al., 1969). The close coupling between phase-locked FSU and RSU responses may ensure that the sensitivity of barrel circuitry to thalamic population firing synchrony is maintained on a moment-to-moment basis, especially when stimuli are changing rapidly. Interestingly, mean firing rates of thalamic and cortical neurons were similar across frequencies and for pulsatile and sinusoidal deflections. Thus, for the stimuli used here, barrel cortex-based distinctions among high vs low velocity periodic deflections likely depend on the sensitivity of thalamocortical circuitry to input timing followed, perhaps, by further

transformation to a mean firing rate code elsewhere in the cortical column (e.g. Salinas et al., 2000; Arabzadeh et al., 2004).

3.0 DETERMINING THE STIMULUS-SPECIFICITY OF ADAPTATION IN THALAMIC BARRELOIDS AND CORTICAL BARRELS

3.1 INTRODUCTION

A ubiquitous property of perception across modalities is that it can be altered by adaptation with repetitive sensory stimulation. The neural basis of perception indicates that the responses of sensory neurons are indeed modifiable. This is the case for the stimulus-evoked responses of cortical neurons after adaptation, which often are significantly suppressed and their tuning curves altered. Responses to particular stimuli can be suppressed or facilitated depending on the particular modality and characteristics of the adapting stimuli. A striking example is found in some macaque area V4 neurons, which prior to adaptation are not direction-selective for the motion of visual stimuli. However, adaptation to a grating moving in one direction causes these neurons to become direction-selective for the opposite direction of motion (Tolias et al., 2005). The modification of tuning properties may be due to the effect of repetitive stimulation on the dynamics of cortical circuits. Adaptation can change the cortical circuit's overall level of excitability and its dependence on feedforward (e.g. thalamic for primary sensory cortices) versus intracortical inputs.

Barrels are an ideal site to further examine the effects of adaptation because they are well-defined cortical circuits. Barrels are anatomically and functionally-defined modules in layer

4 of the rat primary somatosensory cortex that contain excitatory and inhibitory neurons with distinct receptive field properties. The characteristic responses of excitatory and inhibitory neurons are a product of their intrinsic properties as well as their receiving different inputs. FSUs (*fast-spike units*, or putative inhibitory cells) are more responsive and broadly tuned for the angle of whisker deflections than RSUs (*regular-spike units*, or putative excitatory cells) (Simons and Carvell, 1989; Swadlow and Gusev, 2002; Kida et al., 2005). The FSU-RSU dichotomy is established by thalamic inputs (Bruno and Simons, 2002). The properties of FSUs are established by the convergence of many thalamic barreloid cells with different angular preferences. RSUs receive thalamic input from a smaller number of thalamic cells having similar angular preferences. Within a vertical column of the barrel, all excitatory cells have similar angular preferences (see Fig.12) and form *tuning domains*, but horizontally adjacent sites (only 75 μm away) may contain neurons with similar or different angular preferences (Bruno et al., 2003). The rules governing intra-barrel interactions between angular tuning domains are currently unknown and insights may be gained by determining how the angle of adapting whisker deflections affects the response properties of barrel neurons.

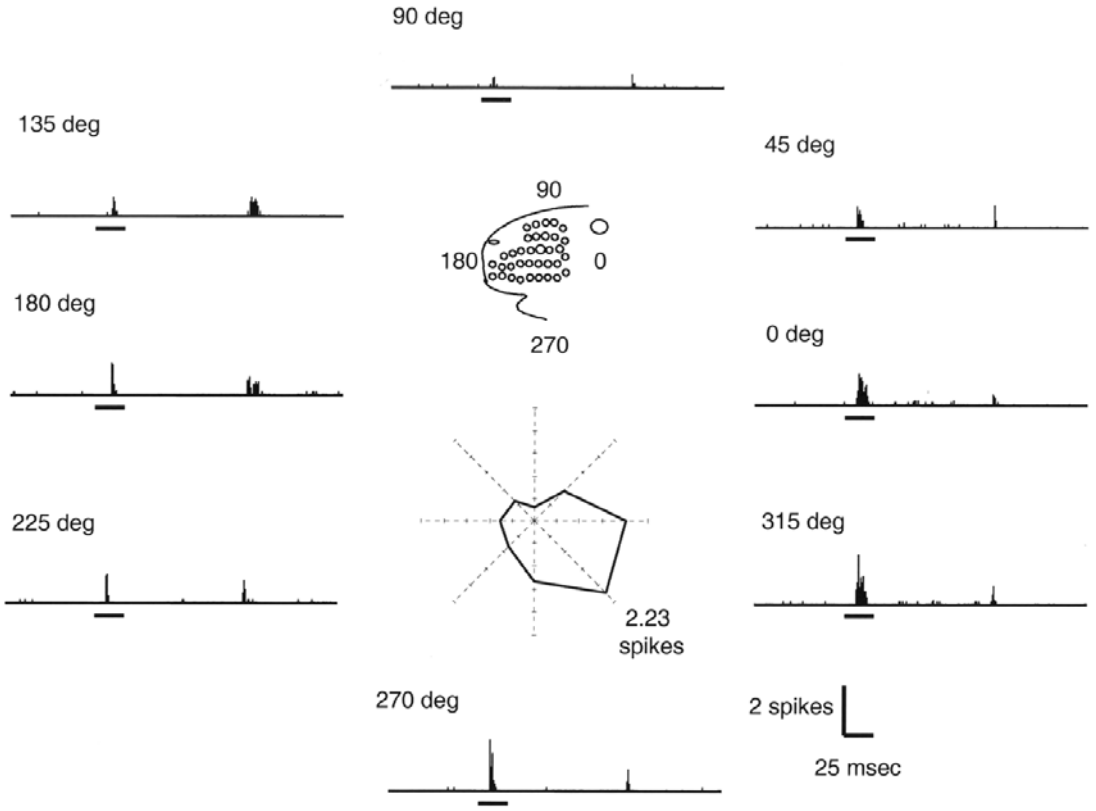


Figure 12. Angular tuning of a putative excitatory cell. PSTHs for each of the 8 directions of ramp-and-hold whisker movements are displayed. The cell's ON response is indicated by the line under each PSTH. The preferred direction is 315^{o} which evoked 2.23 spikes per deflection.

Recently, we demonstrated that FSUs and RSUs display parallel changes in responsiveness during repetitive whisker stimulation (Khatri et al. 2004). Both neuronal types displayed suppression and reductions in their response durations as well. The findings indicate that FSUs and RSUs maintain their distinctive response signatures after adaptation. Here, we investigate this further by determining the effects of repetitive whisker stimulation in different directions upon thalamic barreloid and cortical barrel neurons. Of particular interest is whether FSUs remain broadly tuned after adaptation.

We recorded the responses of RSUs and FSUs in layer IV barrels and those of TCUs (thalamocortical units) in VPM, their primary source of afferent input. Whisker stimuli were systematically varied in deflection angle, preceded by adapting deflections in the same or different directions. In some experiments, layer IV circuitry was rendered unresponsive during sensory adaptation by concurrent electrical stimulation in overlying layer III. These approaches enabled us to determine whether the responses of individual neurons evoked by particular angles of whisker deflection are suppressed most when preceded by deflections in the same direction and whether effects observed in the cortex were strictly of cortical origin. Findings suggest that intra-barrel circuits are dominated by strong intra-barrel angularly-nonspecific suppression, and that angular-specificity is provided by tuned thalamic inputs.

3.2 METHODS

3.2.1 Animals and surgical preparation

Surgical preparation and maintenance of the rats during electrophysiological recording was identical to methods described previously (Simons and Carvell, 1989; Khatri et al., 2004). Adult Sprague-Dawley female rats (200-300 g) were obtained from a commercial supplier. All surgical preparation was performed under halothane anesthesia. A silastic catheter was inserted into the right jugular vein and led out from the nape of the neck for later drug delivery. A short length (~ 40mm) of polyethylene tubing was inserted into the trachea for later artificial respiration, and the left femoral artery was cannulated using an angiocath catheter in order to measure blood pressure. After exposing the skull, small stainless steel screws were placed over the left occipital and frontal cortex for EEG recordings, and a ground screw was placed over the right frontal cortex. Dental acrylic was used to attach a steel post to the skull. The post, which was used to hold the animal's head without pressure points during the rest of the experiment, permitted unimpeded access to the facial vibrissae. In cortical experiments, the bone overlying the right barrel cortex was thinned and a small (< 1 mm^2) craniectomy was made. For thalamic experiments, a craniectomy was made overlying VPM (see Khatri et al). The dura was incised to prevent the brain from dimpling and thus suffering compression damage due to electrode insertion. Lastly, an acrylic dam was constructed around the cranial openings and filled with saline.

Body temperature was maintained at 37°C by a servo-controlled heating blanket (Harvard Apparatus, Holliston, MA). For neural recordings, halothane was discontinued and the rat was maintained in a lightly narcotized, sedated state by intravenous infusion of fentanyl (Sublimaze,

~10 $\mu g \cdot kg^{1} \cdot hr^{1}$; Janssen Biochimica, Berse, Belgium). To prevent spontaneous movement of the vibrissae, which would prevent use of our electromechanical stimulators (below), neuromuscular blockade was induced with pancuronium bromide ($1.6\ mg \cdot kg^{-1} \cdot hr^{-1}$), and the animal respired (90-100 breaths/min) using a positive-pressure ventilator. A computer continuously monitored the rat's EEG, mean arterial pressure, arterial pulse rate, and tracheal airway pressure waveform. Experiments were terminated (see below) if any of the above indicators could not be maintained within normal physiological ranges; this occurred rarely.

3.2.2 Recordings

Data were obtained at a sampling rate of 32 kHz from cortical barrels and thalamic barreloids in the ventral posterior medial nucleus (VPM) using high impedance (5-10 MΩ) stainless steel microelectrodes (Frederick Haer, Brunswick, ME). Signals were amplified and band-pass filtered at 300 Hz -10 kHz. In order to determine the principal whisker (PW), defined as the whisker evoking the strongest response, whiskers on the contralateral mystacial pad were stimulated manually during electrode advancement. Extracellularly recorded neurons were identified by spike amplitude and waveform criteria using a virtual oscilloscope with a triggered delay and an amplitude discriminator produced by custom-made programming written in Labview version 5.1.1 (National Instruments). When multiple units were present, only the one having the largest amplitude was discriminated. Units were further isolated off-line using the cluster cutting program, MClust version 2.0 (A. David Redish, University of Minnesota, Minneapolis, MN). In the cortex, we distinguished two types of neurons based on spike waveform, regular spiking units (RSUs) and fast spiking units (FSUs) (Kyriazi et al. 1996; Bruno

and Simons 2002). These are thought to represent the discharges of excitatory and inhibitory barrel neurons, respectively. In this study, we compared the response properties of RSUs and FSUs with identically studied thalamocortical units (TCUs).

3.2.3 Histology and recording locations

At the termination of an experiment, the rat was deeply anesthetized with sodium pentobarbital (100 mg / kg i.v.) and perfused transcardially for cytochrome oxidase (CO) histochemistry. The cortex was cut tangentially and the thalamus was sectioned coronally. Tissue sections were reacted for CO and all sections were counterstained with thionine. Using microdrive readings, signs of tissue disruption, and/or electrolytic lesions made with metal microelectrodes, recording sites were localized with respect to individual barrels; data are presented only for units recorded in CO-rich barrel centers. Because of the complex geometry of thalamic barreloids, no attempt was made to identify thalamic recording sites with respect to individual barreloids, but all recording sites were confirmed as being located within the ventral posterior medial thalamic nucleus.

3.2.4 Whisker stimulation protocols

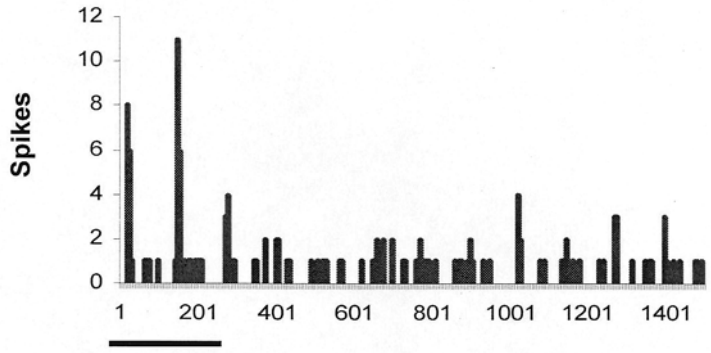
For each unit, we first used hand-held probes to identify the principal whisker (PW). The PW was trimmed to 12-15 mm in length and a multi-angle piezoelectric stimulator was advanced over the terminal 2-5 mm of the cut end of the whisker (Simons, 1983).

As previously described (Simons and Carvell, 1989), a smoothed ramp-and-hold stimulus (peak velocity $\simeq$ 125 mm/sec, peak amplitude = 1 mm, duration = 200 msec) was applied to the PW randomly in each of eight angles spanning 0^o to 360^o in increments of 45^o. Within a trial, the ramp-and-hold was presented at the same angle before and after an adapting stimulus (see Fig.15). The adapting stimulus began with a ramp of velocity and amplitude identical to the ramp of the preceding ramp-and-hold. The adapting stimulus' ramp was followed by a positively-rectified 20 Hz sinusoid in one of four cardinal directions (0, 90, 180, or 270^o) for 100 msec (2.5 cycles). Twenty Hz was chosen because this frequency produces more response suppression in RSUs and FSUs than in TCUs (Khatri et al., 2004). The stimulus design of this study allowed the comparison of responses to a ramp-and-hold deflection angle (e.g. 0^o) before and after adapting stimuli of the same or different angles. The post-adaptation ramp-and-hold followed the adapting stimulus by either 25 or 50 msec. These two time delays were used with the intent of further differentiating the effects of adaptation on cortical and thalamic neurons. For a given delay, there were a total of 32 conditions (8 pre- and post-adaptation ramp-and-holds x 4 cardinal adapting directions). A block consisted of the 32 stimuli presented in pseudo-random order, and ten such blocks were delivered.

