MECHANISMS AND PERCEPTUAL CONSEQUENCES OF EXPERIENCE-DEPENDENT SOMATOSENSORY PLASTICITY

by
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ABSTRACT.

Experience can alter neural responses at early stages of cortical processing. This has been demonstrated in the primary somatosensory cortex (SI), where neural responses undergo plasticity following consistent tactile training. Specifically, animals trained to detect the sequence of simultaneous tactile stimuli delivered across several digits exhibit multi-digit receptive fields (RFs) in SI, area 3b, where RFs are normally confined to a single digit. This finding indicates that neural circuits in primary sensory areas may conform to the statistical properties of stimuli used in training. However, 3b RFs in these studies were quantified using inconsistent hand held stimuli, and the function of such RFs for task performance was unknown. In this thesis we conducted a series of experiments in humans and non-human primates. We sought to understand how similar continuous sensory experience modifies neuronal properties of 3b cells and plasticity's function for tactile perception, as well as the role of attention signals in facilitating these plastic changes in sensory cortex. We characterized 3b RFs with well controlled bar stimuli on individual digits in a naïve animal and in animals trained to detect the temporal pattern of multi-digit tactile stimuli. In the trained animal, we additionally quantified responses while the animal attended to multi-digit stimuli or while its attention was directed to the visual modality. We explored the function of such plasticity for tactile perception, hypothesizing that the features of multi-digit tactile stimuli confer changes in RF properties and tactile acuity. We tested in humans if presumed RF expansion as a result of multi-digit tactile training accounts for improvements in tactile spatial acuity across fingers at the expense of single-digit spatial acuity or temporal acuity between digits. We observed that training subjects on a multi-digit task interfered with single digit spatial

acuity in an orientation and location specific manner and increased temporal acuity across the trained digits. We found that 3b RFs in the trained animal were enlarged, but feature selectivity (e.g. orientation tuning) was unchanged following training. These data suggest that stimulus properties may specify perceptual changes but not 3b plasticity following multi-digit tactile training. We describe many cells, even in a naïve animal and particularly for those with inhibited responses to tactile stimuli, with classical RFs extending over several digits. At the same time, we do not observe that 3b cells exhibit similar feature selectivity across digits, supporting the paradigm that 3b primarily represents tactile features on a single digit. We find that tactile attention modifies the firing rate of 3b cells with RFs covering both attended digits, enhancing responses following stimuli that match cells' RF location. We conclude that cognitive state can alter responses early in sensory processing. Finally, we suggest future experiments to further determine how tactile spatial attention alters 3b neural processing, and its relationship to behavior and experience-dependent plasticity.

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CHAPTER 1. GENERAL INTRODUCTION.

1.1. Adult cortical plasticity due to injury or experience.

It was once believed that cortical sensory processing, particularly in primary cortical regions, was in a fixed state when an organism reaches adulthood. One may trace the origin of this scientific paradigm to the seminal plasticity studies of Hubel and Wiesel, who observed critical periods in development for the establishment of ocular dominance columns in primary visual cortex (Hubel and Wiesel, 1970; Hubel et al., 1977). Indeed, long-term stability of cortical regions that represent fundamental sensory features (e.g. edge detectors) seems necessary to allow downstream processes to successfully integrate these features for the perception of objects or integrate multimodal sensory input. However, flexibility of these circuits given an animal's environment or experience in adulthood could allow for enhanced perception of the most behaviorally relevant sensory information at the earliest level of processing and cope with injury to the nervous system.

We now understand, through decades of studies, that adult sensory cortex does have the ability to adapt and change given the organism's experience. This was established early and most extensively in the somatosensory system, where studies demonstrated that following amputation or deafferation of a body region in the adult that silent regions of primary somatosensory cortex (SI) once connected to the injured body part could come to respond to neighboring body parts (e.g. Merzenich et al., 1983, 1984; Pons et al., 1991; Flor et al., 1995). For example, following amputation of a digit, the deafferented cortex would come to respond to the adjacent uninjured digit (Merzenich et al., 1984). It was first thought this remapping was restricted to areas of cortex 1-2mm

apart, but later it was demonstrated somatosensory remapping could occur over long distances (Pons et al., 1991); even face input could be remapped to deafferented cortex once responsive to lower body input. The mechanisms of the former are thought to arise over a shorter period of time due to enhancement and disinhibition of existing divergent input (e.g. Wall, 1977; Garraghty et al., 1991; for review see Jones, 2000); the latter occurring only after a longer period of deafferation allowing for structural changes, particularly axonal withdrawal (Florence et al., 1998; Jain et al., 2000; Graziano and Jones, 2009).

More relevant for this thesis, later studies established that altered and/or consistent behaviorally relevant inputs to a body region could expand the somatosensory representation of that region in cortex. For example, non-human primates trained to regulate the contact of several digits on a rotating disk (Jenkins et al., 1990), perform consistent dexterous movements of the digits (Xerri et al., 1996), or discriminate vibratory frequencies (Recanzone et al., 1992a) showed increased representation of the trained digit(s). This same representational enlargement has been demonstrated in primary auditory cortex: more cells come to respond to a trained auditory frequency than other non relevant frequencies (Recanzone et al., 1993). Interestingly, this phenomenon does not appear to occur in primary visual cortex (Ghose et al., 2002).

1.2. Receptive field properties in naïve and experience- altered somatosensory cortices.

The classical paradigm of somatosensory processing proposes that receptive field size and feature complexity increases gradually along the somatosensory hierarchy. We will limit discussion to processing of light innocuous cutaneous mechanosensation on the

glabrous skin due to its relevance to this thesis. Cells in the periphery have receptive fields that are punctate (Sripati et al., 2006, though see Pruszynski and Johansson, 2014), confined to the small region of skin that they innervate, and therefore produce an isomorphic representation of stimuli (Phillips et al., 1988). Classes of mechanoreceptors and their associated peripheral afferents have specific types of responses (e.g. sustained or off responses to tactile stimuli). In the glabrous skin, the slowly adapting type I Aβ afferents associated to Merkel complexes and the rapidly adapting type I Aβ afferents associated with Meissner corpuscles have been the most well described. Slowly adapting type I afferents have sustained responses to indented tactile stimuli; rapidly adapting type I afferents exhibit transient responses at the onset and offset of stimuli. We are beginning to understand the molecular properties of receptors (e.g. the presence of the mechanically activated cation channel, Piezo2 in Merkel cells) that confer their physiology as a result of tactile input (see Abraira and Ginty, 2013 and Woo et al., 2015 for reviews). Slowly adapting type I afferents, with their small receptive fields and greater innervation density than other afferent types, produce a spatial image across the peripheral population that accounts for the limits of tactile spatial acuity and roughness perception (Johnson and Phillips, 1981; Phillips and Johnson, 1981; Connor et al., 1990; Connor and Johnson, 1992), while rapidly adapting type I afferents can detect very small asperities related to slip movements on the skin (Johnson and Hsiao, 1992). We acknowledge these are likely simplifications of the full perceptual function of these afferents (Saal and Bensmaia, 2014).

Information from peripheral afferents is carried through the dorsal column medial leminiscal pathway to dorsal column nuclei in the medulla. Cells then project to the

ventrobasal complex of the thalamus, where the body representation is concentrated in the ventroposterior lateral (VPL) nucleus. Somatotopy and modality is maintained, with cutaneous input segregated to the core region of VPL (Jones and Friedman, 1982), but otherwise receptive field properties within these subcortical regions have been greatly understudied. VPL cells project strongly to Brodmann area 3b of the primary somatosensory cortex (SI) (Jones and Burton, 1976) where cells show orientation tuning to stimuli (DiCarlo and Johnson, 2000; Hsiao et al., 2002). Lesions to 3b leave an animal unable to perform most somatosensory discrimination tasks (Randolph and Semmes, 1974). Therefore, this area is thought of as the first cortical region processing innocuous touch and often compared to primary visual cortex as the first step in somatosensory feature detection (though see Pruszynski and Johansson, 2014). The work of Sur and colleagues established that 3b classical receptive fields were most often (>90% of the time) confined to a single finger pad (Sur, 1980; Sur et al., 1980; Iwamura et al., 1983). Later work using random dot stimuli on a single finger pad revealed 3b spatiotemporal RFs and found most cells have inhibitory and excitatory subfields on the finger pad which could account for the orientation tuning properties of these cells (DiCarlo et al., 1998; DiCarlo and Johnson, 2000). Neurons in upper layers of 3b, where orientation selectivity is higher (DiCarlo and Johnson, 2000), project to other areas of SI, including areas 3a, 1 and 2. These have excitatory classical receptive fields that cover multiple digits and respond to mechanosensation as well as proprioceptive input (though see Kim et al., 2015; cell responses in 3b can be influenced by hand conformation). Response properties become more complex higher up the somatosensory hierarchy, including in secondary somatosensory cortex (SII), with cells exhibiting RFs covering the entire hand

or both hands (Fitzgerald et al., 2004). Tuning to more complex tactile features, like curvature (Yau et al., 2013) or motion (Pei et al., 2011) has been demonstrated in these higher-order regions.

Therefore, it was seminal when further work from Merzenich and others established that temporally coincident input to multiple digits could result in excitatory 3b RFs that covered several digits. These studies proposed that the temporal features of tactile stimuli could alter a key feature of somatosensory processing. In one case (Clark et al., 1988; Allard et al., 1991), digits were surgically sutured together (syndactyly), naturally causing the animal to use two digits in concert, and in others (Wang et al., 1995; Blake et al., 2002, 2005), the animal was required to discriminate the temporal pattern of multi-digit input and/or consistently grip a hand holder. RF expansion with coincident input is observed in other species' somatosensory cortex: temporal pairing of input after plucking all but two whiskers also expands RFs in rat barrel cortex (Diamond et al., 1994) and syndactyly of raccoon digits also expands SI RFs (Zarzecki et al., 1993). It had been demonstrated, prior to these studies, that consistent vibratory training on one digit could increase RF size within a digit (Recanzone et al., 1992a), and that temporally inconsistent input could contract SI RFs (Jenkins et al., 1990). It should be noted that the detection of the temporal window between nonconincident input across digits can also expand 3b RFs (Blake et al., 2005). Whether other features of temporally coincident input specificity somatosensory cortical plasticity and the function or mechanism of such experience-dependent plasticity, remained unexplored.

1.3. Attention in the somatosensory system and cognitive states related to experience-dependent plasticity.

After training animals to perform a one-back multi-digit temporal pattern detection task, which only actually amounted to very small periods of synchronous input to the trained digits, the authors observed that multi-digit expansion was present in the cortex but not in the thalamus (Wang et al., 1995). They concluded that it was likely that "active network processes…hypothetically underlying stimulus differentiation, recognition and categorization", and not just synchrony of afferent input, were responsible for remapping. Indeed, there is accumulated evidence that cognitive state can alter responses of cells in somatosensory cortices during a task (e.g. Hsiao et al., 1993), and that tactile stimuli must have behavioral relevance to produce lasting cortical plasticity (Recanzone et al., 1992b; Blake et al., 2006). For example, animals that performed a vibratory frequency discrimination task had expansion of the relevant digit, while animals that received the same tactile input passively while attending to the auditory modality or failed to learn the task did not show this expansion (Recanzone et al., 1992a).

It has been demonstrated that tactile attention can alter responses in somatosensory cortices, both in the overall firing rate of cells and in their precise temporal firing patterns (Hsiao et al., 1993; Steinmetz et al., 2000; Meftah et al., 2002, 2009; Chapman and Meftah, 2005; Roy et al., 2007). As firing rate corresponds to stimulus intensity, the temporal correlation patterns of cell populations is a mechanism by which attentional state can be differentiated from stimulus characteristics (Niebur and Koch, 1994). We have demonstrated that feature-based attention can increase the firing

rate and synchrony of cell pairs with feature selectivity matching the attended tactile feature (Gomez-Ramirez et al., 2014). Previous data suggested that this mechanism of feature-based attention may not be present in SI cortex; texture sensitive cells are specifically enhanced during a texture discrimination task in SII but not in SI cortex (Chapman and Meftah, 2005). Tactile attention has increasing effects along the somatosensory hierarchy (Hyvärinen et al., 1980; Hsiao et al., 1993; Burton and Sinclair, 2000a; Meftah et al., 2002), enhancing or suppressing the responses of more cells in SII compared to SI cortex. Such results have been replicated on the macro level in human subjects using functional imaging (for a review see Burton and Sinclair, 2000). How tactile attention alters primary cortical responses in a multi-digit one-back task like that described to lead to expansion of 3b RFs had not been explored.

1.4. Mechanisms of plasticity.

Many cellular mechanisms that drive plasticity within synapses have been described, though the link between these changes at the cellular level and their relationship with experience-dependent plasticity at the system level have not been fully determined (for review see Buonomano and Merzenich, 1998). We will proceed to review and connect evidence between these fields of study. We hypothesize, as others have (Ahissar et al., 1992), that the combination of firing patterns of cortical cells and the presence of neurotransmitters signaling behavioral relevance, arousal, and reward are necessary for the expression of spike timing dependent plasticity and long lasting experience dependent plasticity in sensory cortices. For example, receptor (e.g. NMDA) activation and intracellular processes (e.g. phosphorylation) necessary for the expression

and maintenance of long term potentiation and depression in synapses are necessary for map plasticity in barrel cortex following whisker trimming (reviews see Feldman and Brecht, 2005; Feldman, 2009). In addition, the release of key neurotransmitters throughout the cortex (e.g. acetylcholine and dopamine), which accompany arousal, selective attention, and reward, are necessary to observe experience-dependent sensory cortex plasticity. This has been most well-defined in the auditory cortex, where nucleus basalis and ventral tegmental activity can alter the representational expansion of a behaviorally relevant tone (e.g. Kilgard, 1998; Bao et al., 2001). In addition, acetylcholine is necessary for barrel and visual cortex experience-dependent plasticity (Ego-Stengel et al., 2001; Chubykin et al., 2013). Neuromodulators alter rules of long term potentiation and depression (Seol et al., 2007), potentially allowing specific patterns of firing to modify synapses in sensory cortices. We also note such neuromodulators may be more important during the acquisition of a behavior and during the plasticity process than for its maintenance (Chubykin et al., 2013). Interestingly, while we described in the previous section that selective attention can change the temporal firing pattern of cells in sensory cortices, and though it is known that synchronous firing patterns can alter synaptic strength *in vitro*, this has not been established as a mechanism that can lead to long term experience-dependent plasticity.

1.5. Functional significance of cortical experience dependent plasticity for perception.

A key question is: what is the function of these described forms of sensory cortex plasticity? That is, do they confer changes in perception for the organism? If so, what changes in neural responses are most related to altered sensory abilities and at what point

are they functional during the learning and plasticity process? Below we review evidence summarizing similarities and differences in perceptual consequences of cortical plasticity following injury and perceptual training. One study suggested that expansion of adjacent body regions into deafferented somatosensory cortex does not lead to increased perception of the uninjured region (Vega-Bermudez and Johnson, 2002); such expansion may instead increase the probability of aberrant perceptions of the missing body part and phantom limb pain (Flor et al., 1995; Grüsser et al., 2001).

Additionally, it is unclear if expansion of cortical areas encoding behaviorally relevant stimuli is truly related to enhanced perception. For example, the degree of representational enlargement of a digit in 3b does not correlate with animal performance on the trained vibratory task (Recanzone et al., 1992a). Instead, the temporal precision of responses to a tactile vibratory stimulus was a better predictor of animal performance (Recanzone et al., 1992b). On the other hand, representational shifts in digit representation as measured by fMRI following passive vibratory stimuli predict improvements in tactile spatial acuity and decrements in vibratory discrimination (Hodzic et al., 2004). More recent data suggests that sensory map expansions may be specifically necessary as an animal learns a task but not for maintenance of a high level of performance (Blake et al., 2005; Reed et al., 2011).

Like plasticity following injury, temporally coincident input across digits can also lead to maladaptive plasticity. Animals who performed a grip task over many months showed symptoms mirroring focal (hand) dystonia, including difficulty performing skilled hand motor tasks (Byl et al., 1996). In addition, individuals with focal dystonia, who often have experienced years of altered and coincident digit use, are more likely to

exhibit overlap of digit representations in the somatosensory cortex (Bara-Jimenez et al., 1998), have decreased tactile acuity (Bara-Jimenez et al., 2000a), and symptom relief corresponds with increased separation of digit representations (Candia et al., 2003). However, it is unknown whether expansion of RFs in 3b helps an animal detect multi-digit input or if short-term training with multi-digit input leads to changes in tactile perception.

1.6. Scope of dissertation.

This thesis focuses and expands upon Wang and colleagues' 1995 finding that training an animal to detect the temporal sequence (one-back task) of multi-digit horizontal bar input leads to expansion of 3b RFs across multiple digits. We sought to examine, in human subjects, if such multi-digit training alters related tactile abilities, such as spatial or temporal acuity (Chapter 2). We quantified area 3b RF properties by recording from single units in naïve non-human primates and those trained on a simplified version of this multi-digit sequence task (Chapter 4). In both these experiments, we asked if features of the multi-digit stimuli the subject was trained with, for example, its location and orientation, conferred changes in perception and/or RF properties. By measuring responses of cells in 3b to oriented bar stimuli presented to multiple digits, we report previously uncharacterized aspects of 3b processing (Chapter 5), including the presence of cells with inhibited responses to tactile stimuli and the absence of similar feature selectivity across digits. Finally, we describe how 3b responses are altered by the attentional state of the animal. We test if cells with expanded RFs are targeted by tactile attention and if tactile attention alters the temporal correlation of such cells (Chapter 6).