For a subset of barrel RSUs, adapting whisker deflections were paired with electrically-evoked cortical suppression. Electrical stimulation was applied with a single microelectrode (<2 MΩ impedance at 1000 Hz) that was either made from pulled and beveled glass pipettes loaded with carbon fibers or from quartz-insulated platinum-tungsten (90-10%) core fibers (Uwe Thomas Recording, Giessen, Germany). The stimulation microelectrode was lowered approximately 600 μm below the pial surface into layer 2/3 overlying the barrel that was targeted for neural recordings. The depth of 600 μm was chosen to minimize the likelihood of stimulating

thalamic afferents as suggested by Chung and Ferster (1998. The stimulation parameters, taken from Chung and Ferster (1998), effectively reduced cortical responses to whisker stimulation in layers 2 through 6 (see Figures 13 and 14). Shocks were delivered 20 msec prior to the estimated arrival time for whisker-evoked thalamocortical inputs. A 20 msec delay was used so that thalamocortical inputs reached the barrel when cortical circuitry was maximally suppressed. Inspection of histologically-prepared specimens indicated that electrical stimulation did not produce visible damage to the cortical tissue (e.g., lesions). Prior to applying shocks with adapting whisker deflections, the effectiveness of the shocks alone was determined. An 8 Hz periodic pulsatile stimulus was applied to the whisker and a single 200 μsec shock of either 150 or 600 μamp was delivered to the cortex. In figure 13, the responses of a cell recorded at ~1550 μm in depth are displayed. The portion of time containing the stimulus artifact produced by the shock has been removed from each PSTH (A: 150 μamp, B: 600 μamp). The black line under each PSTH indicates when the effects of the shock are observed. Comparing the 150 and 600 μamp conditions indicates that the latter produces a larger response reduction. The reduction after 600 μamp shocks was quantified across the sampled cells (n = 13) as a function of depth by taking the average firing rate in the 280 msec period after the shock and its artifact, and dividing that by the average firing rate during that time in a control condition having no shock. Figure 14 shows that effects of the shock could be seen in all cortical layers; the average reduction was ~35%. During the actual experiment, five biphasic electrical shocks (200 μsec at 600 μamp) were applied during the adapting stimulus (see asterisks in Figure 15).

A



B

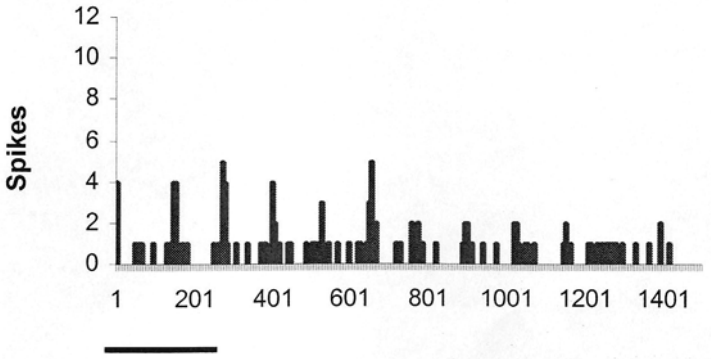


Figure 13. Example of response suppression produced by different amounts of electrical stimulation. A. 150 μamp. B. 600 μamp.

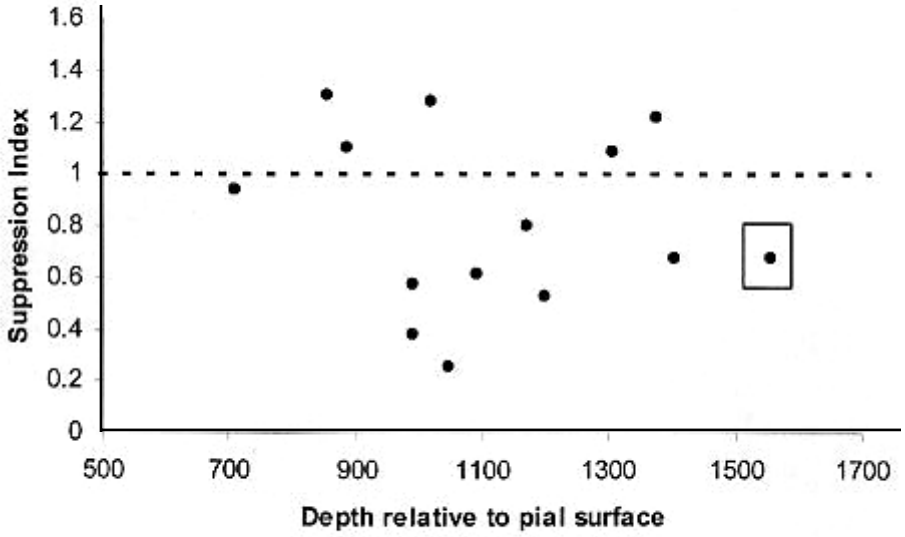


Figure 14. Suppression Index as a function of depth. Boxed point is the same cell from figure 13.

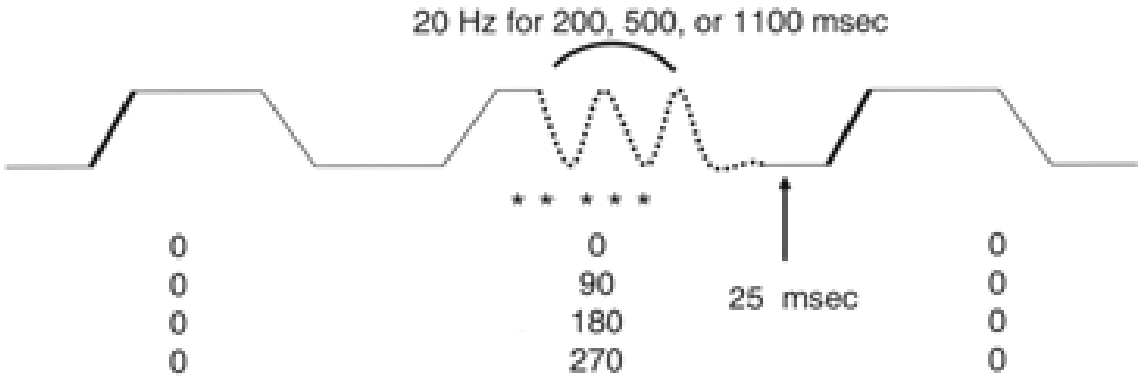


Figure 15. The stimulus. A non-adapted ramp-and-hold is presented in one of 8 directions (0 to 315^{o}, increments of 45^{o}). Five-hundred msec later a 20 Hz adapting stimulus is presented in one of 4 cardinal directions. After a delay of 25 msec, another ramp-and-hold is delivered. The ON responses evoked by the rising phases of the ramp-and-holds (thick line) were analyzed before and after adaptation. Asterisks indicate at what points in the stimulus the cortex was electrically stimulated in a subset of experiments.

3.2.5 Data Analysis

Unit responses were quantified by initially binning spikes with a 1 msec resolution. The ON response magnitude evoked by the rising phase of a ramp (denoted by stars in Fig. 17) was computed by taking the average number of spikes per stimulus occurring during a 20 msec period beginning immediately after the ramp's onset. Effects of adaptation were assessed by comparing pre- and post-adaptation ON responses.

Quantification of angular tuning. The sharpness of angular tuning was determined by representing the ON response for each direction as a vector. First, the response magnitude for each angle (normalized to the mean response across all 8 angles) is converted to a vector of polar coordinates. R_θ is the number of spikes contained within the ON response summed across trials.

$$x = R_\theta * \cos_\theta$$

$$y = R_\theta * \sin_\theta$$

All of the 8 angle's vectors are summed and the magnitude of the resulting vector is computed by:

$$VS = (x * x + y * y)^{1/2}$$

Vector strength (VS) provides a measure of how sharply tuned a neuron is for whisker deflection angle. The preferred angle is equal to arctan (θ) = (sum(y)/sum(x))

Similarity Index (SI). We used a correlation coefficient to quantify the degree to which two polar plots are similar. SI was calculated by correlating ON responses on an angle-by-angle

basis. A value of 1.0 indicates that they are identical whereas -1.0 indicates they are completely opposite.

Due to deviations from normality in the distributions of data, statistical significance is evaluated with non-parametric statistical tests (Mann-Whitney Test, Wilcoxon's Signed Ranks Test, and the Kruskal-Wallace Test). Response distributions are graphically displayed as box plots due to the absence of normality. The upper and lower quartiles are represented by the edges of the box. The extremes are indicated by the length of the lines connected to the top and bottom edges.

3.3 RESULTS

Data are reported from 30 TCUs, 39 RSUs and 9 FSUs recorded from 20 rats. All RSUs and FSUs were located within cytochrome-rich barrel centers. Approximately 2/3 of the RSUs and TCUs were studied using both 25 and 50 msec delay periods following the adapting stimulus; FSUs were examined only with the 25 msec delay. The three populations of sampled units were comparable to those reported previously (e.g., Simons and Carvell, 1989; Kyriazi et al. 1994). As populations, all three cell types displayed distinctly phasic responses to the rising and falling phases of the pre-adaptation ramp-and-hold, i.e., ON and OFF responses. As in previous studies, FSUs were the most responsive and had the largest OFF-to-ON ratios (Kryiazi et al., 1994), whereas barrel RSUs were the least responsive and had the smallest OFF-to-ON ratios. Also as reported previously, FSUs displayed the poorest angular tuning, responding well to all 8 deflection angles. Non-adapted angular tuning of the ON response as measured with VS

was smaller in FSUs (0.56 $\pm$ 0.13, median = 0.52) than in TCUs (3.21 $\pm$ 0.33, median = 3.11) and RSUs (3.36 + 0.30, median = 3.27) (Kruskal-Wallace test, $p < 0.001$) (Fig. 5E). Angular preferences are somewhat biased for caudal whisker deflections in the population of RSUs and TCUs, but not in FSUs (Fig. 16A-C). A bias for caudal directions has also been observed previously in RSUs (Bruno et al., 2003).

The pre-adaptation ramp-and-hold deflection was varied between 0 ^{o} and 360 ^{o} in increments of 45^{o}, followed by a 20 Hz adapting deflection at the same or a different angle, and then a post-adaptation ramp-and-hold stimulus identical to the pre-adaptation one was delivered (see Fig. 15). The 2.5 cycles of 20 Hz whisker deflection led to a reduction in the responses of RSUs to the subsequent ramp-and-hold movement. This is illustrated by the population PSTH in Figure 17 which was constructed from the responses of 39 RSUs; here responses are accumulated for 16 combinations of cardinal angle (0, 90, 180 and 270^{o}) test and adapting deflections. The first ramp-and-hold stimulus evoked a brief response to stimulus onset and a smaller response to stimulus offset. Five hundred msecs later the whisker was deflected with an identical stimulus followed shortly thereafter by 2.5 cycles of a 20 Hz sinusoidal movement; each cycle evoked two responses corresponding to movement away from and back to rest, i.e., ON and OFF responses. Twenty-five msecs after the conclusion of the sinusoid the whisker was deflected again using the same ramp movement as during the prior two stimuli. As illustrated by the population PSTH, RSUs responded less robustly to the third onset ramp than to either the first or second one. Averaged across all stimulus conditions, RSU responses relative to those evoked by the first ramp decreased by 40% (median AI = .57), and FSU responses decreased by 27% (median AI = .75); TCUs, on the other hand, displayed less adaptation, responding at near-control levels (median AI = .86). Adaptation levels differed (Kruskal-Wallace test, $p << 0.001$),

with FSUs adapting somewhat less than RSUs (Mann-Whitney Test, $p = 0.052$) and both cell types adapting more than TCUs (Fig. 16D).

As a measure of overall response reliability, we also examined responses to the first and second onset ramps, matched for stimulus angle (0, 90, 180 and 270^o). For all three populations, first- and second-ramp responses were highly similar to each other in terms of response magnitudes. Angular tuning was quantified with the *Similarity Index (SI*) that compares polar plot shapes, in this case based on the four cardinal directions (see Methods). SIs are close to 1.0 for RSUs (0.85 $\pm$ 0.02, median = 0.88), FSUs (0.73 $\pm$ 0.09, median = 0.88), and TCUs (0.85+0.04, median = 0.95), and are equivalent across cell types (Kruskal-Wallace test, $p = 0.22$) (Fig. 16F). The SIs obtained here contrast with the mean SI of any arbitrary pair of barrel RSUs which is close to 0 (Bruno et al., 2003). The large SIs indicate that responses are reproducible and thus variability will not obscure the effects of adaptation assessed with the third onset ramp. In all three cell populations, the amount of suppression generated by adaptation was smallest for 270^o or downward deflections relative to other directions: RSUs ($p << 0.001$), FSUs ($p<<0.001$) and TCUs ($p < 0.01$) (Mann-Whitney tests). Cells preferring 270^o did not differ, however, from those preferring other angles in terms of either non-adapted angular tuning (VS) or spikes/stimulus.

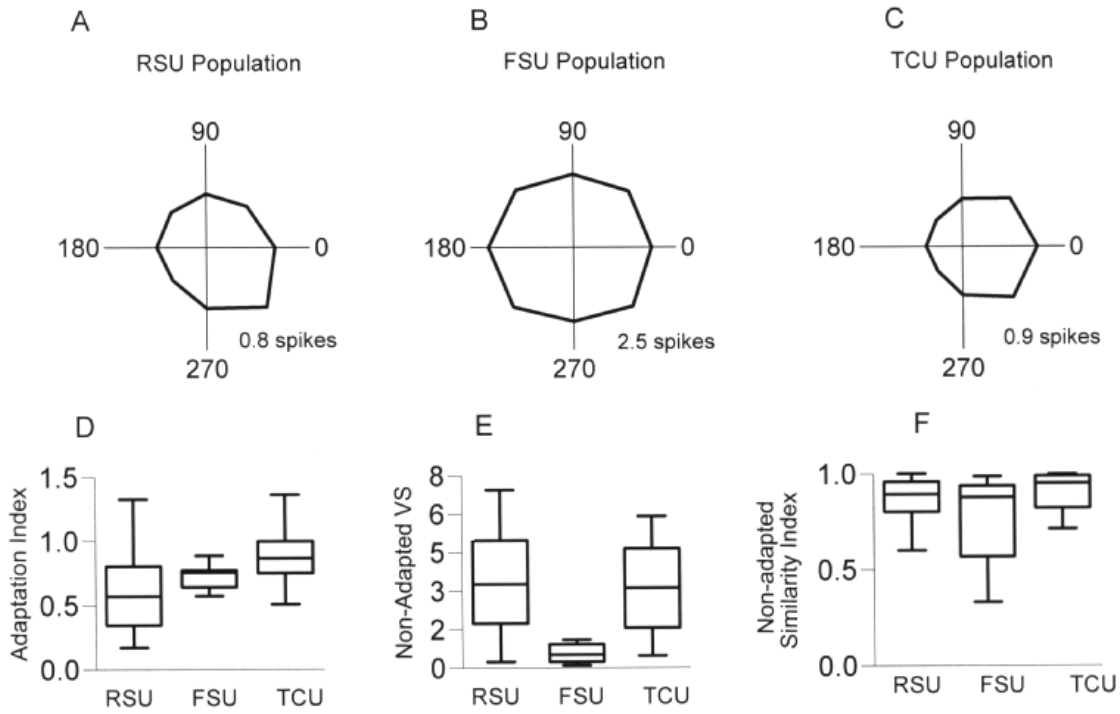


Figure 16. Response properties of RSUs, FSUs, and TCUs. A. Population polar plot for RSUs. A bias is present for caudal (0^o or 315^o) deflections. 315^o deflections evoked 0.8 spikes per deflection. **B.** Population polar plot for FSUs. No angular preference bias is present. **C.** Population polar plot for TCUs. They also display a bias for caudal deflections. **D.** Adaptation indices (post/pre-adaptation ON response magnitude). **E.** Vector strength (VS) of non-adapted angular tuning. **F.** Polar plot similarity indices for responses to first and second ramps.

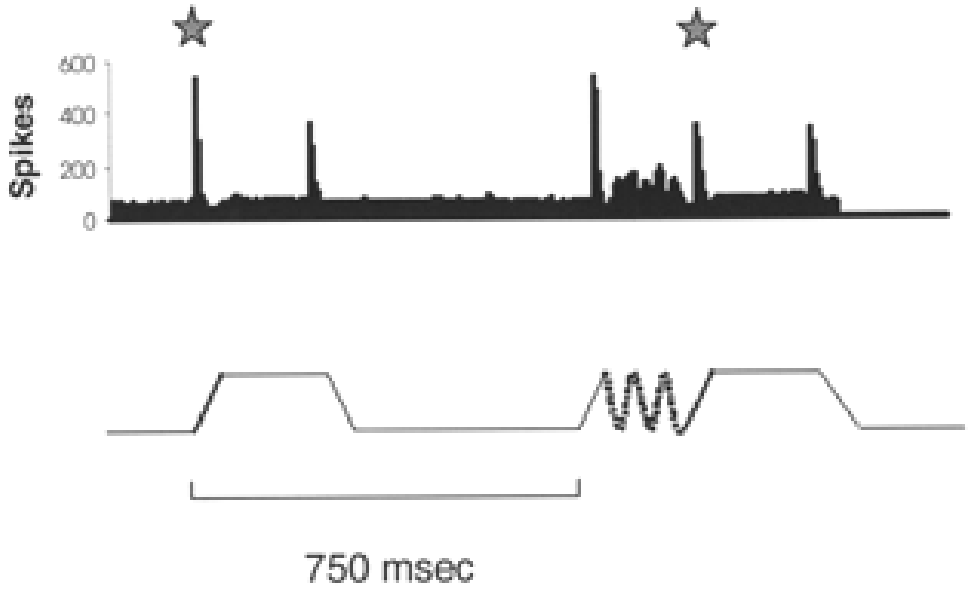


Figure 17. RSU population PSTH. Stars above PSTH indicate non- and post-adapted ON responses, respectively.

3.3.1 Effects of the adapting stimulus angle

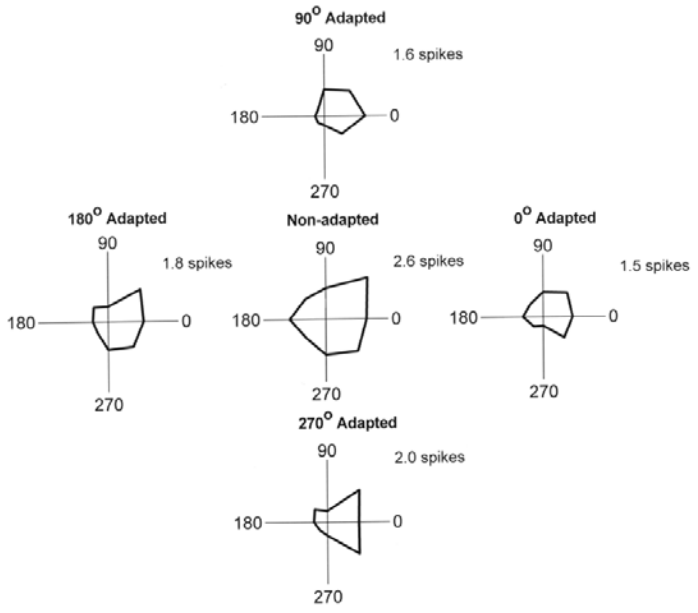
The 20 Hz adapting deflections were equally effective in producing response adaptation, regardless of their direction relative to the angle of the test deflection. Figure 18A shows polar plots obtained from an RSU in the non-adapted condition and following adaptation with the four cardinal directions of 20 Hz stimulation. In the non-adapted state, the unit displayed a preference for caudal (0^o) deflections. This preference is retained following adaptation at any of the four cardinal directions. For example, the RSU's response to 0^o is smaller after adaptation with 0^o deflections, but responses to all other angles are also suppressed. In each case, the unit's tuning as evidenced by the narrowing of the polar plots, is enhanced. The same results hold for the entire RSU population. In figure 18B, the central panel shows a non-adapted RSU population polar plot in which each individual cell's maximal angle has been rotated to 0^o. The surrounding panels contain adapted polar plots for the 0, 90, 180 and 270^o adapting deflections. The graphs illustrate that regardless of the adapting angle, angular preference does not change and tuning becomes sharper.

For individual cells of all three populations, we compared actual spike counts evoked by a test deflection when it was preceded by adapting deflections in the same or opposite (180^o) direction; because responses at some angles were extremely small or 0.0, we did not compute ratios (AI) as in Figure 16. Data for all angles of test deflections were pooled, and analyzed responses thus include combinations of both preferred and non-preferred directions. Adaptation-induced response suppression was largely independent of the directions of the test and adapting stimuli (see Fig. 19). For example, in RSUs, deflections at opposite angles lead to a reduction in the median spike count of ~70%. A small angularly-specific component of suppression is also evident in that when adapting and test stimuli are of the same direction, adapted responses are

even smaller. The distributions of spike counts for same and opposite angle adaptation differed (medians = 0.05 versus 0.10, Wilcoxon signed ranks test, $p < 0.001$). Similarly, in FSUs adaptation at opposite angles reduced medians by ~25% and same-direction adaptation reduced responses an additional 15% (medians = 0.78 versus 1.15, Wilcoxon signed ranks test, $p = 0.003$). A possible concern is that the observed direction-specific effect is a consequence of the already described finding that 270 ^{o} adapting deflections evoked less suppression than other angles. This was not the case because even when 90 ^{o} and 270 ^{o} stimuli were removed from the analysis, RSUs and FSUs still displayed a direction-specific effect ($p = 0.001$ and $p = 0.037$, respectively).

For TCUs adaptation effects were equivalent for adapting stimuli at the same and opposite directions (Wilcoxon signed-rank test, $p = 0.86$). A direction-specific effect was observed, however, when only the first 5 msec of the thalamic response was examined (same versus opposite medians = 0.10 versus 0.20, Wilcoxon signed-rank test, $p = 0.016$). This time epoch has previously been shown to be the critical determinant of cortical responses (see below). This apparent direction-specific effect was a consequence of the fact that as in the cortex, 270 ^{o} adapting deflections evoked less suppression than 90 ^{o} ones. There was no direction-specific effect if responses after 90^{o} and 270^{o} adapting deflections were removed.

A



B

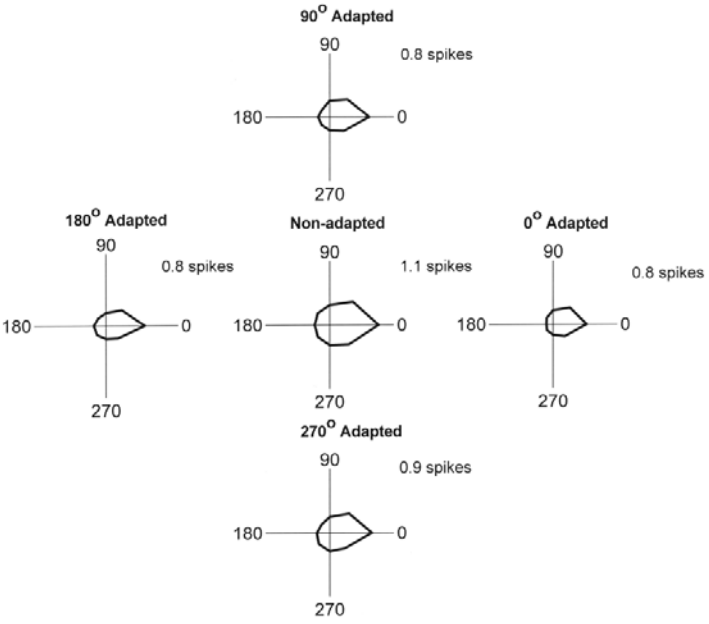


Figure 18. A. Typical RSU behavior. Center panel is the non-adapted angular tuning. 2.6 spikes were evoked on average for each 45^{o} deflection. The right-most panel is the angular tuning after 0^{o} adaptation. **B. RSU population.** Response for each cell has been rotated such that 0^{o} represents the preferred angle.

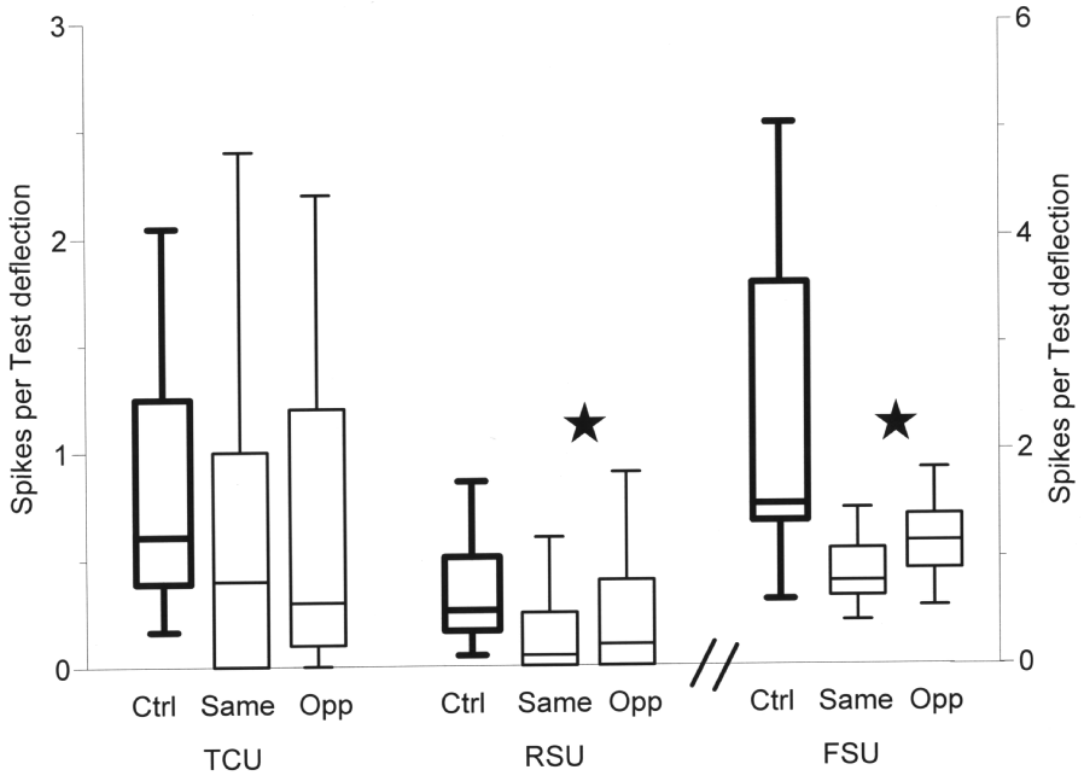


Figure 19. Responses of TCUs, RSUs, and FSUs to same and opposite angle adaptation. Y-axis on the left is for TCUs and RSUs. Right y-axis is for FSUs. Stars indicate significant differences ($p < 0.01$) between responses after same and opposite angle adaptation. Direction-specific suppression is present in RSUs and FSUs, but not TCUs.

3.3.2 Adaptation-induced effects on angular tuning

Because of angularly-nonspecific suppression, the shape of a cell's polar plot remains approximately the same before and after adaptation, as illustrated by the RSU of Figure 18A. To quantify this for each cell, the vector strength measure was used to calculate a phase angle representing each polar plot's overall orientation (see Methods). Vector angles in the non-adapted and adapted conditions were compared by subtraction. Figure 20 shows data for 0^{o} adapting deflections. Following adaptation, vector angles were equivalent to those in the non-adapted condition (Fig. 20A). For 24/38 (63%) RSUs, the preferred angle after adaptation remained within 45^{o} of its non-adapted value. And for 34/38 of those same RSUs, the preferred angle remained within 90^{o} of the non-adapted value. Similar results, not shown, were obtained for the other three adapting angles and for the other cell types (FSUs and TCUs).