CHAPTER 2. FUNCTIONAL CONSEQUENCES OF EXPERIENCE-DEPENDENT PLASTICITY ON TACTILE PERCEPTION.

Continuous training or exposure to a stimulus has been shown to enhance perceptual discrimination (Fahle, 2005; Seitz and Watanabe, 2005; Gilbert et al., 2009). This effect, termed perceptual learning, has been extensively investigated in the visual system. Many studies have shown that individuals demonstrate, over time, enhanced perceptual abilities in tasks requiring discrimination of sensory features such as orientation, motion, and luminance/contrast (Fahle et al., 1995; Goldstone, 1998; Seitz and Watanabe, 2005; Gilbert et al., 2009; Sasaki et al., 2010). Cortical changes that accompany and are thought to underlie such visual perceptual improvement have been termed experience dependent plasticity and have occasionally been observed in visual cortices (Crist et al., 2001; Schoups et al., 2001; Li et al., 2004; Shuler and Bear, 2006). Experience-dependent plasticity following perceptual learning has been observed in other sensory modalities and motor systems (Allard et al., 1991; Recanzone et al., 1992a; Wang et al., 1995; Dahmen and King, 2007; Reis et al., 2009; Reed et al., 2011). For instance, repetitive tactile stimulation on the hand can lead to representational changes in primary somatosensory cortex (SI) of non-human primates. In one study, animals were trained to detect consecutive identical presentations of tactile bar stimuli that spanned digits 2 (D2), 3 (D3) and 4 (D4). After an intensive training regime, the authors observed an expansion of the canonical excitatory receptive fields (RFs) of area 3b cells, from single to multiple

digits (Wang et al., 1995). It was surmised that continuous and synchronized stimulation of neighboring digits promoted synaptic integration of coincident inputs, thus causing RF enlargement of 3b cells (Clark et al., 1988; Allard et al., 1991; Wang et al., 1995). Similar findings have been observed in the auditory modality, where regular exposure to a particular frequency tone increases the representation of that frequency in auditory cortex (Recanzone et al., 1993; Rutkowski and Weinberger, 2005). These are significant findings because they indicate that cortical synapses and cells' RF structures are plastic and can conform to the statistical properties of an organism's environment.

Yet, the behavioral implications of these neural anatomical changes, particularly in the somatosensory system, remain poorly understood. Previous studies have proposed that tactile spatial acuity improves as human participants passively experience vibratory stimuli (Godde et al., 2000; Hodzic et al., 2004; Kalisch et al., 2007) but several of these studies used two point discrimination, an inaccurate measurement of tactile spatial acuity (Johnson and Phillips, 1981; Craig and Johnson, 2000; Tong et al., 2013) and successful replication of these results has been mixed (Gibson et al., 2009). It has also been observed that passive vibratory exposure can interfere with frequency discrimination while increasing cortical representation of the stimulated body region (Hodzic et al., 2004), but this study failed to assay whether changes were specific to features of the stimulus used during training (i.e. does passive exposure to a specific frequency lead to decreased discrimination of that frequency).

A significant outcome stemming from these types of anatomical reorganizations is the alteration of perceptual functions that rely on the cell populations undergoing plastic changes. For instance, in the somatosensory system, one would expect that related

abilities that utilize perception across multiple digits improve with training on tasks that require perception across digits and have been demonstrated to foster multi-digit RF expansion. Thus, in this study we asked whether spatial acuity across multiple digits, using stimuli spanning several digits, would be enhanced following training subjects to detect the temporal sequence of multi-digit oriented bar stimuli. A second prediction about altered perception following perceptual learning, based on neurophysiological principles, is that this topographical reorganization would lead to a reduction in the number of cells with single-digit RFs. We hypothesized this would cause a loss of function in tasks that require discrimination of stimuli within a single-digit or comparisons between single digits. Therefore, we examined whether discriminating the temporal sequence of multi-digit stimuli would come at the expense of spatial acuity at the single-digit level and temporal discrimination between the trained digits. Finally, we investigated whether perceptual learning effects in the somatosensory system are feature-specific, such that changes in spatial and temporal acuity modulate based on the sensory feature(s) (e.g. the orientation of stimuli) experienced during training.

Following our original prediction, we observed that participants' single-digit spatial acuity decreased as subjects trained with multi-digit stimuli and this effect was selective for the stimulus orientation experienced in the training regime. In contrast to our original hypothesis, the data revealed that temporal acuity across the trained digits improved during training, and this effect was not orientation feature specific. These findings suggest that experience-dependent somatosensory plasticity can be utilized across various tactile tasks and can interfere with related tactile abilities.

2.1. Methods and Data Analysis

2.1.1. Participants.

Thirteen healthy human subjects participated in the experiments. Three participants were excluded because TOBT task parameters were not altered during the first day of training to ensure performance was at 60% at the beginning of training, as was done for all other participants (see "TOBT task" for details). The remaining participants were seven females, nine right-hand dominant, and between 18-30 years old. The median age was 20. Five additional participants were recruited to participate in control protocols (two males, all right-hand dominant), ages 18-31, median age 20. All testing procedures were performed in compliance with the policies and procedures of the Institutional Review Board for Human Use of the Johns Hopkins University. Participants were given a small monetary compensation at the end of each day.

2.1.2. Sequence of events

On the first day, we measured participants' baseline temporal and spatial acuity. Participants then returned the following day and trained for nine days over a two week period on a tactile one-back task (TOBT) (Wang et al., 1995). This training took a half hour a day. This two-week time period was chosen as it was demonstrated to produce changes in somatosensory responses as measured by MEG in humans trained on the same TOBT(Spengler et al., 1997). At the end of each training day we measured participants' spatial and temporal acuity functions in separate sessions (Figure 2.1). This testing took another half hour a day. Finally, at least a month after the last day of training, we retested participants spatial and temporal acuities to assay long-term perceptual learning effects in the somatosensory modality.

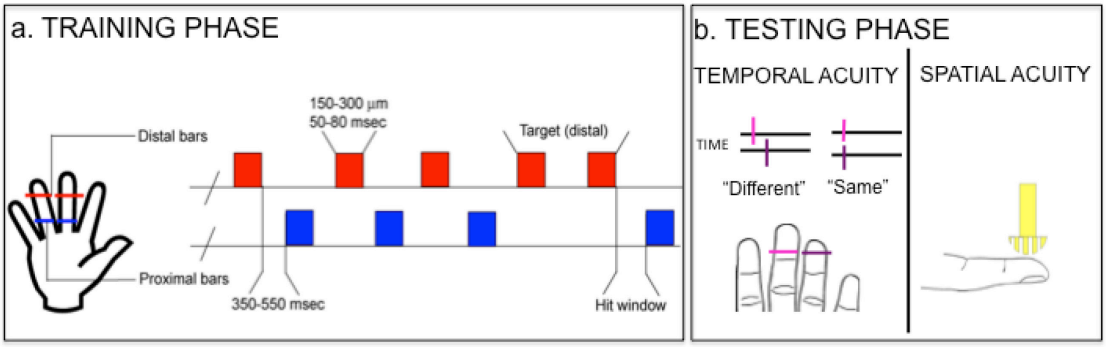


Figure 2.1. Experimental methods. **(A)** Tactile one-back training (TOBT). Participants experienced two horizontal bars that spanned the right middle and ring fingers (D3 and D4). One bar was located distally (near the fingertip) and the other proximally (closer to the palm of the hand). Participants were asked to indicate with a button press when they felt a bar consecutively indent at the same location. They were given feedback if they correctly responded in the designated hit window. Stimulus parameters are indicated, and were adjusted on the first day of training to ensure participants were performing around 60% correct. **(B)** Acuity testing phase. Participants' intra and inter-digit spatial acuity (right panel) and inter-digit temporal acuity (left panel) was tested prior to any training on both hands (baseline acuity). Each day, following TOBT, we tested one hand's temporal and spatial acuity, alternating the hand tested.

2.1.3. Tactile One Back Task (TOBT)

Participants sat comfortably in a quiet room with their right hand supinated in a customized holder device to prevent hand and finger movements. A black curtain blocked the view of the hand and tactile stimulator. On every trial a horizontal bar stimulus that spanned the distal or proximal finger pads of D3 and D4 was indented on participants' right hand to simultaneously contact the two digits. A trial sequence would begin and indentation of the bars alternated between distal and proximal pads. After a random number of stimulus indentations (between three and eight), two consecutive stimuli (target stimulus) were presented at the same location (Figure 2.1A). The target stimuli were not different from any others in any other parameters. Participants were instructed to press a response button in their left hand as quickly as possible in the event of a target stimulus and withhold a response to all other stimuli. Feedback response, in the form of an auditory tone, was provided after every correct response only. After any overt response (correct or incorrect), the tactile stimuli would pause for 1 second to ensure that participants could process subsequent stimuli. White noise was continuously presented to mask auditory cues from the motors. Subjects performed three sets of 80 sequences (~500 stimuli) with five minute breaks. The TOBT task was performed for a half hour each day, which comprised approximately ~1500 stimuli (240 sequences/ target stimuli).

On the first day of training the inter-stimulus interval (ISI), indentation level, and stimulus duration were adjusted during breaks to ensure that participants' baseline performance was approximately 60% (correct responses on about 48/80 sequences). This was done to ensure that task difficulty was equated across individual participants, and to enhance the likelihood of eliciting robust perceptual learning effects. The ISI across all

participants ranged between 350 and 550 msec (steps of 50msec). In particular, seven participants had an ISI between 350-450 msec, one participant had an ISI between 450-550 msec, and two participants had an ISI between 400-500 msec. The stimulus indentation across all participants ranged between 125 and 300 μm. Specifically, the indentation level of the stimulus was 300 μm in four participants, 200 μm in four participants, 150 μm in one participant, and 125 μm in the remaining participant. The stimulus duration was 80 msec in seven participants and 50 msec in the remaining participants. After the first day the experimental parameters were kept constant.

A small portion (5%) of consecutive indentations was presented only on one finger to enhance the likelihood that participants attended to both fingers. Participants were instructed to ignore these trials and only respond to consecutive indentations spanning both fingers. If they responded to consecutive indentations on a single-digit the experimenter provided feedback indicating an incorrect response. No participants responded during these 'catch' trials after the first day of training.

Subjects' d-prime each day was calculated by taking the difference between the z scores of the participant's hit rate and the false alarm rate. Hit rate was defined as the number of correct responses/ number of targets and false alarm rate was defined as the number of times a subject incorrectly responded when a target wasn't present / number of stimuli that were not a target (i.e. all the stimuli alternating between distal and proximal pads).

2.1.4. Tactile spatial acuity

We tested participants' spatial acuity using the well-established grating orientation

task (GOT) (Johnson and Phillips, 1981; Van Boven and Johnson, 1994; Craig and Johnson, 2000). Participants experienced a subset of eight square-wave gratings cut into rounded plastic domes (Altem plastic) with equal ridge and gap widths (0.35, 0.5,0.75, 1, 1.25,1.5, 2, and 3mm, based on design by Stoelting Co., Wood Dale, IL). A dome was indented 2mm into the finger using a linear motor for 1500 msec (see Chapter 2.1.7). The domes were consistently placed 14mm from the end of the fingertip, thus stimulating the upper half of the distal finger pad. Gratings were presented "vertically" (along the long axis of the finger, mediolateral) or "horizontally" (orthogonal, across the finger). Participants were instructed to judge the orientation of each stimulus and reported, with a mouse click using the untested hand, which of the two orientations they felt.

The orientations were chosen randomly on every trial and each grating size was presented thirty times across randomized blocks. On the first day we tested participants on four grating sizes with widths ranging from 1.25 to 3mm. After that point, we used three grating widths that encompassed the participant's previous day's thresholds (four sizes if necessary). This was done to reduce the experimental time. Individual gratings were presented to participants' right hand D3 and D4 (trained) and left hand D2 and D3 (untrained). Our goal was to contrast changes in spatial acuity on homologous and nonhomologous digits across both hands. However, we observed that participants' baseline threshold on the right hand D4 and left hand D2 was significantly different as compared to other fingers (Post-hoc contrasts by Scheffe's, $F(2,18) > 7.1$, $p < 0.05$). This is in line with previous studies that have demonstrated decreasing acuity from index to ring fingers (Sathian and Zangaladze, 1996; Grant et al., 2006; Wong et al., 2011). Therefore, to assay changes in single-digit acuity without the addition of ceiling or floor

effects, we only analyzed data on D3 between both hands. We measured multi-digit acuity in a protocol where two domes of equal grating width and orientation were indented at the same time on two fingers. We instructed participants to judge whether the grooves were horizontal or vertical across both fingers. We did not tell participants that the orientation was the same on both fingers, but asked that they attend to both fingers, in an attempt to create a task that require perception of stimuli across multiple digits. We synchronously indented stimuli on D3 and D4 of the right hand (trained digits) and D2 and D3 of the left hand (untrained digits).

The first day of testing only served to measure participants' baseline acuity. This was done for both hands. From that point, we alternated which hand spatial acuity was measured, beginning with the right hand (i.e. the trained hand). This was done to reduce testing time. We determined participants' spatial acuity threshold as the grating width that elicited correct responses on 75% of trials. Thresholds were estimated for each orientation condition by measuring acuity in trials with horizontal and vertical stimuli separately. We acknowledge that it will be unknown if changes in horizontal and vertical acuity (due to the task being two-alternative forced choice) are truly due to changes in perception or bias.

2.1.5. Tactile temporal discrimination

We measured participants' temporal discrimination threshold (TDT) using two 19mm oriented bars on two adjacent distal fingertips (300 μm indentation, 200 msec duration). The stimulator was the same as the one used in the TOBT task. Bars were either oriented horizontally across the fingertips (congruent), or one bar was indented

vertically while the other was indented horizontally (incongruent). We quantified TDT on D3 and D4 of the right hand (trained digits) and D2 and D3 of the left hand (untrained digits). Participants indicated whether bars were indented at the same or different times (Lacruz et al., 1991). We chose this test, as opposed to a temporal order judgment task such as (Craig and Xu, 1990), given that previous studies had demonstrated changes in TDT using a similar paradigm in participants with focal dystonia (Tinazzi et al., 1999; Bara-Jimenez et al., 2000b; Sanger et al., 2001; Scontrini et al., 2009; Conte et al., 2014). We postulated that similar neural changes in SI RFs and therefore perceptual alterations may occur as a result of TOBT as seen in animal models of focal dystonia (Byl et al., 1996).

The stimulus onset asynchrony (SOA) between the two bars ranged between 0 and 100 msec in steps of 5 msec. We randomized which bar was indented first. Each SOA and orientation condition was presented six times, and this order was randomized. We estimated participants' TDT by determining the stimulus asynchrony that elicited 'same' responses on 50% of trials. TDT for both hands was calculated on the first day (baseline), and then we alternated which hand was tested, beginning with the right hand.

2.1.6. Recovery tests

Participants were asked to return approximately one month after the last day of the TOBT task to reexamine their spatial acuity and TDT thresholds (between 30 and 60 days post training). The goal was to assay long-term perceptual learning effects of the TOBT task.

2.1.7. Mechanical stimulators.

The stimulator used in TOBT and the temporal discrimination task consisted of four custom build linear motors (similar to those used in Killebrew et al., 2007) that have a nominal displacement of 2.9 mm. The four motors were positioned over the hand using four articulated tool holders (Noga Engineering Ltd. Shlomi 22832, Israel) mounted to 4, 2-axis, micro-positioners (Newport Corp., California). Each motor was centered on the to-be stimulated finger pad using magnetic bases. The bars were 19mm in length (Altem plastic), which spanned the entire width of the finger pad, and the short axis of the bar (8mm) was wedge-shaped to produce a smooth edge sensation. Motors were controlled using a National Instruments data acquisition board system (PCI-6229; National Instruments Corp., Austin, TX) and custom software. Motors moved with an on and off linear ramp duration of 20 msec.

The stimulator used in the GOT task consisted of a linear stage (Parker MX80L Miniature Stage; Parker Hannifin Corp, Rohnert Park, CA), mounted vertically and controlled with serial commands via a serial port interface and custom software. Two ARSAPE rotating stepper motors (AM 1020 series, Faulhaber Corp) were attached to the linear stage. The grating domes (Altem plastic, design by Stoelting Co., Wood Dale, IL) were magnetically attached to the stepper motors with custom-designed holders, which allowed for a rapid replacement of stimuli between trial blocks.

2.1.8. Statistics.

We used repeated-measures ANOVA to test for effects, and all results were corrected for sphericity using the Greenhouse-Geisser method. All post-hoc contrasts were corrected for multiple comparisons (Scheffe's method). Non-parametric statistics

were used to test for effects between the small number of control participants who only engaged in the GOT and temporal discrimination tasks and the other subjects.

2.2. Results.

2.2.1 Performance of the one-back task

Ten participants performed the TOBT for nine days over a thirteen-day period. As expected, participants showed systematic increases in d' (D-prime) and corresponding decreases in reaction time (RT) throughout the training period (Figure 2.2). This was confirmed using a one-way repeated measures Analysis of Variance (ANOVA) on d' with Day as the repeated factor ($F(8, 72) = 8.82$, $p < 0.001$, $\eta_G^2 = 0.303$). We observed that d' rose from 2.10 to 3.38 across the training period. This effect was captured by a linearly increasing polynomial contrast ($F(8, 72) = 30.84$, $p < 0.001$, $\eta_G^2 = 0.372$). A separate ANOVA on RT also revealed a significant effect of Day ($F(8, 72) = 10.88$, $p = 0.001$, $\eta_G^2 = 0.201$), whereby RT decreased from 447 to 378 msec throughout training (linear polynomial contrast, $F(8,72) = 44.84$, $p < 0.001$, $\eta_G^2 = 0.199$).

We further quantified the day at which performance on the TOBT task stabilized during training. We performed a series of ANOVAs where the earliest day of training was systematically removed from each test. For example, the first ANOVA was conducted using the full set of training days (9 days), while the second ANOVA was performed with the first day of training removed (8 days). This strategy was continued until the ANOVAs failed to show a significant effect. We found that ANOVAs on d' and RT failed to show significant differences from the fifth day onward (d' ANOVA, $F(4, 36) = 1.61$, $p = 0.215$; RT ANOVA, $F(4, 36) = 2.82$, $p = 0.097$), indicating that training effects on the TOBT began to plateau around the fifth day.

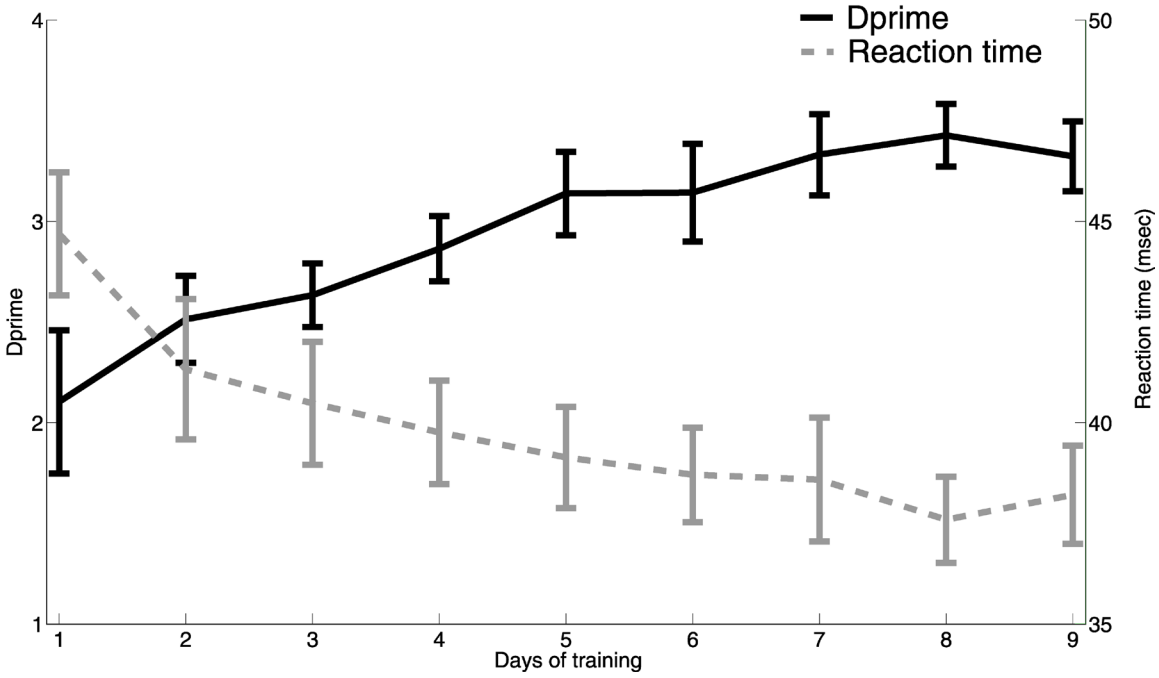


Figure 2.2. Participants' performance during multi-digit tactile one back training (TOBT). Participants' (N=10) d prime (black line, Z(hit rate)-Z(false alarm rate)) is indicated on the left y-axis, and reaction time for correct trials (grey dashed line) on the right y-axis. All error bars are standard error of the mean (SEM), calculated by removing between-participant variability considering this is a within-participant design (see Cousineau, 2005 for a description).

2.2.2. Training-specific changes in spatial acuity

After each TOBT session, we tested participants' spatial acuity using the GOT (Johnson and Phillips, 1981). Figure 2.3 shows changes in threshold across time in single-digit (Figure 2.3A) and multi-digit (Figure 2.3B) for both horizontal (left panels Figures 2.3) and vertical (right panels Figures 2.3) orientation conditions. Note that threshold is the inverse of acuity. Thus, lower threshold values reflect better acuity. Further, the trained hand was tested five times, but only the first four sessions were used in statistical tests to ensure a balanced design. In all graphs, threshold changes are relative to baseline (i.e. baseline acuity measured a day prior to the first session of TOBT task). We ran a four way 2 x 2 x 2 x 4 within-subject ANOVA with factors of Orientation (Horizontal vs. Vertical), Number of Digits (Single vs. Multi-digit), Hand (Trained vs. Untrained), and Training Session (One to Four). We found a main effect of Training Session ($F(3, 27) = 10.429$, $p < 0.001$, $\eta_G^2 = 0.034$), which was captured by a linear decreasing function as assessed by polynomial contrasts ($F(3, 27) = 19.524$, $p = 0.002$, $\eta_G^2 = 0.032$). This is in line with previous studies, which show decreasing thresholds with continuous exposure to the GOT (Johnson and Phillips, 1981; Wong et al., 2013). We also found a significant Orientation x Training Session interaction effect ($F(3, 27) = 7.045$, $p=0.002$, $\eta_G^2 = 0.010$), with vertical threshold decreasing at a faster rate. This was captured by a linear trend (polynomial contrasts, $F(3, 27) = 14.284$, $p=0.004$, $\eta_G^2 = 0.007$). The ANOVA also revealed a significant interaction between Number of Digits and Training Session ($F(3, 27) = 3.611$, $p=0.039$, $\eta_G^2 = 0.010$). This was captured by a quadratic function (polynomial contrasts, $F(3, 27) = 11.757$, $p = 0.008$, $\eta_G^2 = 0.010$), with single-digit threshold increasing on the second testing session, then decreasing across

sessions, and multi-digit threshold decreasing on the first three sessions, then increasing. Finally, we found a significant four-way interaction between Orientation, Number of Digits, Hand, and Training Session ($F(3,27) = 3.72$, $p = 0.042$, $\eta_G^2 = 0.008$). No other significant effects were observed.

This complex four-way interaction was described in two ways. First, we describe the interaction by using a subtraction method. We first subtracted changes in threshold on untrained hand from the trained hand (Figure 2.4). We believe that there were minimal transfer effects in spatial acuity across hands, as we compared the untrained hands' spatial acuity with a separate, small group of control subjects which never experienced the TOBT, but whose acuity was tracked over many sessions. These data are shown in Figure 2.5. Independent samples Mann-Whitney U-tests revealed no significant differences between these two groups for each session and spatial acuity condition ($p > 0.05$ for each session, Figure 1.5).

Figure 2.4 demonstrates subjects' data when the untrained hand was subtracted from the trained hand (Figure 2.4a), and when the vertical threshold was subtracted from the horizontal threshold (Figure 2.4b). This captures the overall trend that the trained (relative to the untrained hand) hand's single digit horizontal threshold (relative to vertical acuity) increased, while the trained hand's multi-digit horizontal threshold decreased across sessions (Figure 2.4b).

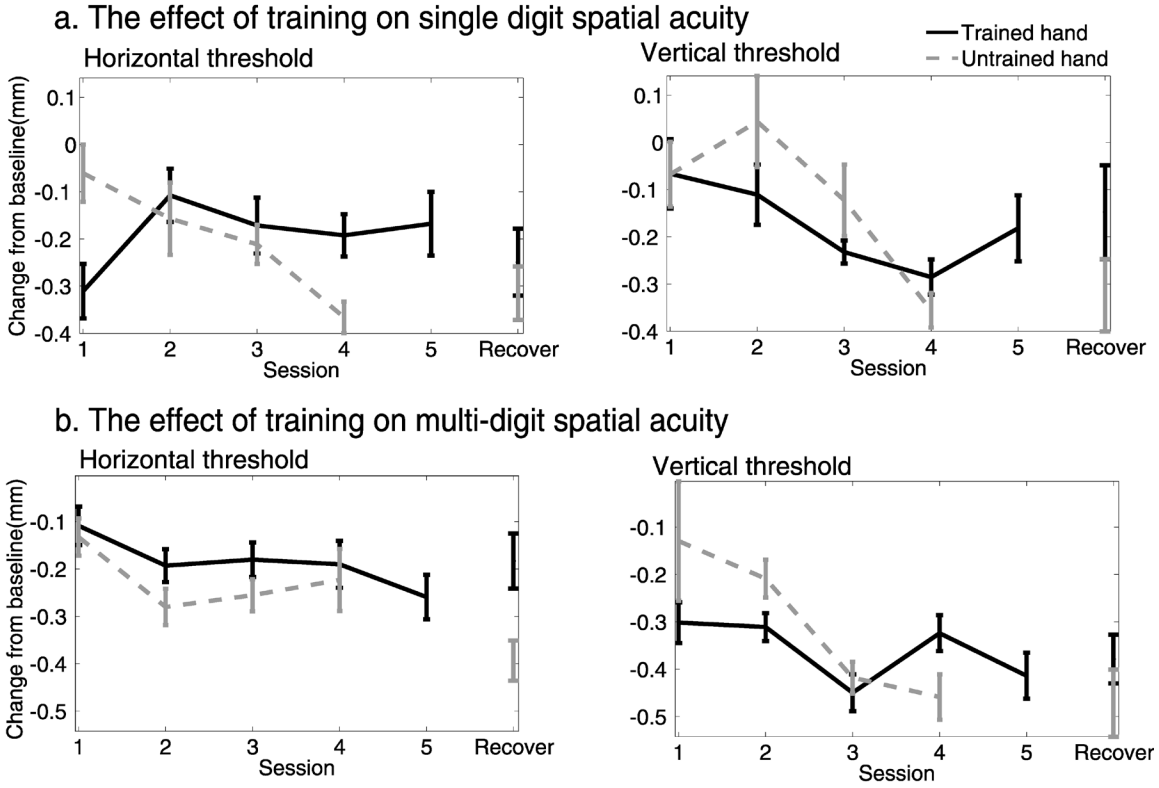


Figure 2.3. The effect of training on spatial acuity. (A) Changes in D3 single-digit grating orientation threshold (relative to baseline) on the trained (black) and untrained (grey dashed) hands. Note that session 1 is day 1 of TOBT training for the trained hand and day 2 for the untrained hand, session 2 is day 3 for the trained hand and day 4 for the untrained hand. The untrained hand was tested five times but only the first four sessions were used in the ANOVA. Left: changes in threshold on horizontal-only trials, Right: changes on vertical-only trials. The trained hand was tested over five sessions, however for statistical purposes (to ensure a balanced design) only the first four sessions were analyzed. Threshold was re-tested one month after session of TOBT (recovery). Error bars are +/- within-participant S.E.M. **(B)** Changes in multi-digit threshold on the trained digits (black, right D3/D4) and untrained hand and digits (grey dashed, left D2/D3). Left: threshold on horizontal-only trials. Right: Threshold on vertical only trials. Error bars are +/- within-participant S.E.M.

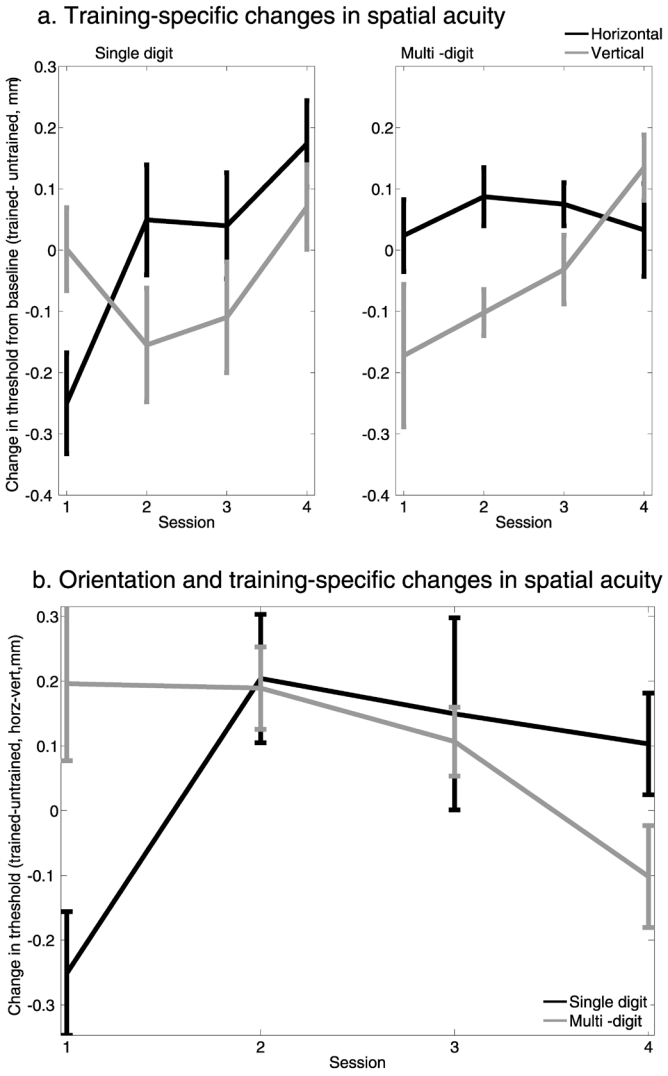


Figure 2.4 Training and orientation specific changes in spatial acuity. (A) Participants' acuity during the first four testing sessions when threshold on the untrained hand was subtracted from the trained hand. Left panel- horizontal threshold, right panel- vertical threshold. Error bars are +/- within-participant S.E.M. (B) Participants intra (black) and inter (grey) acuity across the first four testing sessions when thresholds from the untrained hand was subtracted from the trained hand, and vertical thresholds were subtracted from horizontal thresholds. Error bars are +/- within-participant S.E.M.

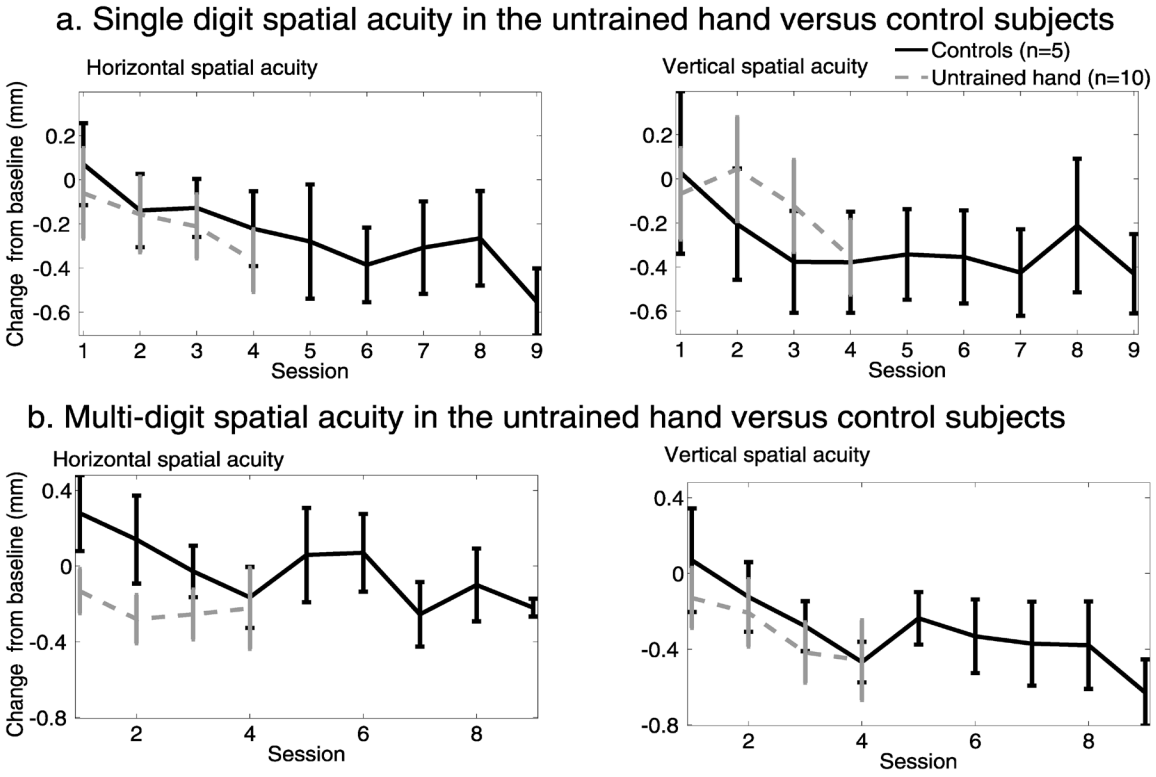


Figure 2.5. Spatial acuity changes on the untrained hand and in control participants. (A) Changes in D3 single-digit grating orientation threshold (relative to baseline) on the untrained hands of participants who experienced TOBT (N=10, grey dashed) and control participants who did not experience TOBT (N=5, black). Left: changes in threshold on horizontal-only trials, Right: threshold on vertical-only trials. The untrained hand was tested over only four sessions and we tested control participants on the GOT over nine days. Error bars are +/- between-group S.E.M. **(B)** Changes in multi-digit threshold on the untrained hand and digits (left D2/D3, N=10 grey dashed) and on the same hand/digits for control participants (N=5, black). Left: threshold on horizontal-only trials. Right: Threshold on vertical only trials. Error bars are +/- between-group S.E.M.