Observation of individual units, such as the RSU illustrated in Figure 18A, suggested that adaptation enhances angular tuning, that is, it makes the polar plots narrower. Again, we used the measure of vector strength to quantify angular tuning. There was no effect of the particular adapting angle, and therefore post-adaptation responses were averaged for all four angles of adaptation. Comparisons of pre- and post-adaptation vector magnitudes showed that for 34 of 40 RSUs (~ 83%), adaptation increased angular tuning (Fig. 20B). On average, vector magnitudes increased ~30% after adaptation (pre versus post medians = 3.27 versus 4.29, Wilcoxon signed ranks test, $p < 0.001$). Adapted responses were also ~30% more sharply tuned in TCUs (medians = 3.11 versus 3.99, Wilcoxon signed ranks test, $p = 0.005$). Tuning was not significantly affected in FSUs, which remained as poorly tuned in the adapted state as they are in the non-adapted one.

The above findings were obtained using a stimulus protocol in which the test deflection followed the termination of the 20 Hz adapting stimulus by 25 msecs. With this delay, TCUs were affected by adaptation. In order to differentiate further the responses of TCUs and RSUs (n = 21 and 25), we also employed a 50 msec delay. With the longer delay, adaptation produced in RSUs effects on angular tuning that were qualitatively similar to those obtained with the 25 msec delay, though the average amount of overall suppression was somewhat less. As with the 25 msec delay, responses were suppressed ~10% more when adapting and test deflections were in the same direction, (Wilcoxon signed ranks test, $p = 0.007$). With the 50 msec delay, suppression was again accompanied by a sharpening of RSU angular tuning ~30% (medians = 2.87 versus 4.13, Wilcoxon signed ranks test, $p < 0.001$). Unlike RSUs, angular tuning in TCUs was not, however, affected by adaptation when tested with the 50 msec delay. Thus with the 50 msec delay, sharper tuning in the cortex can be mediated solely by intracortical processing.

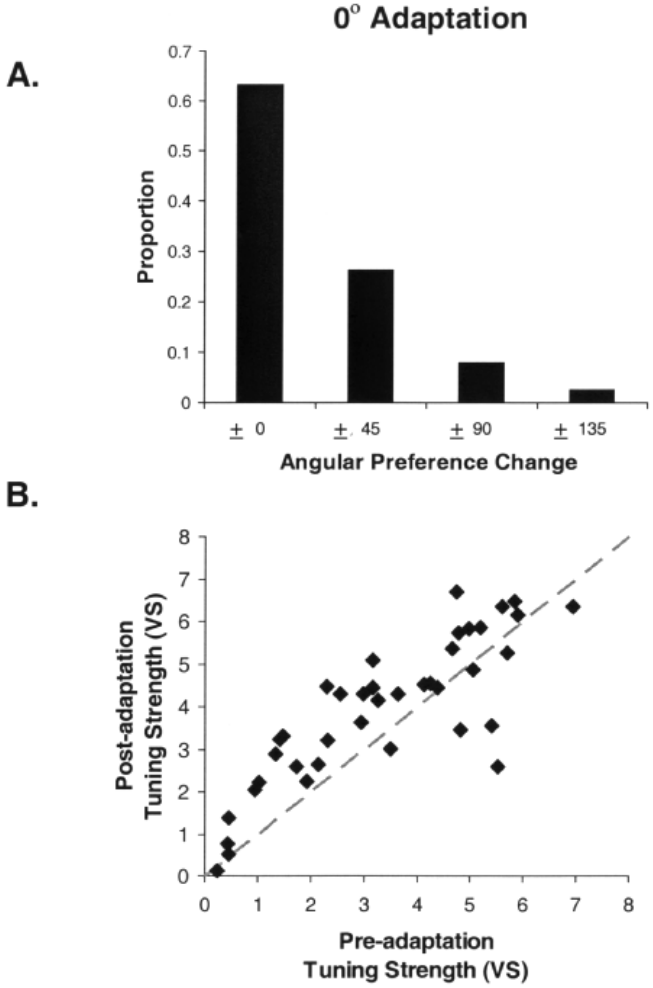


Figure 20. RSU responses after adaptation. A. Distribution of angular preference changes after 0^{o} adaptation. The angular preference of 90% of the post-adapted RSUs stayed within 45^{o} of the non-adapted angular preference. **B.** Angular tuning before and after all angles of cardinal angle adaptation. RSU tuning is sharpened in 33/40 cases.

3.3.3 Angularly-specific suppression

With 200 msecs of adaptation, followed by either a 25 or 50 msec delays, strong adaptation-induced effects were produced in barrel neurons regardless of the angle of the adapting stimulus. To determine the robustness of this finding, we examined effects on RSUs of two longer durations of adapting stimuli, 500 and 1100 msecs, using the same 20 Hz sinusoids and a 25 msec delay. The longer durations were expected to depress thalamocortical input and intrabarrel circuits even further due to more complete synaptic depression and/or summation of inhibition. As shown in Figure 21, firing rates were somewhat higher after the 1100 msec adaptation period but the effect was not significant for either same or opposite angle adaptation (Kruskal-Wallis ANOVA's, p's > .20). Adaptation effects described previously for the 200 msec period were also observed for the 1100 msec case; strong adaptation was produced by both same- and opposite angle deflections, and firing rates with the former stimuli were reduced more. A difference between same and opposite angle adapting effects was not observed for the 500 msec period, perhaps due to large variability in the sample.

3.3.4 Adaptation during cortical inactivation

Thalamic input imparts angular tuning to barrel neurons (Bruno and Simons, 2002; Bruno et al., 2003). Thus, angularly-specific suppression in the cortex could reflect corresponding reductions in thalamic firing and/or depression of the thalamocortical synapse. To test this, we suppressed cortical activity during the delivery of the adapting whisker deflections. We adopted the method of Chung and Ferster (1998) in which electrical pulses are delivered to a site several

hundred microns superficial to the recording electrode in layer 4. Brief trains of electrical pulses are thought to render the cortical network refractory. Cortical activity could not be monitored during electrical stimulation due to the presence of stimulus artifacts, but deflection-evoked activity following the shocks could be measured. After 5 shocks alone (without accompanying whisker deflections), multi-unit activity (n = 23 sites) evoked by deflections in the preferred direction was suppressed by 57 $\pm$ 7 % (see Figure 22), and responses were smaller after 5 shocks than 1 shock (medians = 0.7 versus 0.4 spikes/deflection) (Wilcoxon signed rank's test, $p << 0.001$). Note that in the 5 shocks alone condition, electrical stimulation produced angularly-non specific suppression despite the non-adapted ramp either being in the same or opposite direction relative to the test stimulus. Thus, the electrical shocks produced the desired reduction in overall cortical excitability during the period of sensory adaptation.

Figure 22 shows results obtained when the electrical shocks were delivered with the adapting whisker deflections. Response suppression was greater when the 20 Hz adapting stimulus was paired with 5 shocks versus 1 shock (responses averaged over both same and opposite adaptation conditions, 0.56 $\pm$ 0.11 versus 1.19 $\pm$ 0.20 spikes per stimulus, paired t-test, $p = 0.004$). To evaluate stimulus-specific effects, we calculated same-minus-opposite difference scores for the average response to a test stimulus when preceded by an adapting stimulus of the same versus the opposite angle. Even when activity in the cortical barrel was strongly and globally reduced, the amount of angularly-specific suppression remained constant (same-minus-opposite difference medians: -0.27 versus -0.28, Wilcoxon signed ranks test, $p = 0.14$). Together, the findings indicate that angularly-specific adaptation can be produced without intact intracortical circuits.

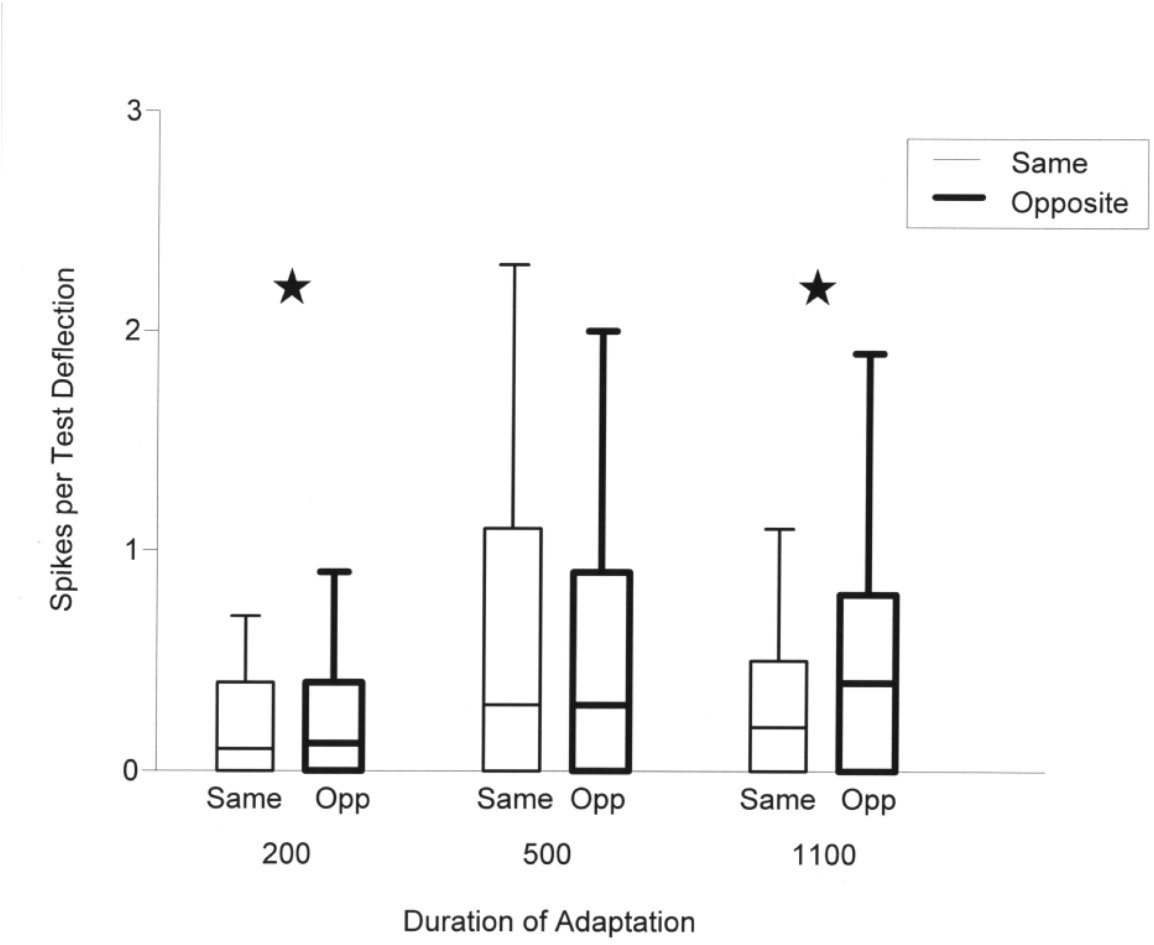


Figure 21. Effect of adaptation duration on direction-specific suppression of RSU responses. The non-adapted preferred direction response is ~1.0 spikes per deflection. The amount of direction-specific suppression did not increase with the duration of adaptation. Stars indicate significant differences ($p < 0.01$) between responses after same and opposite angle adaptation.

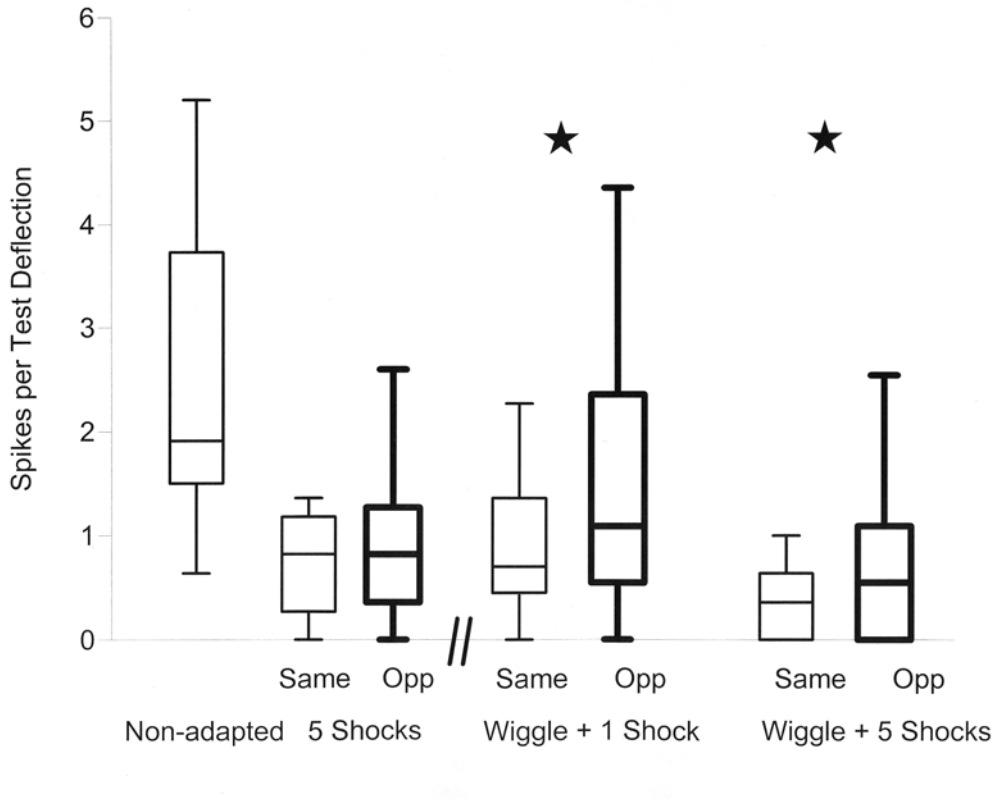


Figure 22. Effect of pairing adapting whisker deflections with electrical stimulation of the cortex. Direction-specific suppression is present whether 1 or 5 cortical shocks are presented. Note the large non-adapted response magnitudes are due to data in this figure only, being from multi-unit recordings.