However, the subtraction method will not elucidate hand and/or orientation specificity of such changes, so we additionally performed linear trend analysis based on specific hypotheses (polynomial contrast analysis on a subset of the data). Our working hypothesis was that training enhances multi-digit acuity over time, at the expense of single-digit acuity, in an orientation and location specific manner. This was partially confirmed by our data. We failed to find a specific decrease in multi-digit horizontal threshold on the trained hand (Figure 2.3B, left panel, black). However, we observed that horizontal single-digit threshold on the untrained hand (Figure 2.3A, left panel, grey dashed) decreased across session, whereas the same measure increased in a quadratic manner on the trained hand (Figure 2.3A, left panel, black). We examined the significance of this specific effect using linear contrast analyses. We assigned orthogonal and equally spaced weights to each data point. Based on Figure 2.3A (left), we assigned a linearly decreasing function over four sessions to the untrained hand, weights [3 1 -1 -3], and a quadratic trend, increasing then decreasing to the trained hand, weights [-1 1 1 -1]. This combination of trends was significant ($F(3,27)=19.02$, $p<0.05$, $\eta_G^2 = 0.016$, corrected for sphericity and post-hoc statistical significance assessed using Scheffe's method) indicating *specifically* that horizontal single digit acuity on the trained hand increased quadratically while the untrained hand's acuity on this exact same measure decreased. Again we contend that the untrained hand represents typical perceptual learning of the GOT (Figure 2.5), as previous authors had shown that regular exposure to the GOT decreases thresholds (Johnson and Phillips, 1981; Wong et al., 2013).

We investigated the persistence of these training effects by retesting participants' spatial and temporal acuity over a month after their last day of the TOBT task. We

performed a 2 x 2 x 2 x 2 ANOVA with factors of Orientation (Horizontal vs. Vertical), Number of Digits (Single vs. Multi-digit), Hand (Trained vs. Untrained), and Training Session (Last TOBT Session vs. Recovery Session). The ANOVA revealed a significant four-way interaction ($F(1,9) = 7.306$, $p = 0.024$, $\eta_G^2 = 0.003$). There was not a significant change at the horizontal, single-digit data (i.e. the trained hand's single-digit horizontal threshold did not significantly change, nor did the untrained hand's from the last day of testing to recovery). We instead believe the significant interaction indicated in the omnibus test was driven by changes in horizontal multi-digit acuity (Figure 2.3B, left panel) during the recovery period, whereby the trained hand's threshold increased between the recovery period and the untrained hand's threshold decreased after a month without training. This specific post-hoc contrast was significant ($F(1,9) = 8.52$, $p<0.05$ corrected via Scheffe's, $\eta_G^2 = 0.006$).

2.2.3. Training-specific changes in temporal discrimination.

We next examined how participants' TDT changed across multiple digits during TOBT (Figure 2.6). We reasoned that the TOBT task would impair temporal discrimination of stimuli between the trained digits due to the continuous and synchronous stimulation experienced during the training period. We predicated this form of stimulation would promote integration across digits thus making tasks that required comparisons between digits more difficult.

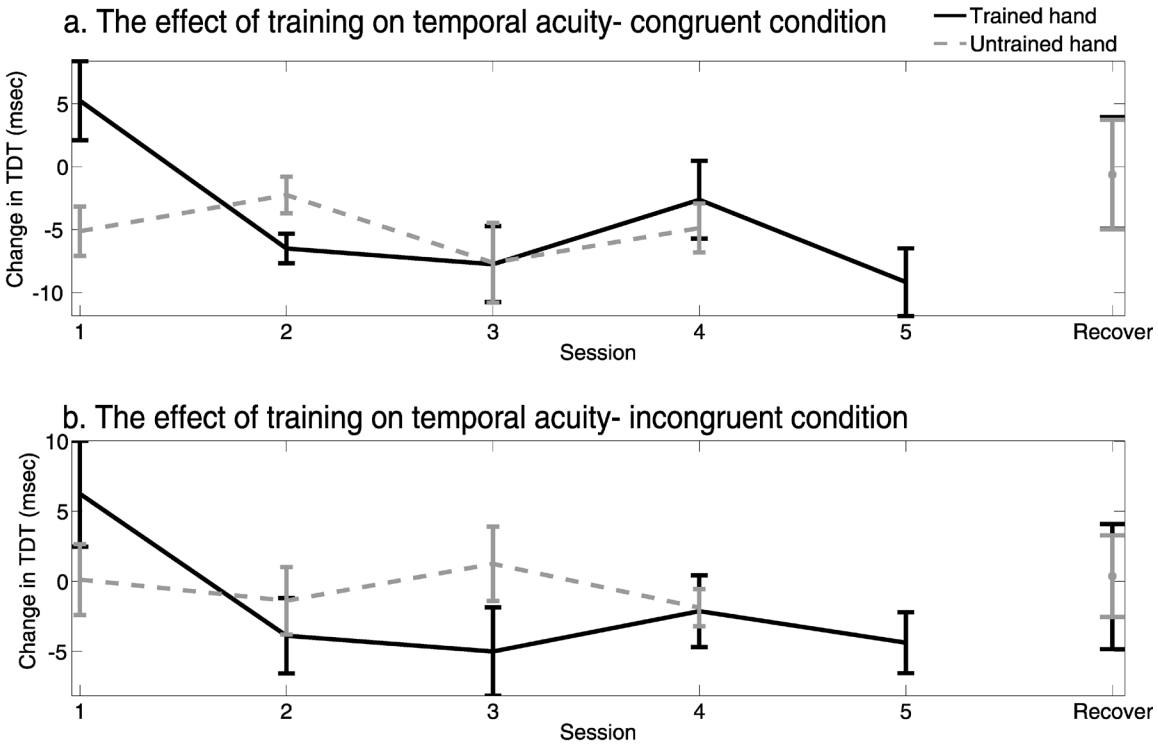


Figure 2.6. The effect of training on temporal acuity. (A) Participants' change in temporal discrimination threshold (TDT) relative to baseline, across the trained digits (right D3/D4) and the untrained hand/digits (left D2/D3). Bars were oriented congruently horizontally (as in TOBT) across digits. Error bars are +/- within-participant S.E.M. **(B)** Participants' change in TDT relative to baseline on the trained and untrained hands. In this protocol, one bar was oriented vertically (medio-laterally) across the finger. Error bars are +/- within-participant S.E.M.

A three-way 2 x 2 x 4 ANOVA with factors of Stimulus Congruency (Congruent vs. Incongruent), Hand (Trained vs. Untrained), and Session was computed to test for significance effects. The ANOVA revealed a main effect of Session ($F(3, 27) = 5.809$, $p = 0.011$, $\eta_G^2 = 0.038$) driven by decreasing thresholds throughout training (i.e. linear polynomial contrast; $F(3,27) = 20.152$, $p = 0.002$, $\eta_G^2 = 0.018$). In addition, we found a significant Session x Hand interaction ($F(3,27) = 3.82$, $p = 0.042$, $\eta_G^2 = 0.034$). Polynomial contrasts revealed that this interaction was best explained by a quadratic function ($F(3,27) = 9.576$, $p = 0.013$, $\eta_G^2 = 0.025$), with TDT on the trained hand decreasing between the first and third sessions and then increasing on the fourth testing session. We again asked if the untrained hand represented typical changes in TDT over time by comparing these data with the separate control group (Figure 2.7). We found that the untrained hand in the congruent condition (Figure 2.7, grey dashed) was significantly lower than the control group in the third testing session (Independent samples Mann-Whitney U test, $p=0.04$). However, the untrained hand and control subjects were not significantly different on any other sessions (Independent samples Mann-Whitney U test, $p>0.05$ for each session). Therefore, while TOBT training may have transferred and enhanced temporal acuity slightly on the untrained hand, overall, the improvement in temporal acuity was specific to the trained hand.

We measured intra-digit temporal acuity a month after training on the TOBT task. We computed a 2 x 2 x 2 ANOVA with factors Stimulus Congruency (Congruent vs. Incongruent), Hand (Trained vs. Untrained), and Session (Last TOBT Session vs. Recovery Session). The ANOVA failed to reveal any significant effects, suggesting no changes in temporal discrimination abilities after cession of training.

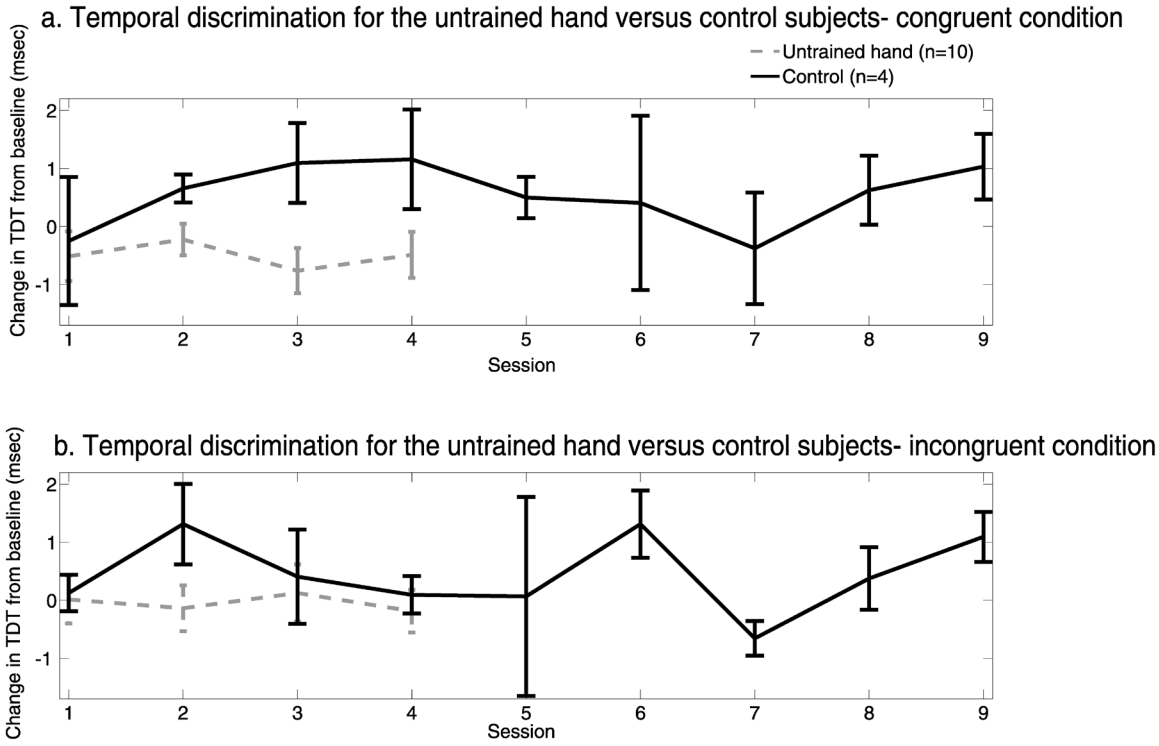


Figure 2.7. Temporal acuity changes on the untrained hand and in control participants. (A) Participants' change in temporal discrimination threshold (TDT) relative to baseline, across the untrained hand/digits (left D2/D3, grey dashed) versus control participants (black). Bars were oriented congruently horizontally (as in TOBT) across digits. Error bars are +/- between-groups S.E.M. **(B)** Participants' change in temporal discrimination threshold (TDT) relative to baseline, across the untrained hand/digits (left D2/D3, grey dashed) versus control participants (black). In this protocol, one bar was oriented vertically (medio-laterally) across the finger. Error bars are +/- between-groups S.E.M.

2.3. Discussion.

We assessed whether training on a task that promotes multi-digit RF expansion would enhance spatial acuity of stimuli spanning multiple digits. In addition, we tested whether enhancements in multi-digit acuity would come at the expense of perceptual discrimination at the single-digit level or between digits. Finally, we investigated whether perceptual learning effects in the somatosensory system are feature-specific.

As participants learned the TOBT, we found location and orientation- specific changes in temporal and spatial acuity. We contend that features of the TOBT predict these acuity changes, in that it used temporal sequence discrimination of horizontal, multi-digit stimuli. We found that participants' temporal acuity across multiple digits improved during training and that participants' horizontal single-digit spatial acuity, relative to acuity on the untrained hand, decreased during the training period. Therefore, these data suggests that experience-dependent plasticity as a result of a tactile regime can both enhance and impede tactile perception in a predicable manner.

2.3.1. Features of TOBT predict changes in spatial acuity

We found that our participants' performance on the TOBT task improved throughout the training period, and that they performed proficiently by the end of training. This improvement plateaued after five days of training (Figure 2). In addition, we observed that spatial acuity changes due to training were specific to the number of digits and stimulus orientation experienced during training. In support of our hypothesis, we observed that single-digit spatial acuity on the trained hand decreased (threshold increased) in a quadratic manner, and this was specific to the orientation of the stimulus

used during training (horizontal). We contend that these modulations in spatial acuity were specific to the hand trained on the TOBT task, as the spatial acuity in participants' untrained hand closely mirrored a separate group of participants who were not trained on the TOBT task, but performed the GOT across a similar timeframe.

2.3.2. Mechanisms explaining changes in spatial acuity due to experience-dependent plasticity

In the field of visual perceptual learning, feature (e.g. orientation) and location (e.g. retinal location or eye tested) specificity has often been cited as evidence for plasticity changes in primary sensory cortex (Fiorentini and Berardi, 1980; Ball and Sekuler, 1982; Karni and Sagi, 1991; Crist et al., 1997; Fahle, 1997, 2004). Studies have observed changes in neural responses in primary visual cortex (V1) as a result of visual experience (e.g. non classical RF modulation with the presence of contextual stimuli, orientation specificity, sustained responses to stimuli which predict reward at a temporal interval) (Crist et al., 2001; Schoups et al., 2001; Li et al., 2004; Shuler and Bear, 2006). However, few, if any, have observed changes in V1 RF properties like size or orientation preference (Crist et al., 2001; Ghose et al., 2002). On the other hand, SI cortical map plasticity and alterations in SI RF properties have been described following learning on a tactile task or a consistent tactile stimulation regime (Jenkins et al., 1990; Recanzone et al., 1992a, 1992b; Blake et al., 2002), but the consequences on perception have not been described. Our study provides significant insight onto the functional implications of RF expansion promoted by multi-digit task training (Wang et al., 1995).

Cells with an excitatory RF confined to one finger are likely to be anatomically

connected to those with input from adjacent fingers (via horizontal or divergent ascending input). Indeed, several studies in area 3b have shown modulatory effects on the classical single-digit RF by stimulation of adjacent digits (Friedman et al., 2008; Reed et al., 2010; Thakur et al., 2012). These anatomical connections between digits in area 3b could be modified by continuous engagement on the TOBT task. Interference in single-digit spatial acuity is possible if TOBT expands horizontally tuned cells' single-digit RFs into excitatory multi-digit RFs in 3b, potentially making horizontal judgments less accurate on a single-digit. We should note that the trained hand's single-digit horizontal spatial acuity did not become worse than baseline spatial acuity; decrements in acuity are only relative to the untrained hand. One can therefore think of TOBT as interfering with perceptual learning in spatial acuity. We also acknowledge that this training could shift participants' bias during the GOT, thereby making them less likely to indicate the horizontal choice on the trained digit. Nonetheless, to the best of our knowledge, we believe this may be the first description of perceptual learning interfering with performance on a different, but related, task in a feature and location specific manner in the somatosensory system. Additionally, our data supports the hypothesis that conscious processing of stimuli features is not necessary to cause perceptual changes (Watanabe et al., 2001; Seitz and Watanabe, 2003, 2009; Seitz et al., 2009), particularly if suppression of sensory stimuli is unnecessary or reinforcement is correlated with a particular stimulus feature (Sasaki et al., 2010). That is, even though performance on the TOBT task was not reliant on judging the orientation of the stimulus, the effect on spatial acuity was orientation-specific.

We found that training on the TOBT task did not improve multi-digit spatial acuity,

particularly for the horizontal orientation, at least as tested with the multi-digit GOT (Figure 2B). One possibility to explain this result is that participants employed different strategies to perform the multi-digit spatial acuity task. For example, as grating stimuli were always oriented in the same manner across adjacent digits, participants may have attended to only one digit to perform the task.

2.3.3. Short-term enhancements in temporal acuity

We found that participants' TDT across trained fingers followed a quadratic trend, decreasing in the first three sessions and then returned to a similar level as the untrained hand, a finding that runs counter to our hypothesis. Indeed, we had originally predicted that the formation of multi-digit representations would make comparisons between fingers more difficult. However, this hypothesis assumed temporal discrimination would rely on a comparison between cell populations conveying the timing of stimuli on a single-digit, and that expansion of RFs would impair such judgments. This need not be the case, as computational studies have demonstrated large RFs could easily convey information about timing of stimuli (Foffani et al., 2008). We found that enhanced temporal acuity was not specific to the orientation of the trained stimulus, as TDT decreased even when participants compared the timing between incongruent oriented bars. This result indicates that temporal and spatial tactile abilities are affected differentially by the TOBT task, and points to separate cortical mechanisms underlying temporal and spatial acuity.

We propose that the quadratic trends seen in both spatial and temporal acuity measures (i.e. increasing horizontal single digit acuity and decreasing temporal acuity

over the first three sessions) may be explained by the sharp increase in performance on the TOBT in the first five days of training (corresponding to the first three testing sessions on the trained hand). This corresponds with data indicating primary sensory experience-dependent plasticity is most prominent during learning as opposed to maintenance of performance (Reed et al., 2011). We did not observe a clear and reliable daily correspondence between each subject's TOBT performance and spatial and temporal acuity, perhaps due to a variable temporal relationship between these measures.

2.3.4. Recovery and clinical implications

While we did not see tactile single-digit horizontal spatial acuity significantly change from the last testing session after a month without training or return to baseline, we did observe shifts that would suggest somatosensory cortex renormalizes after a long period without training (Figure 2A, left panel). This is in contrast to perceptual effects of passive tactile co-activation, which are extinguished after 24 hours (Godde et al., 2000; Hodzic et al., 2004). In our case, the renormalization process seems to take substantially longer than training time. Certainly, better understanding of this process would be of great importance to clinical populations such as patients with focal dystonia for whom experience-dependent plasticity is maladaptive and related to pronounced motor and sensory deficits associated with the disorder. Investigators have implicated somatosensory cortex RF expansion in the development of focal hand dystonia (Byl et al., 1997; Blake et al., 2002), as patients have often experienced a greater degree of multi-digit stimulation throughout the lifespan, thus increasing likelihood of multi-digit representations in cortex. It has been suggested this RF expansion is related to patients'

abnormal tactile spatial acuity (Bara-Jimenez et al., 2000a), and undoing these multi-digit representations may be related to symptom relief (Candia et al., 2003). It is clear that those interested in learning and rehabilitation will need to better understand how training on one task might transfer to other tasks. For example, it has recently been suggested that popular online "brain training" tasks have little bearing on other cognitive skills (Owen et al., 2010) . Our results suggest that one should consider perceptual learning interference as well as enhancement as a result of continuous training on sensory tasks.

CHAPTER 3. GENERAL NEUROPHYSIOLOGY METHODS.

This chapter concerns the neurophysiological techniques employed for the data described in Chapters 4-6. Animal training and surgery, stimulus apparatus and control, data collection, and general principles of data analysis are summarized. Specific data collection and analysis principles are found within the appropriate chapter. Details about the human psychophysics techniques employed in Chapter 2 are found within that chapter.

3.1. Animals, training, and surgery

370 single unit (SU) responses were recorded from the hand regions of primary somatosensory cortex (SI), area 3b from four hemispheres in two male rhesus (*Macaca mulatta*) monkeys. We have only included well isolated 3b cells tested on all relevant conditions with a significant response (see below for description) on at least one distal pad. Both animals were initially trained to perform a visual discrimination task, used during receptive field mapping procedures. Each monkey was then trained on a tactile distal one-back task (see below).

The first animal (MR4358M, average weight 9.7 kg) was trained on a tactile distal one-back task on his left hand and we recorded in the right hemisphere contralateral to the trained hand (referred to as a "trained hemisphere"; 82 cells). We then recorded in the left hemisphere, contralateral to the untrained right hand; this will be referred to as the "control hemisphere" (103 cells). In the second animal (43V, average weight 8.1 kg), we first recorded from the left hemisphere prior to any tactile training and mapped receptive

field properties on the right hand. This will be referred to as the "naïve hemisphere" (91 cells). We then trained the animal on the distal one-back task on his left hand, and recorded in the right "trained hemisphere" (94 cells), contralateral to the trained hand.

All procedures that might have produced pain or distress were minimized. We used operant training procedures and placed animals on water restriction while in their home cage. The animals received ample water consumption in the lab environment. Animals were first accustomed to being brought into the lab, sitting quietly in a primate chair for several hours, and having their hands restrained in arm and hand holders for an hour at a time. This training took approximately one to two months. To train the animals on tasks that required eye fixation and later perform neurophysiology, surgery was performed under anesthesia to implant head restraining posts and recording chambers. Posts were stainless steel and secured to the skull with titanium bone screws (Howmedica Osteonics Corp. Mawah, NJ) and bone cement (Henry Schein, Melville, NY). Recording chambers (stainless steel, 19 mm diameter) were placed on both hemispheres to target the hand region of SI cortex, centered over the Horsley-Clarke coordinates anterior = 6, lateral = 22. All surgical and experimental procedures were approved by the Animal Care and Use Committee of the Johns Hopkins University and conformed to National Institutes of Health and U.S. Department of Agriculture guidelines.

3.2. Experimental paradigm and visual discrimination task

The animal was seated in a comfortable chair with the head restrained. The animal's tested hand was supinated and both hands and arms comfortably restrained in custom-made holders. The tested hand's fingers were placed comfortably in the hand holder and

D2- D5 fingers were secured with cloth tape around the medial pads and nails secured with a small amount of fixative to a nail holder. We used a visual discrimination task to consistently control the animal's attentional state during receptive field mapping and to test the effect of tactile attention in the multi-digit distal one back task (see Chapter 6 for description of these attention protocols).

The visual task began with presentation of a blue square with size of 2.04º. If the animal successfully maintained fixation for 400 msec, two white circles appeared on the left and right of the visual cue (each 2.04º in diameter). These had different luminance levels and the animal was required to make a saccade to the brighter circle. The two visual circles were presented for a maximum of 2000 msec. The inter-trial-interval was 2300 msec. The discrimination difficulty was adapted using an ongoing staircase method based on the animal's performance. The difficulty increased (i.e. the luminance difference decreased, using a logarithmic scale) following three successive correct trials, and decreased after each error. The animal was rewarded with a drop of juice or water after every correct response. All visual stimuli were presented on a Samsung SyncMaster 740b 17" LCD monitor, on a black background with a 60 Hz refresh rate. Eye position was monitored with a PC-60 ViewPoint EyeTracker (Arrington Research - Scottsdale, AZ). It took both animals approximately one month to learn this task.

3.3. Tactile distal one-back task and stimulator

Prior to recording from the trained hemispheres, we trained both animals to perform a distal one-back task on their left hand (note that laterality of hand preference in macaques

has not been established, e.g. Chatagny et al., 2013). An overview of this task can be seen on Figure 3.1.

The rules of this task were based on Wang et al., 1995 , with several modifications, discussed here and throughout the text. The animal sat with its left hand supinated and fingers fixed and both hands fixed in a custom hand and arm holder. This is in contrast to (Wang et al., 1995), which had owl monkeys voluntarily grasp a stimulator, their hands unrestrained (Recanzone et al., 1991). Training owl monkeys to consistently grip the stimulator in a stereotyped manner such that all digits equally contacted the probes took the experimenters several months, and it took over a year to fully train the animals on the one-back task (X. Wang, personal communication).

To begin a trial, the animal would fixate on a central green triangle cue for 100 msec. A white circle response cue above the central cue would appear, while the animal was required to maintain fixation on the green central triangle. Horizontal bar stimuli would be presented to digits 3 and 4 on the distal and proximal finger pads. Note that Wang et al., 1995 stimulated D2-D4 with probes which contacted the distal and proximal pads while the animal grasped the stimulator.

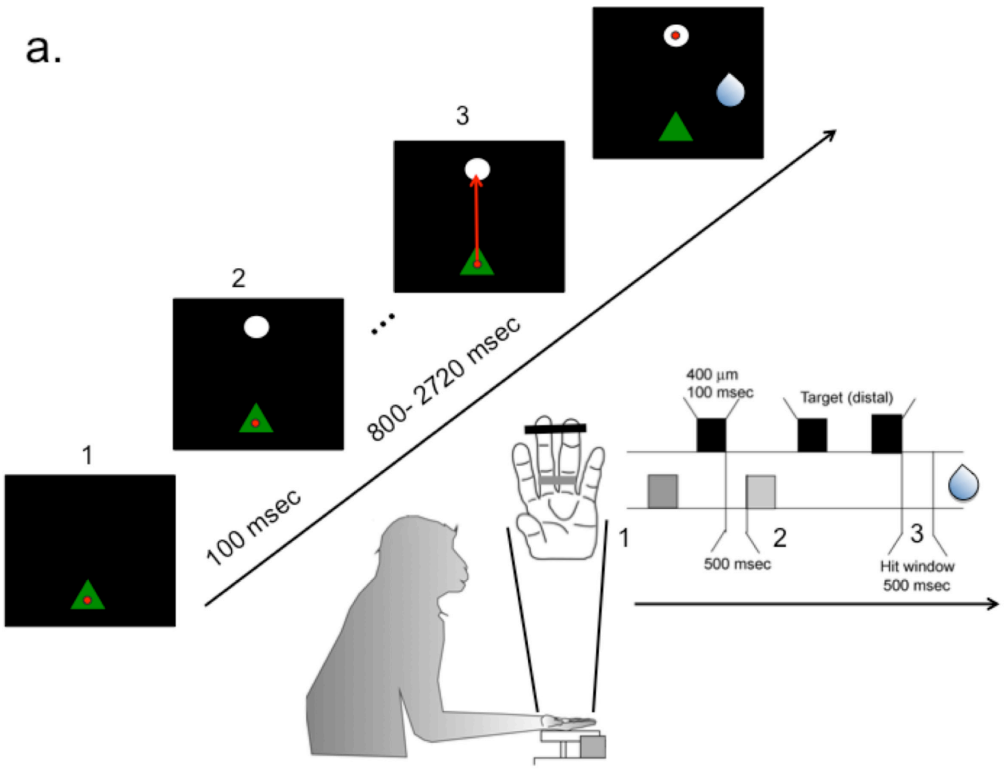


Figure 3.1. Distal one-back task. Both animals were trained to perform this task, which required the identification of two consecutive distally presented stimuli**. (A)** Top left: visual stimuli, Bottom right: tactile stimuli. Arrows indicate trial progression for both modalities. The animal fixated (red point indicates eye position, not actually part of visual stimuli) on a central green triangle to begin a trial (1), and then experienced multi-digit horizontal bar stimuli indenting the D3 and D4 distal and proximal finger pads (2). Grey= proximal stimuli, black= distal. When the animal felt two consecutive distal stimuli, it would break fixation and saccade to a response cue (3), which was present for the entire trial. If it did so in the designated hit window, it received a water or juice reward. **(B)** Tactile sequences/trials presented randomly to the animal.

The stimulator consisted of two custom build linear motors (similar to those used in Killebrew et al., 2007) that have a nominal displacement of 2.9 mm. The two motors were positioned over the left hand using two articulated tool holders (Noga Engineering Ltd. Shlomi 22832, Israel) mounted to 2-axis micro-positioners (Newport Corp., California). Motors were controlled using a National Instruments data acquisition board system (PCI-6229; National Instruments Corp., Austin, TX; software driver version NI-DAQmx 8.10f1) and custom software. A magnetic base could be moved to align one of the motors across the centers of the distal pads of digits three and four and then align the other bar over the proximal pads of the same digits. The bar contacting the distal pads was 35mm in length and the bar contacting the proximal pads 25mm to span the entire width of both finger pads. The bars were 3D printed using VeroWhitePlus plastic (Stratasys, Valencia, Ca.) on an Objet Alaris 30U. The short axis of the bar (8mm) was wedge-shaped to produce an edge sensation.

The animal would need to maintain fixation on the central cue until they felt two consecutive distal tactile stimuli. Wang et al, 1995, had the animal break hand contact with the stimulator when it felt the target stimuli, which could be two consecutive distal or proximal stimuli, or a 50-60 Hz vibration on one of the bars. For our task, if the animal broke fixation at any point the tactile stimuli would stop, the trial would be aborted and the animal would experience a time out period (4000 to 4500 msec). The stimuli were 400 μm, 100 msec indentations and the inter-stimulus interval was 500 msec. In the owl monkey study by Wang et al, 1995, the stimuli were 50-100 μm, 50 msec, and the inter-stimulus interval was 200-300 msec. The animal would need to saccade to the response cue within 500 msec to receive a reward, otherwise if they maintained fixation for the

entire period this was considered a “no response” or “incorrect” trial and the animal would not receive a reward. The animal randomly experienced four trial types: one with two distal stimuli, one with a proximal stimuli then two distal stimuli, a third with the pattern distal-proximal-distal- distal, and a fourth with a pattern proximal-distal-proximal-distal-distal (Figure 3.1B). The intertrial interval was 2300 msec.

As this was the first time our lab had trained an animal on a modified one-back task, we utilized several methods to train the animals such that they would understand the rules of the task. First, we altered the luminance on the response cue during the trial, such that it was low luminance while the alternating stimuli was presented, and would brighten synchronously when the target stimuli (second distal tap) was presented. Therefore, in the very beginning of training, the animal would maintain fixation on the central green triangle until a bright white circle appeared above, causing the animal to quickly saccade up to this response cue. This helped the animal understand the basic fixation rules of the task. Second, we increased the intensity of the target stimuli, specifically the second distal stimuli, to enhance its salience (suggested by X. Wang as it had been successfully used in the owl monkey task). We presented the trial types in blocks (e.g. 5 trials with two distal stimuli, followed by 5 trials with proximal- distal-distal stimuli), and repeated incorrect trials. We then slowly increased the luminance of the response cue during the fixation period until it was a fully illuminated cue during the entire trial. We then removed the other cues and aids until the animal was performing the task with all trial types presented randomly and without any incorrect trials repeated. It took us six months (6 days/week) to train the first animal to consistently perform at over 80% correct on this

task, and five months to train the second animal. Note that this is consistently shorter than the training time by Wang et al, 1995.

3.4. Neurophysiology

Standard neurophysiological techniques were used to collect the data in all animals. Prior to recording, a craniotomy was made in the center of the recording chamber, approximately 3 mm in diameter. Thereafter, the animal was brought in daily to the laboratory (6 days/week), the chamber cover removed, the chamber rinsed with sterile saline, and a positioning stage mounted onto the chamber. Positioning along the anterior/posterior and medio/lateral axes was set on each recording day with a 2D coordinate positioner that provided precision at the micron level. A custom-built microdrive system was then secured to this positioning plate, containing four separate extracellular microelectrodes (2 to 7MΩ, Tungsten FHC Inc, Bowdoin, ME) linearly aligned and spaced 584 μm apart. The animal was transferred to the recording room, and the electrodes advanced through the intact dura and into cortex. At the end of each recording day (5-6 hours later), the electrodes were removed, the chamber cleaned and a small piece of gelfoam with dexamethasone and antibiotic was placed on the dura. The chamber was filled with sterile saline and sealed, and the animal placed back in its home cage. As recordings progressed, the initial craniotomy was expanded to cover the 3b hand region.

3.5. Recording from area 3b

It was crucial that our recordings were always from area 3b, and not from another postcentral gyrus area where cells with different receptive fields properties (e.g. larger receptive field size) have been described. We used the same procedure as previous studies (DiCarlo et al., 1998; Bensmaia et al., 2008; Pei et al., 2011; Kim et al., 2015) to ensure we were recording in area 3b. Response properties as electrodes were driven into cortex were recorded on a central database co-registered with electrode depth and XY location within the chamber. The central sulcus was determined by the depth and transitions of white and grey matter, as well by the presence of motor responses in anterior electrodes. If the array were placed too far laterally, we would encounter responses to the thumb, and if it were placed too far medially, we would encounter responses to the upper arm. The array would be repositioned the next day if this were the case. The array would then be oriented medial-laterally to ensure the most lateral electrodes encountered responses to more lateral digits (i.e. closer to the thumb) compared to anterior electrodes. From that point, the array was oriented anterior-posterior (orthogonal to the central sulcus) such that each electrode encountered responses from approximately the same digit. It took approximately a week to localize the electrode array to the 3b D2-D5 hand region.

Once this cortical mapping procedure was complete, each day electrodes were driven into the cortex until they encountered neurons in area 1 with RFs on D2-D5 fingerpads. As one descends from the cortical surface through area 1, RFs progress from the distal, to middle, to proximal finger pads, and then to the palmar whorls. When electrodes reach area 3b, RFs proceed back up the finger, transitioning from proximal, to medial, and

ultimately to distal pads. Note that this transition was the reason we chose to exclude cells with proximal pad responses, as it would be unclear if such cells were localized to area 1 or to area 3b. This reversal has been verified histologically to correspond to the border between areas 3b and 1 (DiCarlo et al., 1998). Because we never recorded responses from the distal pads in superficial cortex, and all recordings were made 2–3 mm below the top of the neural activity and after observing this progression, there was little to no uncertainty about the anatomical area from which the neurons originated. We recorded from neurons whose RFs were located on the distal pads of digits 2–5 as determined by experimenter probing the glabrous skin with hand held probes. After recording from area 3b at a given location, the next day the electrode array was shifted 100 μm along the postcentral gyrus until the entire 3b representation of digits 2–5 had been covered. The array was occasionally shifted anterior-posterior to track the central sulcus and ensure the maximum number of electrodes reached 3b. This process took approximately 2 months.