3.4 DISCUSSION

Previous studies have demonstrated that repetitive whisker deflections evoke suppression of neuronal responses in barrel cortex (Simons, 1978; Fanselow and Nicolelis, 1999; Ahissar et al., 2000; Garabedian et al., 2003; Khatri et al., 2004). Adaptation effects may differ quantitatively in thalamic barreloid and cortical barrel neurons depending on stimulation frequency, state of arousal, and the type/depth of anesthesia (see Faneslow and Nicolelis, 1999; Castro-Alamancos, 2004). Here we examined whether response suppression in the thalamocortical circuit is selective for a specific characteristic of the adapting whisker deflection, its angular direction. We found that activity in both thalamocortical units (TCUs) and cortical neurons (RSUs and FSUs, presumed excitatory and inhibitory neurons) is diminished following repetitive whisker deflections in any direction. For any given unit, adapting stimuli diminished responses regardless of the directions of the adapting and test stimuli. Suppression was not uniform however in that responses to stimuli in non-preferred directions were diminished more. This parallels findings that weaker responses are disproportionately affected by changes in the excitability of barreloid-to-barrel circuit (Kyriazi et al., 1996; Pinto et al., 2003). Together, the two major effects of angularly global response suppression and the disproportionate reduction of initially weaker (non-preferred) responses led to a sharpening of angular tuning in the thalamocortical circuit. In the cortex, the angular preference of RSUs was maintained despite marked reductions in overall evoked firing rates, and concomitantly, angular tuning was enhanced. These findings suggest that the impact of angularly-nonspecific suppression

outweighs that of angularly-specific suppression. Otherwise, polar plots would have become consistently rounder and/or their orientations would have shifted systematically away from the direction of the adapting stimulus.

Adaptation effects observed in the cortex could reflect intracortical processing and/or changes in the thalamic inputs themselves. We attempted to disambiguate these possibilities by choosing a frequency of the adapting stimulus that more strongly suppresses cortical than thalamic firing and by varying the time interval between the termination of adapting stimulus and the onset of the test deflection. Our previous study using similar whisker deflections and recording conditions showed that 40 Hz stimuli strongly adapt both thalamic and cortical neuron responses, whereas 20 Hz stimuli produced strong response suppression in RSUs and FSUs but not TCUs (Khatri et al., 2004). Under the assumption that the period of these periodic stimuli is critical, here we used two different delays between conditioning and test stimuli, a 25 msec delay which corresponds to the period of a 40 Hz stimulus and a 50 msec delay which corresponds to a period of 20 Hz. We found that with the 25 msec delay, angular tuning of both TCUs and RSUs increased by ~30%. Thus, under this stimulus condition, TCUs themselves displayed adaptation effects which could in turn be reflected in the cortical responses independent of any additional intracortical mechanism. However, with the 50 msec delay, only the tuning of RSUs was sharpened, again by ~30%. Thus, adaptation-induced sharpening of RSU angular tuning can occur independently of parallel changes in thalamic inputs.

3.4.1 Mechanisms of response suppression

We classified suppression effects as angularly-specific and angularly-nonspecific in terms of whether response suppression occurs when adapting and test stimuli are at the same or different (e.g., opposite) directions. We propose that both angularly-specific and angularly-nonspecific effects are mediated largely by local inhibition (see also below), whereas angularly-specific suppression reflects an additional contribution of short-term depression at thalamocortical synapses.

A distinctive feature of fast-spike units in barrel cortex is that almost all of them are broadly tuned for deflection angle (see Fig. 16B and Simons, 1978; Kida et al., 2005); this property reflects highly convergent inputs from multiple thalamocortical neurons having a range of preferred deflection angles (Swadlow and Gusev, 2002; Bruno and Simons, 2002). Recently, we showed that FSUs retain their characteristic high firing rates and temporally focused responses after adaptation with repetitive whisker stimulation similar to that used here (Khatri et al., 2004). Present findings indicate, further, that in the adapted state, barrel FSUs also retain their broad angular tuning. It is therefore likely that the pervasive, angularly-nonspecific adaptation effects observed in the layer IV barrel reflect synaptic inhibition mediated locally by FSUs. Because FSUs are non-selective for deflection angle, the inhibitory network within the barrel is as likely to be engaged when adapting and test angles are similar as when they are different. Thus, to the extent that inhibition underlies response suppression observed in cortical neurons, angularly-specific effects and angularly-nonspecific effects can be mediated by the same mechanism.

Adaptation-induced reductions in neuronal firing could also be produced by short-term depression of excitatory thalamocortical synapses (Chung and Nelson, 2002). If so, such changes

should occur independently of the (post-synaptic) cortical network. In order to evaluate possible contributions of thalamocortical synaptic depression, we disrupted the cortical circuitry by applying electrical shocks to layer III at the time that the adapting whisker stimuli occurred. The shock had a magnitude of 600 μamp and a duration of 200 μsec, a charge (1200 nC) more than sufficient to affect cells within a 450 μm radius (Butovas and Schwartz, 2003). We assume therefore that the electrical stimuli non-selectively discharged neurons in the underlying barrel and that the cortical circuit remained refractory during the adapting whisker deflections (Boudreau and Ferster, 2005). Evidence for this is that test responses were strongly reduced in magnitude when the shocks were presented alone, without accompanying adapting whisker deflections, and the reduction was greater with 5 shocks than 1. When paired with adapting deflections, angularly-specific response suppression was still observed. Assuming that network affects were eliminated by the shocks, the angularly-specific suppression originates with the thalamic inputs, either as changes in the responses of the thalamocortical input cells and/or as a reduction in the efficacy of their synapses. This conclusion is consistent with previous findings that the directional specificity of RSU responses is inherited from their thalamic inputs which unlike the case for FSUs, is based on selective convergence of TCUs having the same or similar angular preference (Bruno and Simons, 2002).

A possible criticism of our usage of electrical stimulation to inactivate the cortex is that the shocks may have also antidromically activated thalamic neurons. Additionally, the shocks may have orthodromically activated corticothalamic cells, and consequently, thalamocortical neurons could have been affected. The large electrical shocks used here most likely did activate thalamic neurons, both antidromically and orthodromically. However, these effects upon thalamic neurons should not be a concern in interpreting the results. If thalamic neurons were

affected by electrical stimulation, barreloid neurons of different angular preferences should have been influenced in a directionally-nonspecific manner. Such an effect could not explain the direction-specific suppression that was observed in barrel neurons after inactivation. Electrical stimulation of the cortex without any adapting whisker deflection only produced angularly-nonspecific suppression (see 5 shocks alone condition in Figure 22).

Our interpretation of a contribution of thalamocortical synaptic depression to the direction-specific effect is based on the assumption that those synapses can undergo stimulus-dependent depression. In the present recording conditions, TCU's fire spontaneously at rates of 10-15 Hz (Simons and Carvell, 1989; Kwegyir-Afful et al., 2005), presumably placing the thalamocortical synapse in a tonic state of partial depression. In geniculocortical circuits under conditions where thalamic cells fire spontaneously >20 Hz, thalamocortical synaptic depression can be produced, though at lower levels than those obtained when spontaneous LGN firing is decreased (Boudreau and Ferster, 2005; see also Swadlow et al., 2002). Thus, in our experiments it is likely that the adapting whisker stimuli cause further reductions in thalamocortical synaptic efficacy. Low levels of spontaneously thalamic activity could be associated with somewhat different effects. For example, Garabedian et al. (2003) reported that angularly-specific suppression dominated the responses of barrel neurons in barbiturate-anesthetized animals, resulting in marked alteration of polar plot shape and hence angular tuning. Thus, depending on the level and pattern of thalamic background firing, a whisker's initial deflection could render barrel circuitry differentially responsive to subsequent deflections of that whisker in the same or a different direction. Receptive field size, another spatial property of barrel neurons, is also affected by tonic activity levels in the thalamocortical circuit (Alamancos, 2002; Kwegyir-Afful et al., 2005).

3.4.2 Are interactions among barrel angular tuning domains angularly-specific?

A barrel contains angular tuning domains or minicolumns in which RSUs lying in vertical register within layer IV share the same angular preference. Tuning domains for different angles may be represented at multiple locations with a barrel and appear to be randomly dispersed throughout it. They are estimated to be ~75 μm wide and thus smaller than the dendritic/axonal arbors of barrel spiny stellate cells, which can span ~200 μm (Simons and Woolsey, 1984; Lubke et al., 2000); the dendritic and axonal process of many inhibitory neurons are considerably larger (Erickson et al., 2004). These anatomical features of barrel circuitry raise the issue of whether functional angular tuning domains are synaptically interconnected in an angularly-specific manner. Individual barrels in rats contain morphologically identifiable subunits (e.g, cytochrome oxidase blotches: Land and Erickson, 2005), but there is no evidence to date that these - or other subdivisions - of the barrel neuropil are interconnected in a systematic fashion. *In vitro* studies have shown that any given pair of excitatory barrel neurons located within a 300 μm radius of each other may be synaptically interconnected, leading to the suggestion that the barrel is a "spatially homogeneous processing unit" (Petersen and Sakmann, 2000). We attempted to probe these connections functionally by determining whether adaptation of barrel neurons is angularly-specific; the rationale is that angularly-specific suppression would be mediated by angularly-specific circuits. On average, the RSUs collected here spontaneously fired spikes at a rate of 2.5 Hz and in conjunction with the *in vitro* findings, this suggests that the suppression of RSU-RSU synapses is only ~15% complete (Petersen, 2002). Thus in our recording conditions, intra-barrel excitation should be functionally intact and subject to further short-term depression. We found pervasive angularly-nonspecific suppression. This suggests

either that excitatory cells in neighboring angular tuning domains interconnect nonspecifically or that the effects of adaptation-induced depression at excitatory-to-excitatory cell synapses are weak relative to those of global inhibition (see above).

3.4.3 Feedforward inhibition and the specificity of sensory adaptation

Present findings are consistent with feedforward models of thalamocortical circuits in which stimulus-specific responses of layer IV neurons derive largely or entirely from specific patterns of thalamic input onto cortical cells (e.g., Hubel and Wiesel, 1962; Simons and Carvell, 1989). In terms of angular sensitivity in the whisker system, excitatory barrel neurons (RSUs) receive synchronously active, convergent inputs from thalamocortical neurons having similar angular preferences. Circuit dynamics, mediated by local interconnections among and between excitatory and inhibitory barrel cells, contribute to angular tuning by enhancing the intrinsic, non-linear sensitivity of RSUs to the rate of rise of the thalamic input signal (Pinto et al., 2000; Wilent and Contreras, 2004). Similarly, in cat visual cortex, orientation selectivity is thought to depend on feedforward geniculocortical inputs (Ferster et al., 1986) with local circuitry serving to reinforce or maintain the tuning provided by the thalamic inputs. Interestingly, orientation-specific or orientation-nonspecific adaptation effects may predominate depending on a cell's distance from a pinwheel center (Dragoi et al., 2001). In auditory cortex as well, strong frequency-specific adaptation effects can be observed (Ulanovksy et al., 2002). One difference in local circuit function that may underlie these strikingly dissimilar effects of adaptation is the degree to which feedforward inhibition is stimulus-specific. In the barrels FSUs are typically broadly tuned for deflection angle, whereas available evidence suggests that inhibitory neurons in cat primary visual cortex are sharply tuned for orientation (Azouz et al., 1997; Anderson et al.,

2000; Martinez et al., 2002).The degree of correspondence between the stimulus-specificity of local inhibitory interneurons and that of the excitatory neurons upon which they synapse may be an important factor in determining how ongoing sensory information affects the moment-to-moment activity of cortical circuits.

4.0 THE EFFECT OF ADAPTATION ON STIMULUS-EVOKED FIRING SYNCHRONY AMONG BARRELOID AND BARREL NEURONS

4.1 INTRODUCTION

Efficient sensory processing by a neural system requires the ability to deal with a continuous stream of rapidly changing inputs. Except upon waking, it is rare that stimuli occur in isolation. Hence, there is a need to investigate the effects of adaptation on neural processing. Previous studies have examined how adaptation affects the response properties of single neurons, but there has been no examination of how adaptation affects the behavior of a neural network. The importance of investigating the effects of adaptation on multiple neurons simultaneously was recognized by Barlow (1961) when he proposed that for the brain to remove the inherent redundancy present in the natural world, adaptation is needed to decorrelate the activity of cortical neurons (Barlow, 1961). The idea being that if certain features always occur together, then the brain can assume that and does not need to devote processing to the redundant input. For example, if several whiskers are simultaneously deflected during object contact, adaptation could focus the processing by individual neurons to their preferred whisker. Alternatively, Wainwright (1999) has suggested that correlated neural activity is required due to the fact that neural responses are variable; the same stimulus can evoke varying numbers of spikes on different trials.

No previous experimental work has directly tested Barlow's hypothesis for two major reasons. The first is that recording from more than one neuron in a network is not a trivial task, but as technologically has advanced, simultaneous recordings have become more common. The second issue is that synchronous activity can only be meaningfully interpreted if there is an understanding of the underlying circuits. An advantage of studying the rat whisker-to-barrel pathway is that this thalamocortical circuit is relatively well-defined.

Each of the rat's major whiskers are primarily represented by distinct cytoarchitectonically-defined modules in the thalamus and the cortex: *barreloids* in VPM and *barrels* in the cortex. Both individual barreloids and barrels can be subdivided into local processing units with distinct receptive field characteristics. Within the barreloid (Timofeeva et al., 2003) and barrel (Bruno et al., 2003), columns of cells respond best to the same direction of whisker movement. In a barreloid, preferred directions of whisker movement continuously vary from forward/upward in the dorsal region to backward/downward in the ventral region (Timofeeva et al., 2003). In a barrel, there is no obvious map for preferred directions though putative excitatory cells (regular-spike units, RSUs) in a single vertical penetration tend to share the same angular preference (Bruno et al., 2003).