3.6. Mapping receptive fields and stimulator

When we had successfully isolated single units with RFs centered on the distal pads of digits 2-5, we mapped receptive fields using a rotating bar protocol similar to that described in (Fitzgerald et al., 2004). The tactile stimulator consisted of a custom made linear motor as used in (Killebrew et al., 2007) mounted on the shaft of a rotating stepper motor (Arsape AM 1020, 10 mm diameter, 15.9 mm length, Faulhaber, Clearwater, Fl.). The oriented bar was 3D printed from plastic (VeroWhitePlus plastic, Stratasys, Valencia, Ca.) on an Objet Alaris 30U. The bar was 10mm long, approximately the

width of a monkey's finger; its short axis was 3 mm, and a 90° wedge. A wedge-shaped bar was used because it produced a crisp sensation of the edge of a surface. The motors were attached to an articulated tool holder (Noga Engineering Ltd. Shlomi 22832, Israel) mounted to a micro-positioner (Newport Corp., California) and a magnetic base. During stimulation, the bar was indented into the skin at the center of the distal pads of D2–5 of the hand contralateral to the recorded cortical hemisphere. The animal's digits were slightly spread apart such that there was never a possibility of the bar contacting adjacent digits. The center of each distal digit was marked on the skin by the experimenter using a 3D plastic positioner that corresponding to the dimensions of each digit, and the stimulator positioned over this point. For each stimulus, the bar was presented for 500 msec, with a ramp time of 20msec, at one of eight 22.5° separated orientations (0 to 157.5°). The bar was indented with a depth of 1mm and the interstimulus interval was 700 msec. In the second animal, we additionally tested indentation depths of 500 and 200 μm. There were eight repetitions of each of the eight orientations, and eight blank trials. Therefore, there were a total of 72 stimuli per digit pad in the first animal and 200 stimuli per pad in the second animal. After stimulating a randomly chosen pad with a random sequence and covering all repetitions of each of the eight orientations and indentations, the experimenter moved the bar to another randomly chosen finger pad. This sequence was repeated until each pad experienced the oriented bar stimuli. Only cells where D2-D5 distal pads were fully mapped were analyzed. We believe this to be the first study to characterize 3b receptive fields to oriented bar stimuli delivered to several individual digits in a well-controlled manner; a similar procedure characterized SII RFs (Fitzgerald et al., 2004). Figure 3.2 depicts the stimulator and setup for mapping 3b receptive fields.

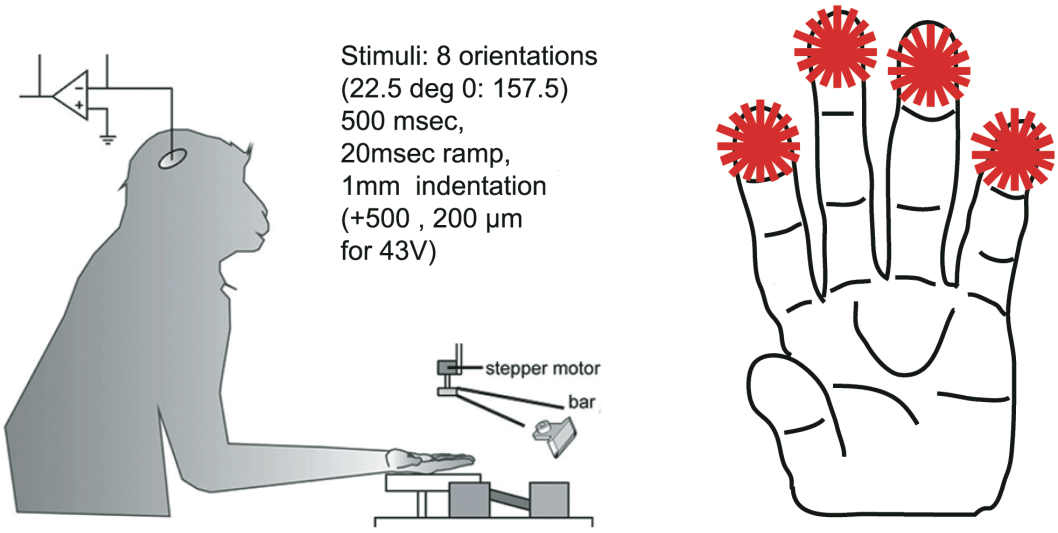


Fig 3.2. Mapping 3b receptive fields across several digits. The animal sat in a primate chair with their tested hand comfortably supinated, with the hand and digits held in place. A stimulator with a linear and stepper motor indented a small bar onto a single digit in a particular orientation. After all orientations were tested, the stimulator was moved from one distal digit pad to another, eventually covering D2-D5d. (Modified from Kim et al, 2015). Note that additional indentation depths were tested in one animal.

3.7. Ensuring single-unit isolation and neural acceptance while mapping RFs

We utilized several methods to ensure that a single-unit was recorded from during the entire recording session. This was essential, as a multi-unit recording of cells with inputs from several digits would have a larger RF than a single well-isolated cell with input from a single digit. Single units (SUs) were isolated using a template-based spike sorter and only one neuron per electrode was recorded at a time. The shape and timing information of each action potential (AP) was stored, and additional SU isolation analyses were performed offline to ensure that SU activity was well isolated. First, spikes occurring within 3msec of one another were excluded, as it would be unlikely to observe such spikes from the same neuron. Next, the shape of the AP was subjected to principal component analysis (PCA), and shapes that were more than three standard deviations away from the center of mass of the two most principal components (using the normalized Euclidean distance method) were deleted. Next, the experimenter visually inspected each block of trials and manually deleted AP shapes that were deemed outliers. Finally, we sorted the mean firing rate (FR) of a cell across trials within one protocol and fitted with power function. Trials from the tail ends were deleted until the fit produced a non-significant fit ($p > 0.05$). Since the experimental conditions were uniformly randomized, a negative or positive slope of the sorted trials would be indicative of cell loss or inclusion of APs from nearby cells, respectively. Cells with less than 30 trials were not analyzed.

3.8. Definition of significant responses in RF characterization

We only included cells with a significant response to the 1mm indentation depth on at least one tested distal digit pad, assessed in the following manner. Baseline was the inter-trial interval 250 to 450 msec after stimulus offset. The response to the stimulus was separated into 20 msec blocks: from stimulus onset to 160 msec after stimulus offset (to ensure off responses were included). We only considered cells and bins where the response in that bin or in the baseline period was at least 5 Hz. We asked if the response in the tested bin was significantly different than baseline (as responses were often not normally distributed, we used a Wilcoxon rank-sum test, $p<0.01$). If a cell had two consecutive bins with a significant response in the same direction with respect to baseline (two consecutive negative or positive bins), we considered that cell to have a significant response on that digit. We used this procedure rather than significance of the overall firing rate across the entire stimuli period to ensure that cells with rapidly adapting-like, or transient but strong responses were included in the analysis.

3.9. Definition of RF center/ hotspot

For each cell and at each digit tested, we calculated the cell's response, defined as the average response, across all orientations, from the stimulus onset to 100 msec after the stimulus offset. Baseline response, 250-450msec after stimulus offset, was subtracted from this response. We defined a cell's "receptive field center" or "receptive field hotspot" as the significantly responsive digit with the highest absolute response index. The sign of the response at the hotspot determined if a cell was classified as "excited" or "inhibited". We should note that it was often the case that cells had various temporal

responses across digits (e.g. one digit had a transient response to the oriented bar, and on another digit a sustained response to the oriented bar, see Chapter 5.2.5 and figure 5.9-5.10 for more information). Therefore we acknowledge taking this average response may be an imperfect measure of "strength" of response across digits, particularly for a cell with various temporal response patterns across digits.

CHAPTER 4. EXPERIENCE-DEPENDENT PLASTICITY IN PRIMARY SOMATOSENSORY CORTEX, AREA 3B.

We sought to quantify how tactile training altered receptive field (RF) properties in primary somatosensory (SI) cortex of trained rhesus macaques. We examined the RFs of cells in area 3b, as previous authors had proposed that multi-digit training expands RFs in this region, whose classical RFs are typically defined as confined to single digits (Iwamura et al., 1983). We hoped to replicate these findings and test which stimuli properties used in multi-digit training alter RF feature selectivity. A major implication of Wang and colleagues (1995) was that cortical plasticity was at least partially input dependent, and therefore one prediction would be that *all* features (e.g. orientation, location) of the stimuli used during training confers RF changes. Alternatively, only the stimulus properties relevant to the animal, that is, stimulus properties that must be discriminated upon (e.g. the timing of stimuli), impact cortical representations.

We quantified RF size using well controlled oriented bar stimuli across all the distal digits, in contrast to inconsistent hand-held probe stimuli used in previous studies to indicate RF expansion (Clark et al., 1988; Allard et al., 1991; Wang et al., 1995). This allowed us to systematically vary orientation and indentation depth and record corresponding 3b neural responses. We examined orientation tuning (in this analysis, restricted to a single indentation depth) and the effect of intensity on RF size. We tested if animals trained on a distal one-back task with horizontal bar stimuli would exhibit an

overrepresentation of this orientation in 3b cells. We hypothesized that stimulus location during training specifies plasticity, testing if responses across the trained digits (digits three and four) were greater than on untrained digits in trained animals. We examined if increased responses to trained digits were specific to trained animals, comparing responses to homologous digits in a naïve animal and the untrained hemisphere of a trained animal.

Finally, we hypothesized that RF plasticity following multi-digit training would conform to task demands. In the one back task, the animal must quickly respond when he feels a second consecutive distal tap in a tactile sequence; he must respond within 500msec of the end of this stimulus but cannot respond before it is off the finger pad. We hypothesized that the rules of the one-back task would promote the expression of cells with transient responses to the on and offset of tactile bar stimuli. Cells with transient responses at the on and offset of stimuli may provide an advantage over those with sustained responses to a tactile indentation. We therefore also tested whether training alters the temporal properties of responses. Note that this chapter and Chapter 5 only describe RF properties quantified measured outside of the distal one-back task. Chapter 6 examines responses while the animal performed the trained tactile task.

4.1. Specific Methods.

4.1.1. Multi-digit (MD) index

In the following analysis, we wished to quantify the similarity of a cell's response across the (four) tested distal digits. We therefore developed a measure, which we call a

multi-digit (MD) index, which could quantify equality of responses across digits for each cell with a significant response on at least one tested digit.

MD index was assessed using the following equation:

$$\mathrm{MD_{index}} = 1 - \frac{\sum_{i=1}^{N} |D_i - \bar{x}_N|}{\max SS_N}$$

Where:

N = number of tested digits (in this case, four tested distal digit pads)

$D_i = {|R_i|}/{\sum_{i=1}^{N} |R_i|}$ Or, how much the current digit accounts for the sum of total response over all four digits. Note that we took the absolute value of the response (strength not dependent on if responses were negative or positive compared to baseline).

R_i was the average response of the cell on a specific digit at the 1mm indentation across all orientations, from stimulus onset to 100msec after stimulus offset, with baseline response (250-450 msec after the stimulus) subtracted.

$\mathrm{maxSS_N} = 2(N-1) * \bar{x}_N$, in this case, $\bar{x}_N = 0.25$. The proportion of one digit's response to the total response for an ideal multi-digit cell, which would respond equally over all tested digits

$\mathrm{maxSS_N} = 2(N-1) * \bar{x}_N$, in this case, max $SS_N = 1.50$. The deviation of digit responses, as a proportion of the sum of all digits' response, in an ideal single digit cell compared to an ideal multi-digit cell. That is, the deviation between digit responses of ideal single

digit cell tested over four digits is: [(1-0.25) + (0-0.25) +(0-0.25) + (0-0.25)] compared to an ideal multi-digit cell with equal responses over all four digits [(0.25-0.25)*4].

Values could range from 0, indicating a multi-digit cell with minimal responses outside the hotspot, to 1, a cell with exactly equally strong (regardless of sign) responses across all significantly responsive digits.

4.1.2. Orientation tuning

Orientation tuning was assessed in the same way as described in Bensmaia et al., 2008. Only cells with a minimum of 5 repetitions per condition were considered (N=339). Orientation selectivity was computed by using vector strength assessed by the equation (Ringach et al., 2002b; Bensmaia et al., 2008):

$$OI = \frac{\sqrt{[\sum R_i \sin(2\theta_i)]^2 + [\sum R_i \cos(2\theta_i)]^2}}{\sum R_i}$$

where R_i is the average response of the neuron to the bar at orientation θ_i at the 1mm indentation level. We used the average response over the entire stimulus period (onset to 100msec after offset), as it has been shown that orientation selectivity can vary over the stimulus period (Bensmaia et al., 2008). To allow for similarity with previous authors' calculation of orientation selectivity in somatosensory and visual cortices (Ringach et al., 2002b; Shapley et al., 2003; Bensmaia et al., 2008), we choose to assess response as the average absolute response to the stimulus (i.e. baseline rate was not subtracted). Implications of this will be discussed.

Values of OI range from 0, where a neuron has an exactly uniform response to all orientations, to 1, where the neuron has a non-zero response to only 1 orientation. For

each neuron, we determined the statistical significance of OI by randomizing responses across repetitions 5000 times and recalculating OI each time to obtain a distribution of values expected by chance (Yau et al., 2009). A separate randomization distribution was calculated for each cell. We defined tuning to be significant when the actual OI value exceeded 95% of the values in the randomized distribution.

Preferred orientation was determined in two ways: by assessing the exact tested orientation with the highest absolute mean FR (baseline subtracted), and by calculating the mean vector response $\bar{r}$, (circ_mean, Circular Statistic toolbox for Matlab, Berens, 2009), where $\bar{r} = (atan2 \sum_i R_i e^{i2\theta})/2$. Angles were transformed twice to cover the entire unit circle and to determine the mean orientation within the tested angles (0-157.5 deg.)

4.1.3. Submodality specificity of responses

We examined submodality specificity of responses similar to Pei et al 2009. We asked if cells had significant offset responses, suggesting more input from rapidly adapting peripheral afferents, and/or a significant sustained response, indicating more input from slowly adapting afferents. Cells with only a significant off response were categorized as “RA-like”, those with only a sustained response as “SA-like”, and those with both periods significant as “Mixed cells”. We also encountered cells with significant responses at the onset of the bar stimuli but without a significant off or sustained response, which we call “Transient” cells.

To determine which category a cell fell into, we assessed the significance of the sustained and off responses. The sustained response was calculated as the average

response in the 200-400msec period following the onset of the tactile stimuli, and was compared to the baseline response to determine its significance (Wilcoxon rank-sum, $p<0.05$). This is in similar to Pei et al (2009), who consistently used a 300msec period 150 msec after onset of the stimulus for every cell to assess sustained responses. We used a slightly smaller window to ensure off responses were not included. Significance of the offset response was calculated by subtracting response 20msec before offset of the stimulus from the response in the period 200msec after offset. This ensured that a cell's sustained response was subtracted from the offset response. We then asked if this response was significantly different from baseline (Wilcoxon rank-sum, $p<0.05$).

4.1.4. Adaptation index.

We calculated an adaptation index (AI) for every cell with a significant sustained and/or offset response. Transient cells were not included, as these cells had negligible sustained and off responses. AI was assessed by the equation (Pei et al., 2009):

$$\mathrm{AI} = \tan^{-1}\left(\frac{|\mathrm{R_{OFF}}|}{|\mathrm{R_{SUSTAIN}}|}\right)\mathrm{X}\frac{2}{\pi}$$

$R_{sustain}$ was the average response in the 200-400msec period following the onset of the tactile stimuli, with baseline response subtracted. To calculate R_{off} we determined the maximum during the 200msec after offset and the period 40msec around this maximum was used for R_{off} (with baseline response subtracted). This is in contrast with Pei et al, which used the 40 msec window after stimulus offset for each cell and a 40msec period 90 msec before offset of the stimulus for every cell to assess sustained responses. We believe our changes to Pei et al's (2009) procedure better take into account neurons' variable responses during the sustained and off periods, as opposed to choosing arbitrary time windows for all cells and ensuring that small fluctuations do not obscure the

measure. These values were normalized by dividing by the grand mean across the population, as R_{off} was often orders of magnitude greater than $R_{sustain}$. That is, an individual cell's sustained response was divided by the average sustained response for all recorded cells. Adaptation indices ranged from 1, for a cell with only an off and lacking a sustained response, considered an ideal RA- like neuron, to 0, an ideal SA-like neuron, with only a sustained response and no off response.

4.2. Results.

4.2.1. Animal performance on the distal one-back task

The animals' performance was assessed in 21 sessions (40 trials each) for 43V and 24 sessions for MR4358M, over five days at the end of recording when performance was very stable. This is shown in Figure 4.1. MR4358M performed significantly better than 43V, at 88% correct compared to 81% correct ($F(1,43)=6.9$, $p=0.012$, Figure 4.1a). 43V had significantly faster reaction times, responding at an average of 175 msec after the final stimulus offset on correct trials, versus 232 msec for MR4358M ($F(1,43)=90.1$, $p<0.001$, Figure 4.1b).