The presence of distinct local processing units in barreloids and in barrels necessitates an examination of whether and how local these local processing units interact, cooperatively or competitively. Here, we explore this issue by determining how adaptation affects the *response synchrony* of neurons in two major structures of the whisker-to-barrel pathway: thalamic barreloids and cortical barrels. *Response synchrony*, as defined here, is a quantification of the tendency of two neurons to both respond or fail to respond to a stimulus on a trial-by-trial basis. This measure has been used in past studies to examine stimulus-evoked correlations among pairs

of neurons (Gawne et al., 1996; Kohn and Smith, 2005). Interpretation of response synchrony among pairs of thalamocortical units (TCUs) is guided by results from Bruno and Simons (2002). They demonstrated that any given barreloid thalamocortical unit (TCU) makes a monosynaptic connection upon a barrel RSU with a probability that varies with the similarity of the TCU and RSU's angular tuning. Even ~15% of TCU-RSU pairs that are oppositely tuned evidence a monosynaptic connection. These findings allow one to meaningfully compare the response synchrony of any two pairs of TCUs because both neurons have the capability of synapsing on the same post-synaptic RSU. As for layers 2/3, a major target of barrel layer 4 excitatory cells, data relating receptive field parameters and monosynaptic connections is lacking. However, extensive horizontal interactions in layers 2/3 (Gottlieb and Keller, 1997; Lübke et al., 2003) suggest that even cells with opposite angular tuning should be able influence each other.

The major result of the present study is that adaptation decorrelates the activity both of thalamic and of cortical neurons, that is, repetitive whisker stimulation makes the local processing units within barreloids and within barrels act more independently. Additionally, the neuronal responses of cells with dissimilar angular tuning are decorrelated the most, allowing correlated firing between similarly-tuned cells to be more salient in the adapted state.

4.2 METHODS

4.2.1 Animals and surgical preparation

Surgical preparation and maintenance of the rats during electrophysiological recording were identical to those described previously in Chapter 1. Ten Sprague-Dawley adult female albino rats (200-300 g) were used here.

4.2.2 Recordings

Single and multi-unit extracellular recordings were obtained from neurons in cortical barrels and thalamic barreloids in the ventral posterior medial nucleus (VPM) using 2-6 M electrodes (60 um shank diameters) made from pulled and beveled quartz-insulated platinum-tungsten (90-10%) core fibers (Uwe Thomas Recording, Giessen, Germany). The recordings were obtained with a multi-electrode array microdrive (Uwe Thomas Recording) used to independently control two to four recording electrodes. To insert several electrodes into the same barreloid or barrel, first the brain was mapped with a carbon fibre electrode (~1MΩ) while manually brushing the whiskers. After localizing VPM barreloids or barrel cortex, 2-3 electrodes were inserted ~100 um apart and into the same barreloid or barrel.

4.2.3 Histology and recording locations

At the termination of an experiment, the rat was deeply anesthetized with sodium pentobarbital and perfused transcardially for histological processing. The cortex was cut tangentially and the thalamus was sectioned coronally. Tissue sections were reacted for CO (Land and Simons 1985), and all sections were counterstained with thionine. Using microdrive readings and signs of tissue disruption, recording sites were localized with respect to individual barrels. Because of the complex geometry of thalamic barreloids, no attempt was made to identify thalamic recording sites with respect to individual barreloids, but all recording sites were confirmed as being located within the ventral posterior medial thalamic nucleus. Due to minimal damage to the cortex by the quartz electrodes, histological verification of all recordings sites could not be verified in some experiments.

4.2.4 Whisker stimulation protocols

As previously described (Simons and Carvell, 1980), a ramp-and-hold stimulus (peak velocity $\simeq$ 125 mm/sec, peak amplitude = 1 mm, duration = 200 msec) was applied to the PW randomly in each of eight angles spanning 0^o to 360^o in increments of 45^o. Within a trial, the ramp-and-hold deflection was presented at the same angle before and after an adapting stimulus that was presented at 0^o. The adapting stimulus began with a ramp of velocity and amplitude identical to the ramp of the preceding ramp-and-hold. The adapting stimulus' ramp was followed by a positively-rectified 20 Hz sinusoid for 100 msec (2.5 cycles). The post-adaptation ramp-

and-hold followed the adapting stimulus by 25 msec. Each of the 8 stimulus conditions were presented for 20 repetitions.

4.2.5 Data analysis

Unit responses were quantified by initially binning spikes with a 1 msec resolution. The ON response magnitude evoked by the rising phase of a ramp was computed by taking the average number of spikes per stimulus occurring during a 20 msec period beginning 5 msec after the onset of the ramp.

Similarity Index (SI). The measure SI indicates the degree to which the angular tuning of neuron's ON responses are similar. SI is calculated by correlating ON responses on an angle-by-angle basis. A value of 1.0 indicates that they are identical whereas -1.0 indicates they are completely opposite.

Response synchrony (RS). Due to the rapid nature of the rising ramp (~20 msec) and its correspondingly brief response duration (~10 msec), traditional cross-correlation analysis is not an appropriate means of analysis for the data collected here. Instead we examined response synchrony, or correlated firing, by determining whether the number of spikes evoked in both units changed in a similar manner on a trial-by-trial basis. The extent to which increases and decreases in spike counts covaried between units was quantified with a correlation coefficient. The measure RS indicates to what extent trial-by-trial changes in spike counts were correlated in simultaneously recorded units. RS can range from -1.0 (anti-correlated) to 1.0 (correlated). A similar measure would be to correlate spike counts as has been done is previous studies (Gawne et al., 1996; Kohn and Smith, 2005).

4.3 RESULTS

Prior to determining how adaptation affects the response synchrony of neurons within individual barreloids and barrels, we first examined the non-adapted response synchrony of 19 pairs of single thalamic neurons and 81 pairs of barrel multi-unit activity. Due to lack of spontaneous activity and relatively sharp angular tuning, the barrel recordings are likely to be predominantly from regular-spike units (RSUs), or putative excitatory cells (Bruno and Simons, 2002). The examined waveforms were also characteristic of RSUs (see Bruno and Simons, 2002). All members of pairs from here on will be referred to as units. The stimulus used was a ramp-and-hold whisker deflection, and only the ON response evoked by the rising phase of the ramp has been examined.

To determine whether correlated firing or response synchrony has a stimulus-nonspecific (i.e. angularly-nonspecific) influence, we examined whether units within single barrels and within single barreloids were more likely to respond (produce at least 1 spike) or fail (produce 0 spikes) together on a particular trial than would be expected from each unit's individual response or failure probability (see Fig.23). The number of observed joint responses was divided by the expected number of joint responses to derive a value of excess synchrony (ES). Similarly, to derive the measure excess failures (EF), the number of observed joint failures was divided by the expected number. In the barrel, 79/82 unit pairs (96%) fired together more than expected by chance (median ES = 1.07). Similarly in the barreloid, 15/19 (79%) unit pairs had ES values >1 (median ES = 1.05). As for excess failures in the barrel, 79/82 unit pairs (96%) had EF values >1

(median EF = 1.2). In the barreloid, 16/19 (84%) unit pairs had EF values >1 (median EF = 1.79). Thus, in both the barreloid and barrel, simultaneously recorded units responded or failed to respond together more than would be expected by chance.

The finding that barrel and barreloid neurons respond or fail together more than chance on a trial-by-trial basis suggested that angular tuning or polar plot similarity (SI, see methods), of simultaneously recorded units could be greater than those recorded at different times. In barrel units, simultaneously recorded units had higher SIs than units recorded at different times (medians = -0.18 versus 0.49) (Mann-Whitney Test, $p<<0.001$). The SI distributions of barreloid units were similar whether or not the units were recorded simultaneously or separately. Thus, the response properties of barrel neurons are more similar on a single trial than recordings from individual neurons at different times would imply.

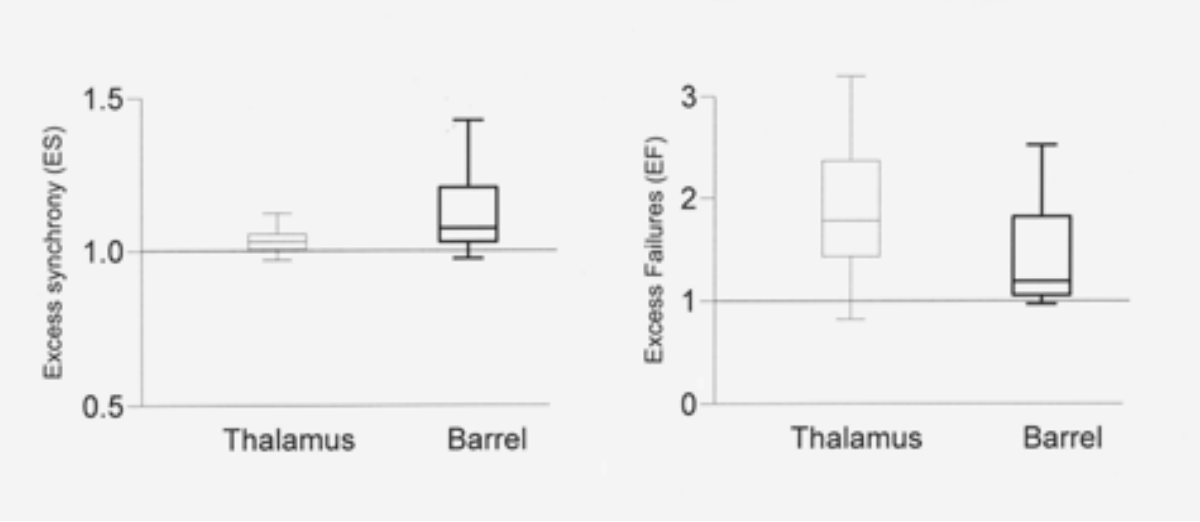


Figure 23. Excess synchrony and Excess failures for thalamic and barrel pairs. Dashed line of each graph indicate what values should be if joint synchrony or failure could be predicted from individual probabilities.

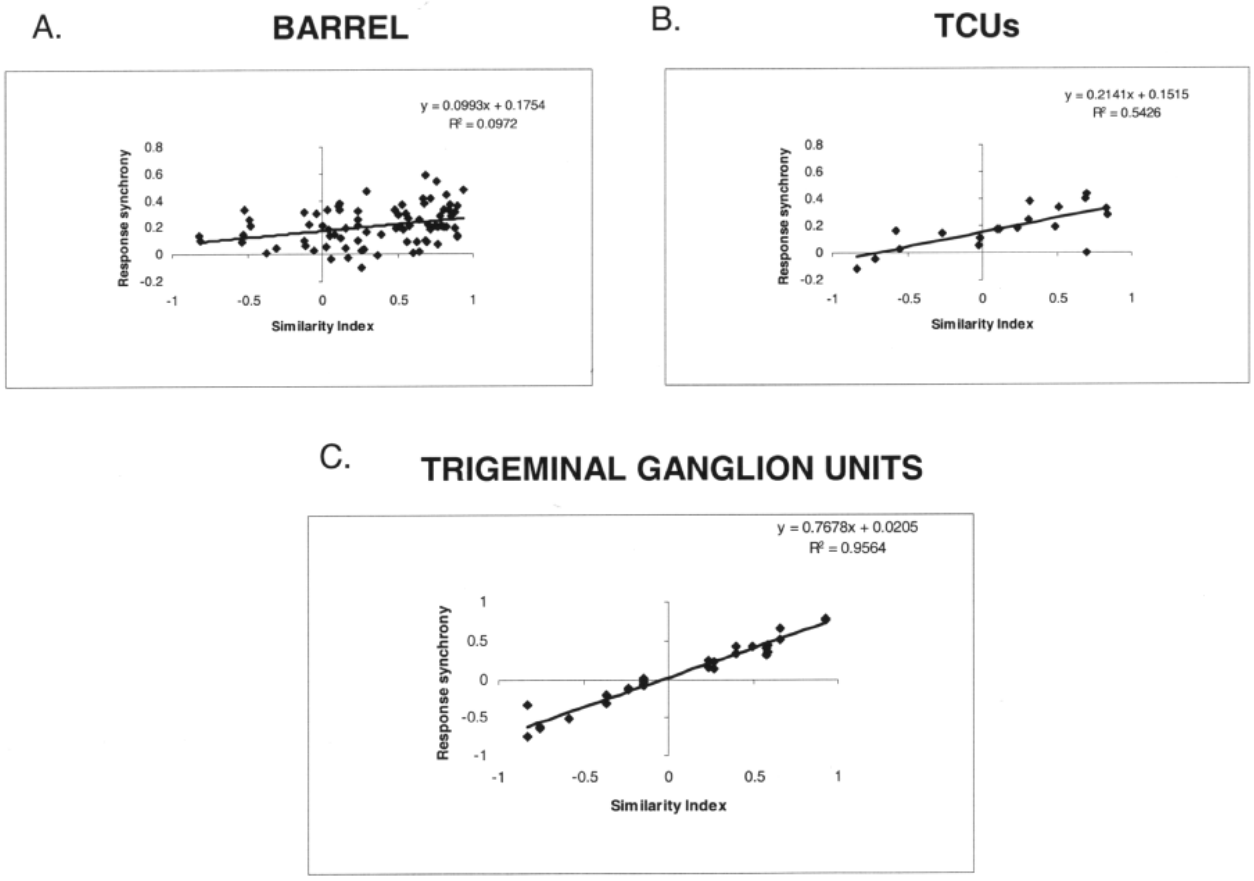


Figure 24. Response synchrony versus SI in non-adapted barrel, thalamic, and trigeminal units. Note that slopes and correlations decreases as the whisker-to-barrel pathway is ascended.

4.3.1 Response synchrony versus polar plot similarity in the non-adapted state

Next, we determined whether response synchrony was correlated with the Similarity Index (SI) for angular tuning between a pair of units. Note that response synchrony is based on individual trials while SI is a measure of trial-averaged behavior.