We additionally tested MR4358M over six sessions (40 trials each) on the untrained/ control hand prior to recording in the control hemisphere (Figure 4.2c). We asked if performance on the one-back task would transfer to the untrained hand. We found it did not, as the animal performed at only 20% correct on this hand. The animal appeared to understand the basic rules of the task: that is, to maintain fixation for a set period (and likely retained that there were four fixation windows) and then saccade to the response cue, but did not seem to be using the pattern of tactile stimuli to determine when to respond.

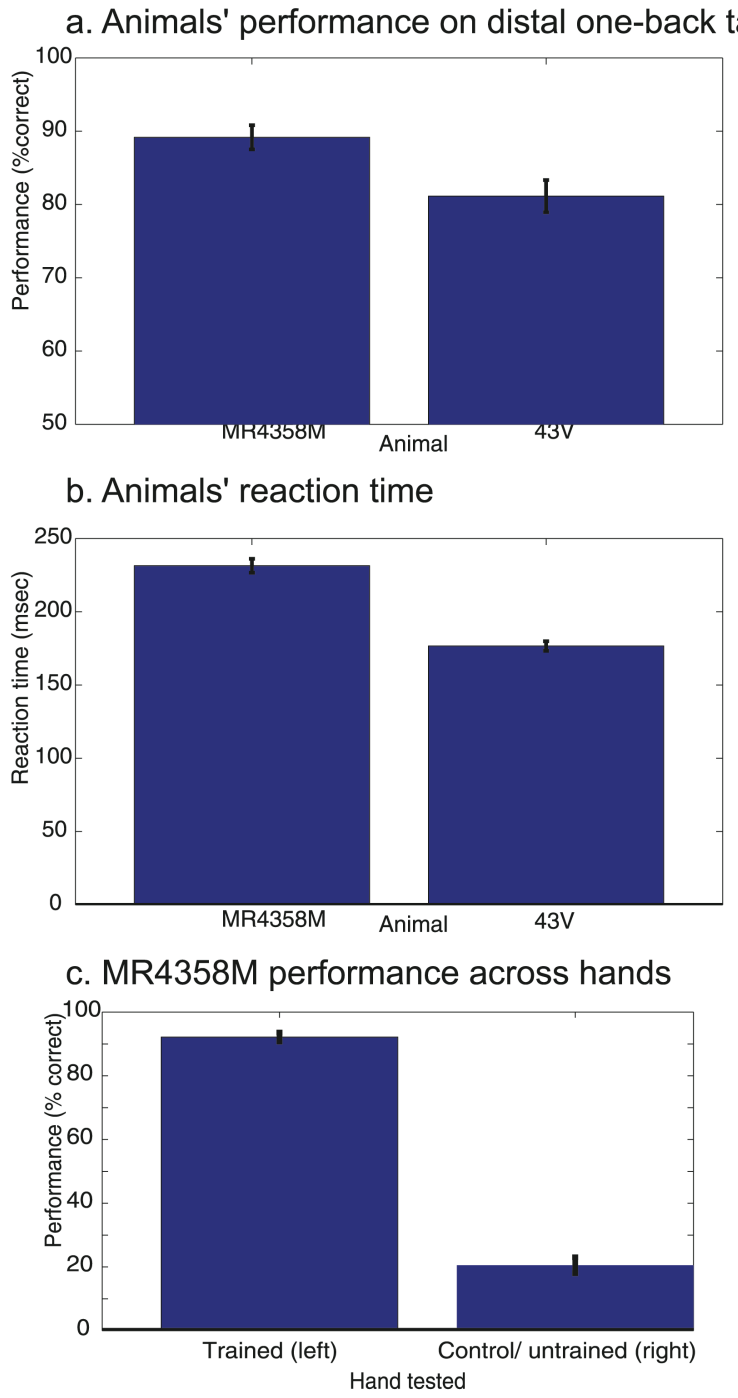


Figure 4.1. Animals' proficiency on the one-back distal tactile task at the end of training. (A) Average performance of both animals. Performance measured as percent correct over ~20 blocks (40 trials each). **(B)** Reaction time on correct trials of both animals, measured after stimulus offset. **(C)** Performance of MR4358M on the untrained right hand, this corresponds to the control hemisphere.

4.2.2. RF size.

We observed cells in all hemispheres with significant responses to more than one digit. Figure 4.2 shows two example cells observed in the trained (Figure 4.2a) and naïve hemisphere (where no tactile training had occurred for either hand, Figure 4.2b) with multi-digit responses to the stimulus indented at 1mm. The right panels of these Figures demonstrate that the action potential waveforms did not change as the stimulator was moved between digits; we are confident that recording stability was maintained across protocols.

Figure 4.3 illustrates the proportion of cells confined to a single digit or exhibiting multi-digit responses, to the stimulus indented at 1mm. Figure 4.3a includes cells with a significant response to at least one tested digit; RF size was quantified as the number of digits with a significant response (two consecutive 20msec bins with statistically different responses compared to baseline, Wilcoxon sign-rank, $p<0.01$), regardless of if excited or inhibited compared to baseline. As predicted by (Wang et al., 1995), we found that the trained hemispheres had a higher proportion of cells with multi-digit responses (57%, N=176) than the completely naïve hemisphere (42%, N=91). These proportions were significantly different ($\chi^2(1,N=267)=5.07$, $p=0.02$). The control hemisphere (untrained hemisphere of the trained animal) exhibited a similar proportion of multi-digit cells (56%, N=103) to the trained hemispheres ($\chi^2(1, N= 279)=0.03$, $p=0.86$). The latter finding was not explored nor predicted by Wang et al, 1995, as only naïve animals were compared to trained animals.

This initial analysis included cells with inhibited responses to the stimuli. When using hand-held stimuli to probe RFs in somatosensory cortices, as performed by (Wang

et al., 1995), excitatory responses to stimuli are often only considered by the experimenter. Therefore we further examined cells where the hotspot had an excitatory response (response to stimulus greater than baseline, N=317), and only considered significantly excited digits to be part of a cell's RF. We reason that this was a similar procedure to that used by Wang and colleagues (1995) to quantify RF size. These data are shown in Figure 4.3b. In the trained hemispheres, 44% of cells were classified as multi-digit (N=141), whereas 25% of cells in the naïve hemisphere were multi-digit (N=79). This difference was statistically significant (χ^2 (1, N=220)=7.54, p=0.006). Forty-seven percent of cells in the control hemisphere were classified as multi-digit (N=97); this proportion was not statistically different than the trained hemisphere (χ^2 (1, N=238)=0.28, p=0.60). We also note the two trained animals did not exhibit statistically different proportions of multi-digit cells in their trained hemispheres (39% of 71 cells for 43V and 49% of 70 cells in MR4358M, (χ^2 (1, N=141)=1.19, p=0.27). Overall, these data replicates Wang and colleagues' 1995 findings that training with multi-digit stimuli corresponds with a greater proportion of cells with multi-digit RFs in area 3b when RF size is only quantified by excited responses to tactile stimuli. Cells with inhibited responses will be discussed in the following chapter. These data also adds the finding that multi-digit expansion is found in both the trained hemisphere and the contralateral untrained hemisphere of the trained animal. Since the animal could not perform the task on the untrained/control hand (Figure 4.1c), it also suggests that RF expansion is not sufficient for performance of the distal one-back task.

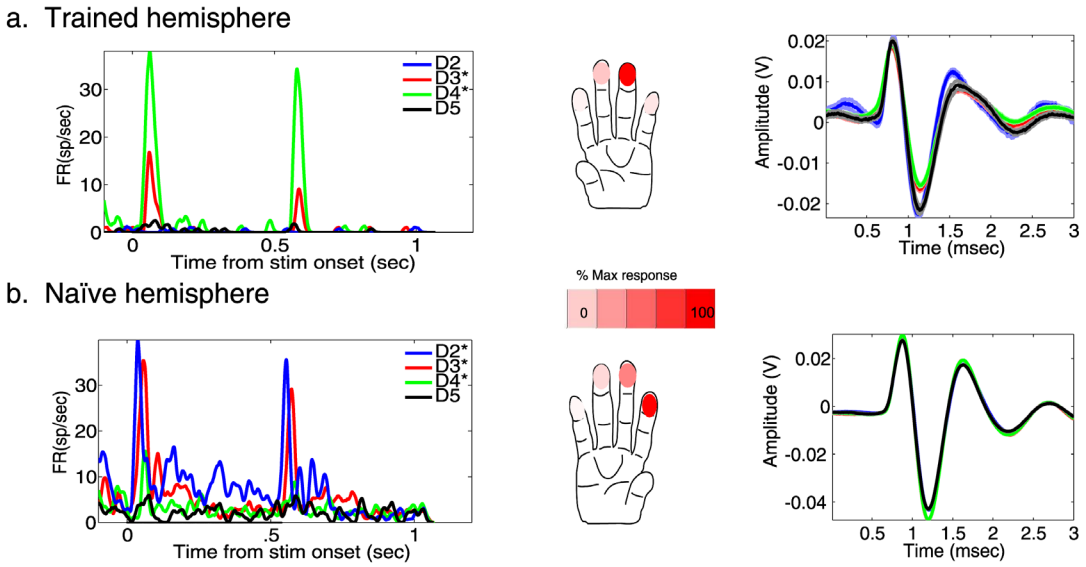


Figure 4.2. Example cells from the trained and naïve hemisphere with multi-digit responses. (A) Cell from a trained hemisphere, significantly (see methods) responsive on digits 3 and 4, with its hotspot on digit 4. Left panel: Instantaneous firing rate profiles to bar stimuli across all tested orientations at the 1 mm indentation depth. Colors correspond to response on various digits. Middle panel: Heat map of responses across digits compared to cell's digit hotspot. Right panel: Average action potential (AP) waveform on each tested digit (colors match digits in left panel), demonstrating that the cell AP shape was consistent across digits and protocols. **(B)** Cell from the naïve hemisphere, significantly responsive on digits 2, 3, and 4, with its hotspot on digit 2.

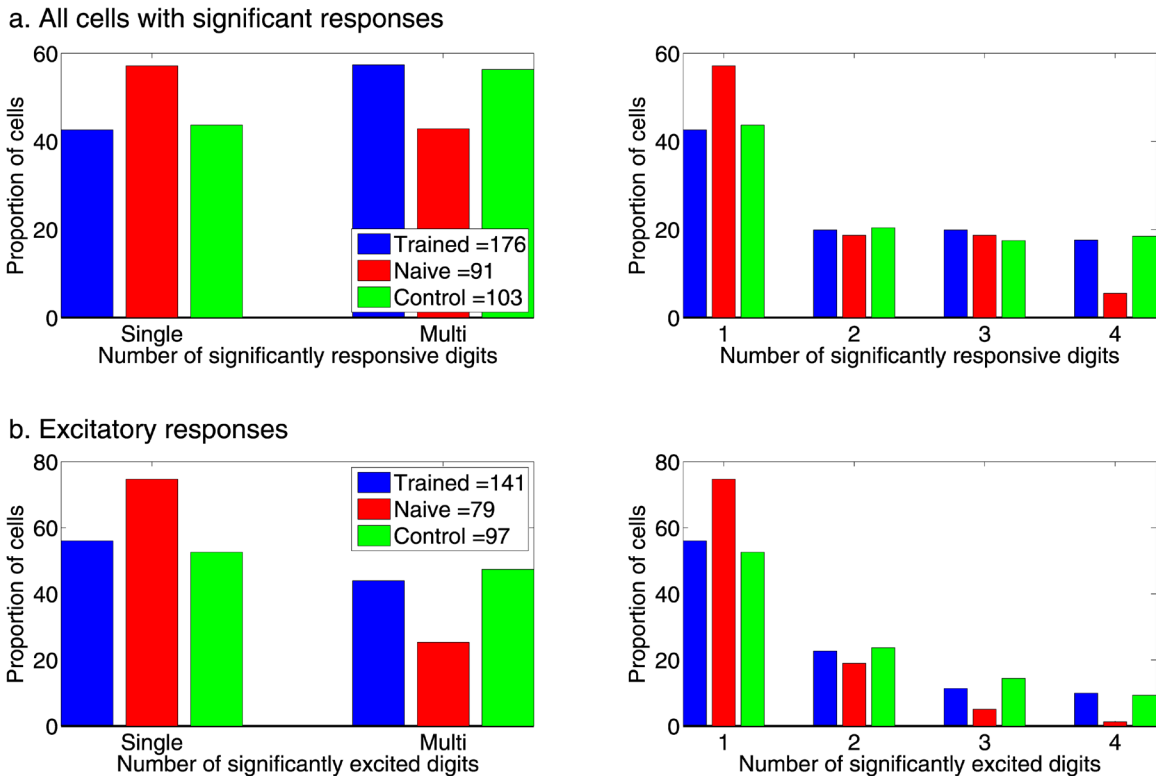


Figure 4.3. Higher proportions of multi-digit cells in trained and control hemispheres. (A) Left: Proportion of cells with significant responses on only one digit versus several digits. Right: Distribution of cells with responses to one, two, three, or all four tested digits. All cells with a significant response included. **(B)**. RF size defined by significantly excited responses and for cells with an excited hotspot response.

We followed up this result by asking if responses across digits were more similar for cells in the trained than naïve hemispheres, as our protocol had quantified responses on each digit to the oriented bar stimuli. We again choose to examine cells with a significantly excited hotspot response (N=141, trained hemispheres, N=79, naïve hemisphere). Unless noted, all future analysis in this chapter will focus on such cells, in an effort to offer new insight on the results of Wang and colleagues (1995). Chapter 5 describes properties of those cells whose hotspot had a negative response relative to baseline.

We calculated for each cell the other digit responses as a proportion of the hotspot response (i.e. the "redness" of digit 3 in Figure 4.2a, middle panel). A scatterplot of this measure on the second and third most responsive digits is shown in Figure 4.4a. The extremes represent responses closer to the hotspot response; cells on the left and lower quadrants had responses below baseline on the adjacent digits. One can see that cells in the trained hemisphere (blue) lie more at the extremes than those in the naïve hemisphere (red), and that cells with significant responses (two consecutive 20msec bins with statistically different responses compared to baseline, Wilcoxon sign-rank, $p<0.01$) across multiple digits are also found at the extremes (filled circles). Figure 4.4b is a density plot of the ratio of the second most responsive digit relative to hotspot response, comparing cells in trained (blue) versus the naïve (red) hemisphere. Mann-Whitney U tests were performed to determine if this difference was significant. This test revealed a significant difference between the two populations ($Z=-2.03$, $p=0.04$, Cohen's $d=0.13$). The mean of this ratio for cells in the trained hemispheres was 0.21 (that is, 21% of the hotspot response), and 0.09 for cells in the naïve hemisphere.

As this analysis only takes into account the relative response of the second most responsive digit, we developed a measure to indicate how similar responses were across all four tested digits. We called this measure a cell's MD index (see specific methods for a description of the calculation). Values could range from 0, for a cell that responded only on the hotspot digit, to 1, where a cell had equal responses (absolute value of response) across all four tested digits. Figure 4.5 is a density plot of MD index for cells in the naïve (red) and trained (blue) hemispheres. The mean of this index for excited cells in the trained hemisphere was 0.49 (N=141) and 0.43 in the naïve hemisphere (N=79). We performed a Mann-Whitney U-test, which revealed that MD index was significantly higher in excited cells in the trained hemisphere than for cells in the naïve hemisphere ($Z=-2.22$, $p=0.03$, Cohen's $d= 0.15$).