In Figure 24 (above), the relationship between response synchrony and SI is depicted for barrel units, thalamic units, and trigeminal ganglion units. The trigeminal ganglion units were acquired in another study and not recorded simultaneously, but illustrate what the relationship would be between response synchrony and SI if correlated firing was only due to the stimulus and not any network effects, trigeminal ganglion neurons do not interact. For trigeminal ganglion units (see Fig. 24C), the correlation between response synchrony and SI is ~ 1.0 ($r^2 = 0.96$) with a slope of 0.77; almost the full range of response synchrony values (-1 to 1) is present. Also, units with SIs ≤ 0 (e.g. unit 1 has a preferred direction of 0^o and unit 2 has a preferred direction of 90^o) display a lack of response synchrony, or in other words, their evoked responses are independent or even anti-correlated. In the barrel the correlation between response synchrony and SI is only 0.10 ($p = 0.004$) with a slope of 0.10. Units with larger SIs display more response synchrony (Fig. 24A), but unlike trigeminal units, barrel units with SIs of ≤ 0 have response synchrony values that are larger than 0. This reflects overall firing synchrony, as in Fig.23. Also barrel units with highly similar angular tuning (SIs ≥ 0.5) only display a response synchrony value of ~ 0.3 whereas the value is ~ 0.8 in trigeminal units. Thus, in the barrel, response synchrony is still stimulus-specific, but less so.

In order to determine whether the weaker relationship between response synchrony and SI originates intracortically, we examined thalamic units as well. In thalamic units, intermediate values for the correlation coefficient ($r^2 = 0.54$) and slope (0.21) were observed (Fig. 24B). As in the cortex, even cells with opposite angular tuning displayed some response synchrony and cells with highly similar angular tuning only have response synchrony values of ~ 0.3. Thus as one ascends the whisker-to-barrel pathway, response synchrony is still stimulus-specific, but progressively less.

4.3.2 Response synchrony after adaptation

The weak relationship between response synchrony and SI in thalamic and barrel neurons in the non-adapted state motivated investigation of whether adaptation could strengthen the relationship. The same ramp-and-hold stimulus was delivered to the whisker, but preceded by 2.5 cycles of a 20 Hz adapting stimulus which terminated 25 msec prior to the ramp's onset. As in the non-adapted state, post-adaptation response synchrony is still only weakly related to SI (see Fig.25 below). In pairs of barrel units (n = 41), the correlation between response synchrony and SI is 0.35 with a slope of 0.19. In thalamic units the correlation is 0.39 with a slope of 0.18.

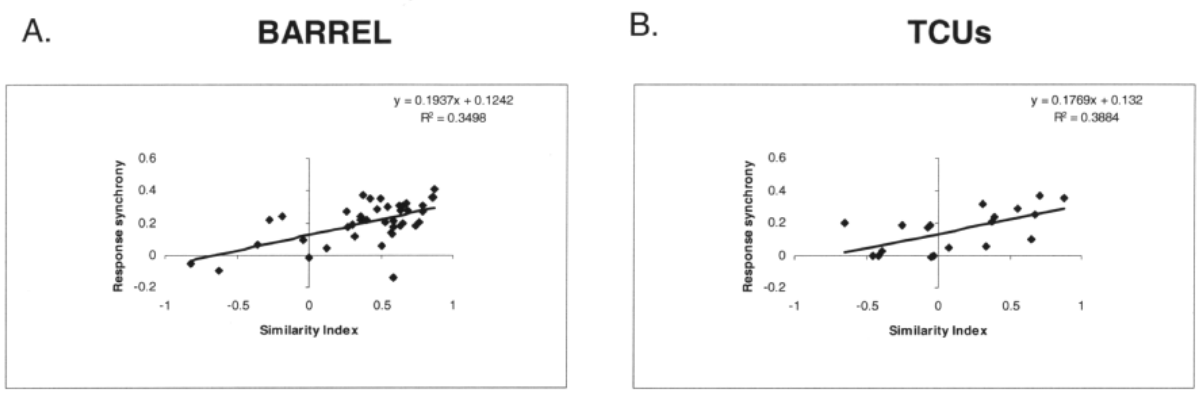


Figure 25. Response synchrony versus SI in adapted barrel and thalamic units. Units with similar trial-averaged angular tuning did display somewhat more trial-by-trial correlated firing.

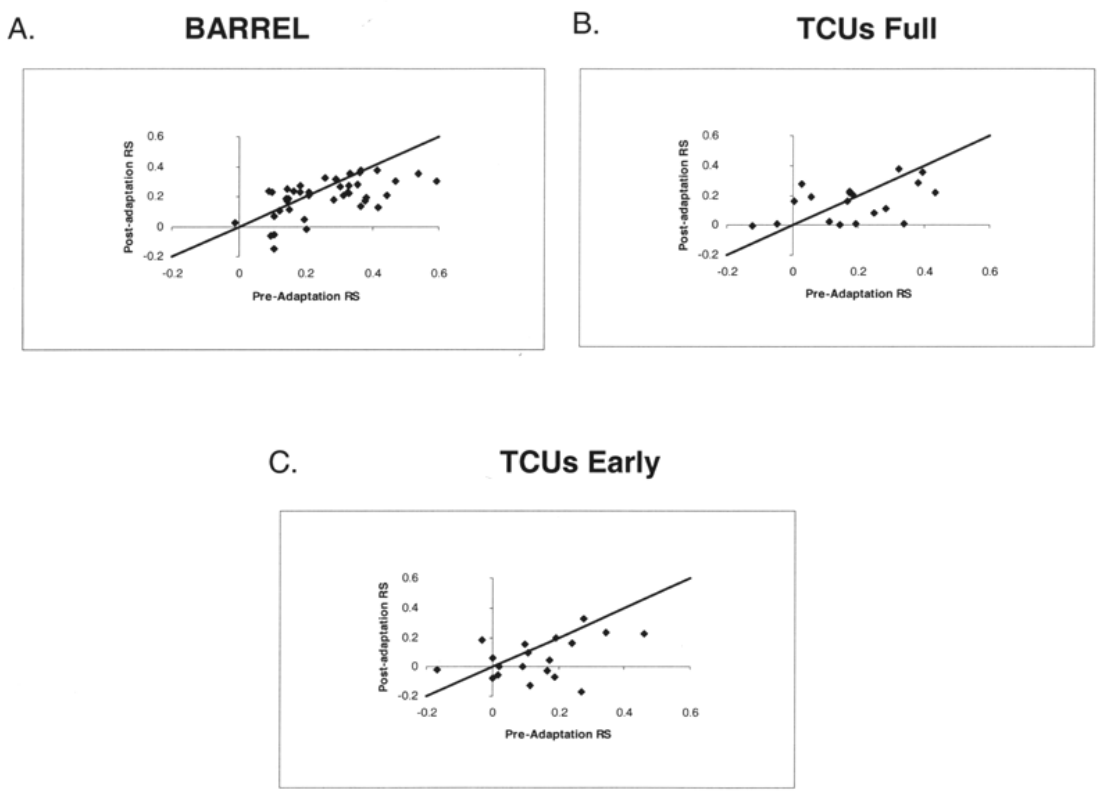


Figure 26. Post-adaptation versus pre-adaptation response synchrony. Adaptation decreased correlated firing in barrel units for their entire response, but for thalamic units, response synchrony only decreased for the early response component (first 5 msecs).

In barrel units, adaptation clearly lessens response synchrony (decorrelates responses) as can be seen in Fig. 26A above. Prior to adaptation the amount of response synchrony is 0.26 $\pm$ 0.02 (median = 0.26) and afterwards it is lessened by ~20% to 0.20 + 0.02 (median = 0.22) (Wilcoxon's signed ranks test, p = 0.002). In thalamic units (Fig. 26B), when examining the entire response, adaptation does not significantly affect the amount of response synchrony (pre vs post: 0.18 $\pm$ 0.04, median = 0.18 versus 0.15 $\pm$ 0.03, median = .16) (Wilcoxon's signed rank test, p = 0.24). The first 5 msec of thalamic responses were also analyzed since previously it has been shown that this portion of the thalamic response encodes velocity, a stimulus characteristic to which cortical neurons are preferentially sensitive (Pinto et al., 2000). If only the first 5 msec of thalamic responses are analyzed (Fig. 26C) then it can be observed that adaptation does decorrelate activity (pre vs post: 0.13 $\pm$ 0.03, median = 0.12 versus 0.06 $\pm$ 0.03, median = 0.05) (Wilcoxon's signed rank test, p = 0.04). Thus, decorrelation observed in the barrel could be in part a consequence of the reduced response synchrony of thalamic inputs.

To determine whether changes in response synchrony (delta RS) were related to changes of SI (delta SI), we correlated both measures for early thalamic activity and barrel units (Fig.27 below). Delta RS is the change in response synchrony before and after adaptation, and delta SI is the change in polar plot similarity. Importantly, SIs of pairs did not change significantly before and after adaptation. What we examined here is whether decorrelation occurs for all units equivalently or more so if the angular tuning of units remains or becomes more dissimilar. In thalamic and barrel units, delta response synchrony and delta SI were correlated (barrel: r^2 = 0.16, thalamus: r^2 = 0.53) as would be predicted. More interestingly, if delta SI is 0 (no change in tuning similarity) then response synchrony decreased significantly in both thalamic and barrel

units as evidenced by the negative y-intercepts in the linear regression (thalamus: -0.07 $\pm$ 0.03, barrel: -0.07 $\pm$ 0.02). In other words, the responses of neurons with dissimilar angular tuning are decorrelated the most.

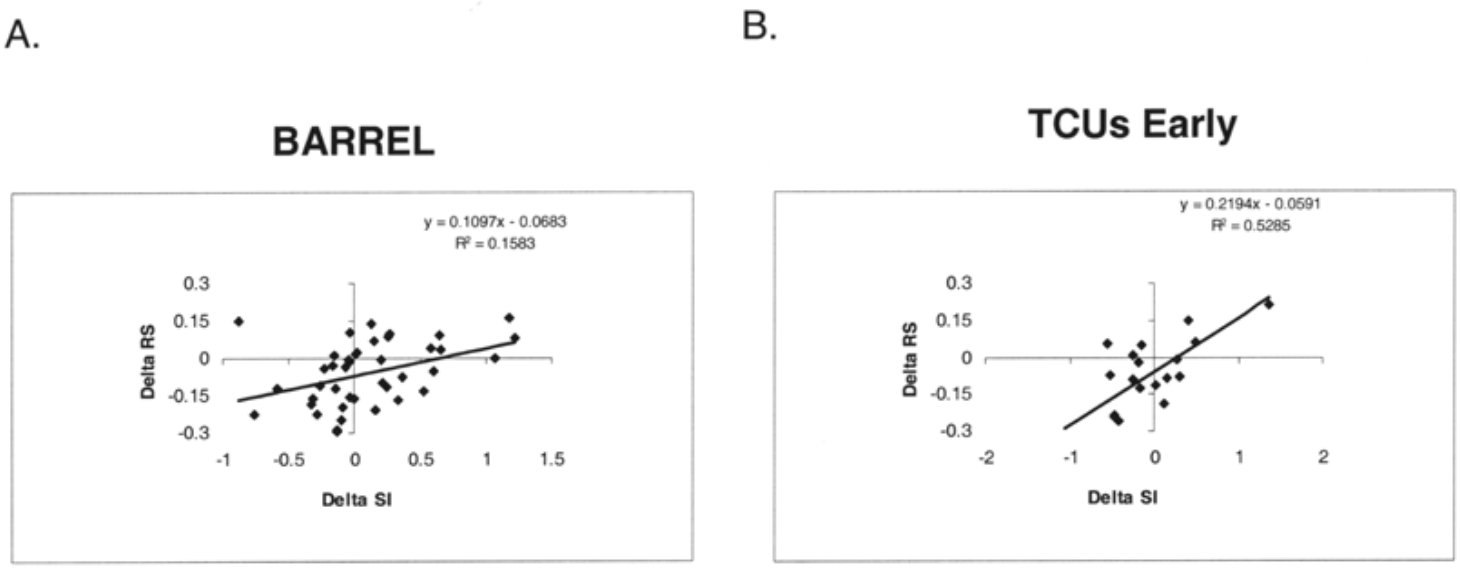


Figure 27. Changes in response synchrony versus delta SI. As expected, unit pairs which increased in SI after adaptation displayed more correlated firing, and those that decreased in SI showed less correlated firing. The interesting finding is that pairs with angular tuning relationships that did not change, displayed less response synchrony as can be seen for the negative y-intercepts of the linear regressions.

4.4 DISCUSSION

The goal of this investigation was to examine how adaptation affects response synchrony within a specific neuronal circuit, the barreloid-to-barrel pathway. Prior to investigating the effects of adaptation, non-adapted response synchrony was characterized. As one ascends the whisker-to-barrel pathway from the trigeminal ganglion to the barreloid and barrel, the amount of correlated firing in neurons becomes less dependent on the characteristics (e.g. deflection angle) of the stimulus itself. Within a barreloid and a barrel, neurons responded or failed to respond together more than would be expected by their individual response probabilities. This suggests that a 'neuronal response' is a product of the interaction between the stimulus and the background state of the thalamocortical network. Our results parallel the work of Arieli et al. (1996) in cat area 17 which showed that only 20% of a neuron's response is determined by the stimulus itself. The other 80% is due to the background state of the cortex.

The background state of the thalamocortical network is influenced by several factors. One source of the stimulus-nonspecific correlated firing could be related to spontaneous oscillations, these are present even in the visual cortex of awake, behaving primates (Leopold et al., 2003). Another influence on the stimulus-evoked responses of thalamic and cortical neurons could be whether the former is in tonic or burst mode. In our recording conditions, the thalamus is likely to be in tonic mode <60% of the time (Hartings et al., 2003). Possibly, cortical and thalamic neurons failed to respond when the thalamus was in burst mode and this could be the source of any correlated firing that is not stimulus-specific. We can rule this out since for

thalamic neurons, the similarity of angular tuning between any two thalamic neurons did not vary if they were recorded simultaneously or not. If the thalamus was in burst mode when thalamic neurons failed to respond and in tonic mode when they did respond, then polar plots should have been more similar when units were recorded simultaneously.

Unlike thalamic neurons, the angular tuning similarity of barrel pairs varied according to whether or not the neurons were recorded simultaneously. Simultaneously recorded barrel cells displayed more similar angular tuning than units that were not recorded at the same time. This finding has major implications for interpreting the importance of receptive field specificity. If an animal is able to discriminate a sensory stimulus on a single trial, then it may be utilizing neurons with much more similar tuning than recording of individual units at different times would suggest.

The weak relationship between stimulus-specificity and response synchrony of thalamic and cortical neurons could also be a consequence of the large amount of convergence/divergence as one ascends from the trigeminal ganglion where cells do not interact synaptically, that is, there is no network. Regardless of how the range of correlated firing and its stimulus-specificity are decreased, the results obtained here indicate the overall amount of response synchrony within a given neural structure must be accounted for when interpreting the functional relevance of correlated firing.

After adaptation, response synchrony among barrel and barreloid units is reduced. The greatest decrease in response synchrony occurred for unit pairs in which cells had dissimilar angular tuning. Cells with dissimilar angular tuning are likely to be within different local processing units in barreloids and barrels. Adaptation makes the local processing units act more independently of each other. A consequence of this is that the correlated firing of similarly-tuned

cells could be more effective in driving downstream neurons due to there being less overall response synchrony in the system. Both barrel neurons receiving converging thalamic inputs as well as the post-synaptic targets of barrel neurons could benefit from this effect of adaptation.

There are several possible sources for the observed response decorrelation. Possible network mechanisms include inhibition and synaptic depression. Additionally, acetylcholine may be involved. Fournier et al. (2004) has shown that after tactile stimulation, the amount of acetylcholine was found to increase in primary somatosensory cortex. Additionally, another study demonstrated that acetylcholine redistributes the response synchrony evoked by electrical stimulation in a cultured network of cortical neurons (Tateno et al., 2005).

As for Barlow's hypothesis that adaptation decorrelates the responses of cortical neurons, we find evidence for this in our recordings from the barrel. However, as Wainwright suggests response correlations are preserved. We found a preservation of response correlations among neurons that are similarly-tuned and likely to be influencing the same post-synaptic targets. Thus, rather than stating that adaptation decorrelates cortical neurons, a more accurate statement would be that adaptation redistributes correlated firing in a stimulus-specific manner.

5.0 DISCUSSION

I have used single and multi-electrode recordings to examine how local stimulus history affects the barreloid-to-barrel thalamocortical response transformation. Previous work from our laboratory has demonstrated that the non-adapted thalamocortical response transformation can be modeled as a *window of opportunity* in which strong stimuli (e.g., high velocity or preferred direction whisker deflections) generate enough thalamic firing synchrony to engage recurrent excitation before being 'damped' by slightly delayed, but strong feedforward inhibition (Pinto et al., 2002). However, this model of the thalamocortical circuit has been formulated with data obtained from responses to single, isolated whisker deflections. In order to acquire tactile information, rats repetitively sweep their whiskers across a surface. Consequently, the question is raised as to whether and how adaptation with repetitive whisker stimulation affects neuronal processing. The present experiments indicate that in the barreloid-to-barrel pathway, the history of the stimulus itself modifies this thalamocortical response transformation without fundamentally changing it. These findings should have parallels in other thalamocortical response transformations since both the barreloid-to-barrel and the lateral geniculate nucleus-to-area 17 transformation behave similarly in the non-adapted state (Miller et al., 2001).

The Effect of Adaptation on the Window of opportunity

As in the non-adapted state, after adaptation there is a strong influence of feedforward thalamic inputs upon the response properties of cortical neurons. In both the adapted and non-adapted states, FSUs are more responsive than RSUs, and they possess more broadly-tuned receptive field properties (e.g. angular tuning). Bruno and Simons (2002) have shown that FSUs receive inputs from thalamic cells with a wide range of angular preferences, and this strong convergence results in their broad angular tuning. I have observed that the same FSU-RSU dichotomy is retained after adaptation with repetitive whisker stimulation. After adaptation, relative to RSUs, FSUs are still more responsive, better able to track high-frequency whisker deflections, and have broader tuning for the angle of whisker deflections. Randy Bruno and I demonstrated that the tuning of barrel RSUs is established by their thalamic input. After adaptation, I have observed that the angular tuning preference of a barrel RSU remains the same, regardless of the angle of the adapting stimulus (see below for further discussion of angular-nonspecific suppression). The most parsimonious explanation for the maintenance of an RSU's angular tuning preference is that after adaptation, the same thalamic input imparts angular tuning to barrel RSUs.

The adapted barrel can also be modeled as having a window of opportunity in which recurrent excitation attempts to withstand strong, local inhibition. Despite FSUs still being more responsive after adaptation, both FSUs and RSUs adapt equivalently, and in both cell types repetitive stimulation results in responses becoming briefer. However, the balanced decrease in excitation and inhibition strongly suggest that adapted barrel circuitry still creates a window of opportunity. Consistent with this is that both FSUs and RSUs were better able to respond to later cycles of high-frequency whisker deflections if they were rapid. Additionally, the tuning of

RSUs can significantly sharpen after adaptation for conditions in which thalamic responses are not affected as was the case here for both the 25- and 50-msec delay conditions. Thus, even in the adapted barrel, inhibition still appears to suppress the barrel's output produced by intracortical recurrent excitation. Our findings suggest that adaptation narrows the window of opportunity, raising the threshold for the activation of barrel recurrent excitation by synchronous thalamic inputs. One of several possible mechanisms whereby the activation threshold is raised could be that adaptation produces synaptic depression between barrel excitatory neurons.

In layer 4 of cat area 17, there also appears to be a brief time duration in which thalamic synchrony can engage recurrent excitation (Miller et al., 2001) before being suppressed by strong inhibition. As in the whisker-to-barrel pathway, orientation-selectivity and the receptive field structure of simple cells are determined by thalamic inputs. There have been previous adaptation studies of area 17, but none have focused on layer 4 neurons. My current findings strongly suggest that future studies of the visual system should examine how adaptation affects computations in layer 4 of area 17. Do area 17 RSUs and FSUs adapt equivalently? Do response durations of RSUs and FSUs both become briefer after adaptation?

Dominance of Angularly-nonspecific Suppression and its Implications for Intra-barrel Interactions

As mentioned above, the tuning preference of RSUs is unaffected by the angle of the adapting stimulus. This result constrains the plausible wiring diagrams that could represent how barrel angular tuning domains interact functionally. The presence of angularly-nonspecific suppression indicates that if barrel RSUs are only connected to other similarly-tuned RSUs, then short-term depression of these synapses contributes little to the adapted responses. Angularly-

nonspecific response suppression must be mediated by angularly-nonspecific circuitry. One possibility is that response suppression is largely a consequence of broadly-tuned intracortical inhibition. Alternatively, RSUs may be interconnected in an angularly-nonspecific manner. Regardless of the rules governing intra-barrel interactions, the present results suggest that stimulus-specificity is provided by the thalamic inputs and intracortical interactions are a stimulus-nonspecific source of response modulation. The modulation of layer 4 output could be crucial for response generation in downstream superficial cortical neurons which may not be as sensitive to input timing due to the fact that in the latter, excitation and inhibition occur simultaneously (Wilent and Contreras, 2004).

Despite the fact that tuning preference is maintained regardless of the angle of the adapting stimulus, there was a small but significant amount of angularly-specific suppression in barrel RSUs and FSUs. Our cortical shocking experiments strongly suggest that the direction-specific effect either originates in thalamic neurons (see below) or from thalamocortical synaptic depression. Regardless of the source of the direction-specific suppression, it is weak relative to the nonspecific effects. Interestingly, the nonspecific suppression observed here differs from other sensory systems (vision, audition, and olfaction). In these other modalities, the suppression of neuronal responses is largely stimulus-specific. After adaptation, tuning preferences can change and originally weak responses can be facilitated. The presence of largely stimulus-specific suppression in other systems may be due to the modality-specific nature of intracortical circuitry. For example in cat primary visual cortex, intracortical inhibition appears to be very strongly tuned to the orientation of an edge (e.g., Anderson et al., 2000). In this circuit, adaptation can produce the greatest response suppression when the adapting and test orientations are the same. By contrast, in barrel cortex, putative inhibitory neurons are poorly tuned for

whisker deflection angle. Consequently, whisker deflections produce inhibition that outlasts the duration of adaptation, and test deflections are suppressed in a directionally-nonspecific manner.

The presence of stimulus-specific suppression might also be related to the modality-specific statistics of behaviorally relevant stimuli. For example in natural visual scenes, a small region of space contains edges of the same orientation and a saccade to a new fixation point will likely result in edges of a different orientation to fall within a given cell's receptive field (Dragoi et al., 2002). Under these conditions, orientation-specific suppression might be beneficial in that it would make the cortex maximally sensitive to the novel orientation. In terms of whiskers, the characteristics of natural whisker deflections are unclear, but it is known that during tactile exploration, rats sweep their whiskers back-and-forth in the rostral-caudal plane. Directionally-nonspecific suppression might be optimal here so the system can still respond to rostral and/or caudal whisker deflections that are not self-generated. Thus, the specificity of response suppression could be determined by the modality-specific demands imposed by the environment.

Implications for Intra-barreloid Processing

An unexpected result of the present studies is that adaptation can significantly affect the response properties of the thalamic units themselves. This is the case even though repetitive whisker stimulation evokes much less suppression in the thalamus than in the cortex. As in barrel RSUs, for TCUs the majority of stimulus-evoked suppression is angularly-nonspecific and weaker responses are suppressed most, thereby sharpening angular tuning. There must be underlying circuitry that mediates the angularly-nonspecific suppression. One likely possibility is inhibition provided by neurons in the reticular thalamic nucleus.

Future investigations will need to determine whether changes in the thalamus are influenced by corticothalamic (CT) feedback. A study did show that ventrobasal thalamic neurons could follow high-frequency electrical stimulation of the skin with greater fidelity if the cortex was intact (Yuan et al. 1986). Responses to low-frequency electrical stimulation were shown to be independent of CT feedback. Additionally, Temereanca and Simons (2004) have demonstrated that CT feedback sharpens the receptive field focus of thalamic neurons by enhancing principal whisker responses and suppressing adjacent whisker responses. Adaptation may modulate the amount of CT feedback that is received by thalamic neurons (see Castro-Alamancos and Calcagnatto, 2001). Nonetheless it is currently not known whether CT cells are tuned for the direction of whisker deflections, and whether CT cells exert a net excitatory or inhibitory influence on TC cells having the same or different angular tuning. Thus, it remains an open question as to what role corticothalamic feedback plays in the angularly-nonspecific suppression observed of thalamic neurons.

Adaptation Redistributes Firing Synchrony among Thalamic and Barrel Neurons

The findings discussed above were all obtained from single electrode experiments, but adaptation could have additional effects on the firing synchrony of thalamic and/or barrel neurons. Synchrony of neuronal firing is crucial for evoking responses in post-synaptic targets. Thalamic firing synchrony has been shown to be efficacious in evoking spikes from common target cortical neurons (Usrey et al., 2000). I*n vitro* results suggest that synchronous inputs from many barrel neurons are needed to produce a spike in a superficial cortical neuron (Feldmeyer et

al., 2002). Barlow proposed that the decorrelation, or the reduction in firing synchrony, of cortical neuronal responses by adaptation could remove redundant inputs. As Barlow predicted for cortical neurons, we determined that adaptation decorrelates the firing of barrel neurons (presumably mostly excitatory neurons). Alternatively to Barlow, Wainwright has suggested that correlated firing is needed due to the fact that neural systems are noisy. As Wainwright suggested, correlated firing is still present after adaptation for barrel neurons with similar angular tuning. Additionally, we found that adaptation decorrelated the early response component of pairs of thalamic neurons. This suggests that decorrelation of barrel neurons could result from changes in the thalamic inputs themselves. Regardless of the source of decorrelation, the present findings indicate that adaptation can redistribute correlated firing within a population of thalamic or cortical neurons. The overall decreases in correlated firing and remaining presence of correlated firing among similarly-tuned neurons could facilitate detection by downstream targets that are subject to less synaptic depression and local inhibition

Unresolved Issues/Future Studies

Many issues need to be examined in future studies. For example, it is open question as to how much of a contribution neuromodulators could make to the adapted responses of thalamic and cortical neurons. Repetitive sensory stimulation (e.g. whisker deflections) might lead to the exposure of thalamic and cortical neurons to neuromodulators (e.g. acetylcholine) which can produce response suppression. Fournier et al. (2004) have shown that in urethane-anesthetized rats, acetylcholine is selectively released in visual or somatosensory cortex if the corresponding modality is stimulated. Support for this hypothesis is also provided by the finding that stimulation of the reticular formation spatially focuses the receptive fields on neurons in barrel

cortex (Castro-Alamancos et al., 2002). Similarly, repetitive stimulation of the forepaw focuses receptive fields of forepaw cortical neurons (Armstrong-James and George, 1988). Thus, future investigation of sensory adapted neuronal responses must consider the role of neuromodulators.

Also unknown is how adaptation affects the sensitivity of thalamic and cortical neurons to whisker deflection velocity. The result that barrel neurons can follow high-frequency whisker deflections better if they are of high velocity suggests that adaptation affects sensitivity to velocity. But the proper experiment for examining the interaction of adaptation and velocity-sensitivity would be to systematically vary the relative velocities of adapting and test deflections as has been done for contrast-sensitivity of cat area 17 neurons (Ohzawa et al., 1985). This study has shown that the after adaptation to a specific grating contrast, the contrast thresholds of cortical neurons shift and they are maximally sensitive to changes from the adapting contrast. Perhaps, a similar thresholding mechanism is operating for velocity in rat barrel cortex.

Another important issue that has not been directly addressed is the nature of intra-barrel excitatory connections. Cellular anatomy and the dominance of angularly-nonspecific suppression suggest that in the barrel, excitatory neurons are connected regardless of the similarity of their angular tuning. Another possibility is that barrel excitatory neurons only communicate if they share the same angular preference, but that local inhibition dominates in the production of response suppression. Only paired recordings from barrel neurons might be able resolve the issue, therefore necessitating the use of dual intracellular recordings.

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