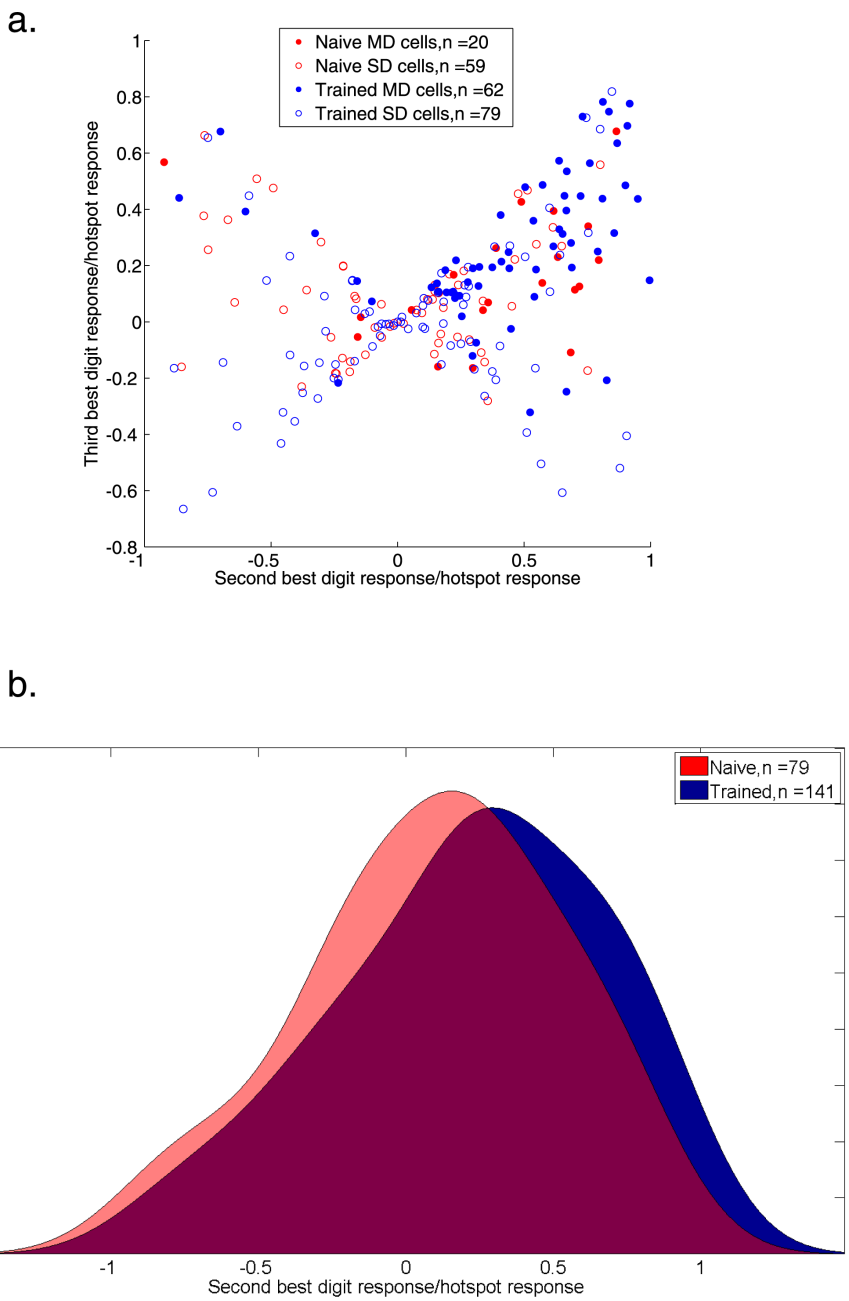


Figure 4.4. Cells in the trained hemisphere exhibit more equal responses across the most responsive digits. (A) Scatterplot of ratio of second and third most responsive digit to hotspot response. Blue: cells from trained hemispheres with excited hotspots, Red: Cells from naïve hemisphere with excited hotspots. Filled circles, cells with significant responses on multiple digits. **(B)** Probability density function of distribution of ratio of second most responsive digit response to hotspot response.

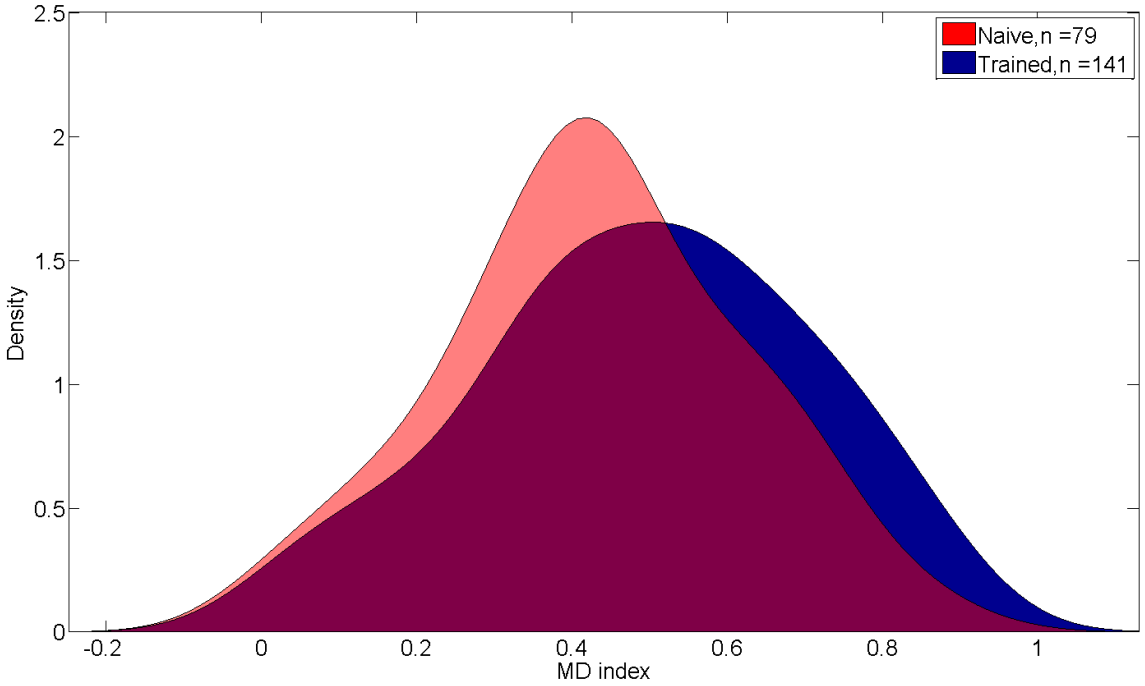


Figure 4.5. Cells in the trained hemispheres exhibit more equal responses across all four digits (MD index). Probability density function of MD index in trained and naïve hemispheres. Calculated for cells with an excited hotspot.

One concern is that increases in multi-digit cells are a byproduct of overall increased excitability of cells. For example, in whisker barrel cortex, it has been observed that spontaneous firing rate correlates with responsiveness to whisker deflection (Clancy et al., 2015). We therefore tested if baseline responses (as measured on the cell's hotspot) were greater in cells from the trained hemisphere than the naïve hemisphere, and found this was not the case (Mann Whitney U-test, Z=-0.33, p=0.74, Figure 4.6a). However, we found a significant correlation between MD index and baseline response (R^2=0.07, p<0.001), in that as baseline response increased, MD index also increased (Figure 4.6c). Therefore, to confirm that an increase in RF size was not just due to increased excitability, we ran an analysis of covariance (ANCOVA) with baseline as a covariate, and found that there was still a significant effect of training condition on MD index (adjusted means: 0.50 for cells in the trained hemispheres, 0.43 for cells in the naïve hemisphere, F (1, 218) =4.84, p=0.03, η_P^2= 0.02). Relative response of the second most responsive digit to the hotspot response was still higher in the trained cells (adjusted mean, 0.22) than naïve cells (adjusted mean, 0.09) when controlled for baseline response (F (1, 218)=3.74, p=0.05, η_P^2= 0.02). Overall, these data supports the finding that multi-digit training enhances responses across digits, and is not just an effect of overall enhanced excitability of cells.

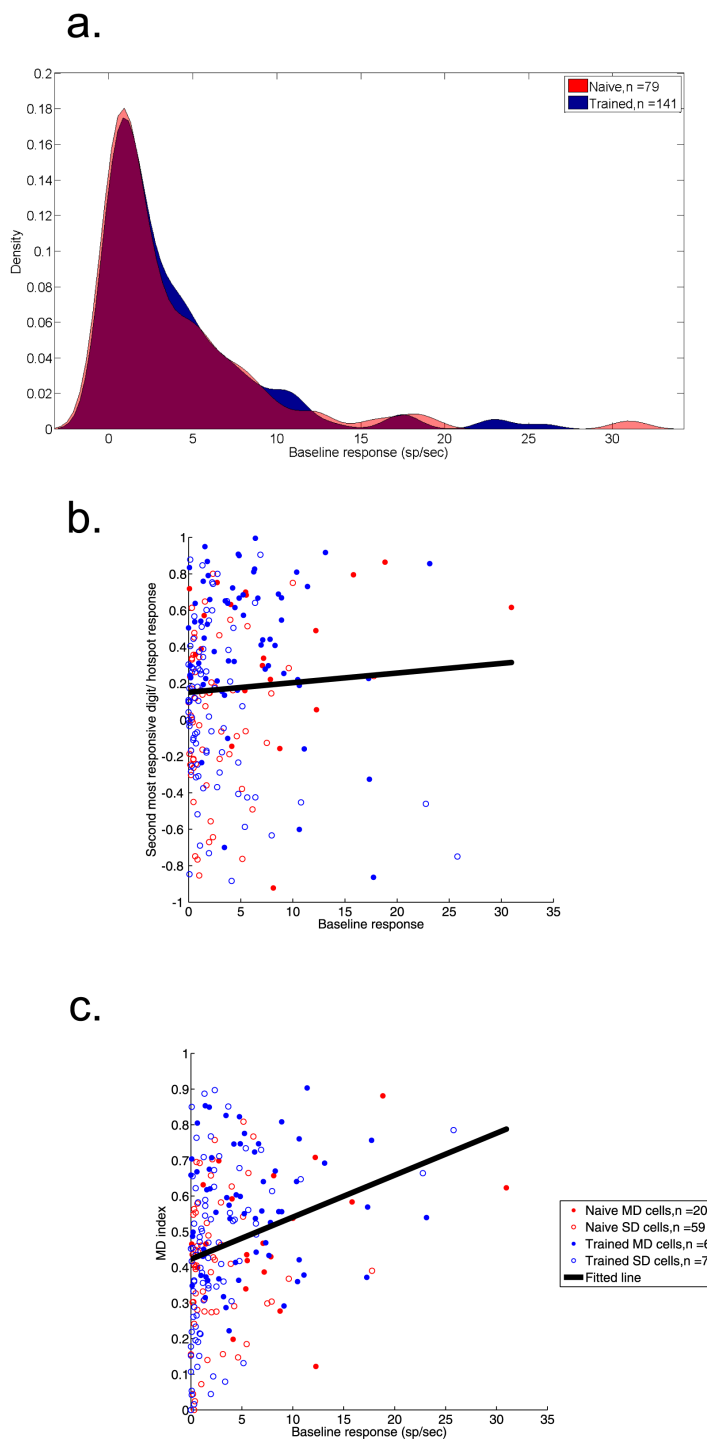


Figure 4.6. Relationship between measures of RF size and spontaneous rate. (A) Probability density function of baseline responses for cells in trained and naïve hemispheres. **(B)** Scatterplot of cells' baseline response and ratio of second responsive digit to hotspot (linear relationship not significant, R^2=0.003, p=0.40). **(C)** Scatterplot of baseline response and MD index. Black lines indicate fitted linear curve.

4.2.3. Intensity and RF size

In one animal (43V), we measured responses to indented bars on single digits at various indentation depths (1mm, 500 μm, and 200 μm) on a subset of cells to examine if the effect of training would be more evident when tested at smaller indentations. We hypothesized that weak interdigit connections in 3b are more likely to be activated by suprathreshold stimuli in naïve animals and are strengthened by this training; therefore smaller indentations will expose a stronger effect of multi-digit training. Figure 4.7 is an example cell from the naïve hemisphere with significant responses on two digits to all tested indentations.

Figure 4.8 shows the proportion of cells with excited RFs and responsive at 1mm: (1) confined to a single digit, (2) responsive across several digits, or (3) not responsive at the smaller tested indentations. One will note that in both hemispheres, many cells had a significant response only at the largest indentation depth tested.

To further test if RF size varies with indentation depth and training, we quantified for each cell how many digits had a significant response at various tested indentations. We examined excited cells with a significant response on one tested digit at the 1mm indentation and tested on all indentation depths (N=47, trained hemisphere, N=79, naïve hemisphere). These data are illustrated in Figure 4.9. We ran a 2 X 3 mixed- factor ANCOVA with a between group factor of training (trained and naïve) and a within-subject factor of indentation depth (1mm, 500□m, 200 □m) and controlled for baseline responses (as measured on the cell's hotspot). The ANCOVA revealed a significant effect of indentation level on number of responsive digits ($F(2, 246)= 47.65$, $p < 0.001$, Greenhouse-Geisser corrected, $\eta_P^2=0.28$), as the number of responsive digits decreased

with smaller indentations. The ANCOVA also indicated a significant interaction between indentation level and training ($F(2, 246) = 2.25$, $p = 0.001$, Greenhouse-Geisser corrected, $\eta_P^2 = 0.07$), whereby RF size was greater in the trained hemisphere than the naïve when tested at the largest indentation.

4.2.4. Digit specificity of plasticity

We next asked if the effects of training on cells' responses were greater in the digits that experienced repeated stimulation (digits 3 and 4) as compared to those that did not experience the stimuli. We averaged a cell's responses to the bar stimuli at the 1mm indentation on digits 3 and 4 and compared this to the average response on digits 2 and 5. Figure 4.10 is a scatter plot of these responses, with responses to the untrained digits (or comparable homologous digits, D2 and D5) on the x-axis, and responses to the trained digits (or homologous digits, D3 and D4) on the y-axis. We first compared cells with an excited hotspot from the trained hemispheres (N=141) to excited cells in the naïve (N=79) and ran a 2 x 2 mixed factor ANOVA with a between group factor of training condition (trained, naïve), and within-subject factor of response on trained (D3 and D4) versus untrained (D2 and D5) digits. The ANOVA did not find there to be a significant effect of digit tested, that is, among all cells, the average response on the middle two digits was not different than the average of the index and ring finger responses, nor was there a significant interaction between digits' responses and training condition. We additionally tested if responses for the trained digits in the trained hemisphere were different than homologous digits of the untrained hemisphere. A 2 x 2 mixed factor ANOVA with between group factor of training condition (trained, N=141, control= 97)

and within-subject factor of digit specific response (average D3 and D4 response to average D2 and D5 response) also did not reveal significant effects. This suggests that training did not significantly increase responses on the trained digits for cells in the trained hemisphere.

4.2.5. Training and orientation tuning properties

Another prediction is that features of a stimulus used throughout training will specify cortical plasticity effects; indeed, this was based on our data from Chapter 2 where we found orientation and location specific changes in tactile spatial acuity following multi-digit training. We asked if training with consistent horizontal bar stimuli would alter the number of cells tuned to the horizontal orientation or orientation selectivity. We examined this across the entire population of excited cells as well as those with their hotspot on the trained digits to assess if changes in orientation tuning may be location specific. The tuning curves measured over the four tested distal digit pads in example cells are shown in Figure 4.11, demonstrating variability of tuning curves and relative weak orientation selectivity, particularly on non-responsive or non-hotspot digits. See Chapter 5.2.6 for further discussion of similarity of tuning across several digits. We examined the preferred orientation and orientation selectivity on the hotspot digit (e.g. green curve for Figure 4.11a)

Figure 4.12 plots the distribution of preferred orientations for all cells with excited hotspots (Figure 4.12a) and for cells with their hotspot on the trained digits (Figure 4.12b). Preferred orientation was calculated in two ways: by determining the exact tested orientation (0 to 157.5 degrees, 22.5 degree steps) that elicited the highest response, or by taking a vector average of the responses. The former data is shown in the left histograms

of 4.12a and b. Probability density functions were calculated over the vector averaged preferred orientations (Figure 4.12, c and d). We performed two-sample Kolmogorov-Smirnov tests to determine if distributions from the trained hemisphere's population were different than distributions from the naïve hemisphere. These tests found no significant differences in distributions across populations (Kolmogorov-Smirnov tests, $p>0.05$). These data suggests that training does not alter the distribution of preferred orientations. We found that all distributions were uniformly distributed; that is, all orientations were equally represented (Rayleigh tests, $p >0.05$).

We hypothesized that training with multi-digit stimuli may impact cells' overall orientation selectivity on a single digit, as expansion of excitatory RFs would disrupt the balance of excitatory and inhibitory RF components thought to confer spatial selectivity (DiCarlo and Johnson, 2000). We therefore predicted that training with multi-digit bar stimuli would decrease orientation selectivity on single digits as cells expand their RFs. We also asked if there was a relationship between RF size and orientation selectivity.

We found weak negative relationships between measures of RF size and orientation selectivity on cells' hotspot; that is, as responses across digits became more similar, orientation selectivity slightly decreased (Figure 4.13a and 4.13b). However, we did not observe any changes in orientation selectivity with training (Mann Whitney U test, $Z=0.47$, $p=0.64$, Figure 4.13c).

4.2.6. Training and temporal pattern of responses

Finally, we sought to examine if training would alter the temporal pattern of cells' responses to single digit bar stimuli, hypothesizing that the overall nature of the distal

one-back task requires the animal to attend to the location of stimuli and identify two consecutive distal stimuli, waiting until the stimulus was off the finger pads to respond. Therefore, a shift in the population towards rapidly adapting temporal responses, which respond transiently at the onset and offset of tactile stimuli, may be advantageous.

We quantified this property in two ways. First, we asked if cells had significant responses at the offset of the bar stimuli or during the sustained portion (see specific methods and Pei et al., 2009). If they only had the former, they were classified as "RA-like", if only the latter, "SA-like", and if both, "Mixed". Cells with significant responses but neither a significant offset nor sustained response were classified as "Transient" responding cells (these cells were presumably not found in the dataset of Pei et al., 2009 , as all recorded cells in their analysis had either a significant sustained or off response). We did not find that the proportions of cells falling into these four categories were significantly different for cells in the trained versus the naïve hemisphere (Figure 4.14a, χ^2 (3)=1.08, p=0.78). We also calculated for each cell an adaptation index on its hotspot digit, which quantifies relative offset and sustained responses. Cells with only sustained responses have adaptation indices of 0, while those with only offset responses have adaptation indices of 1. We chose to exclude cells with neither a significant onset nor sustained response ("transient" cells, 11 in the naïve hemisphere and 22 in the trained hemispheres), as AI is calculated based on the relative responses at these time points. We found there was no difference in adaptation index for cells in the trained versus the naïve animal (Mann-Whitney U test, Z=-0.18, p=0.85, Figure 4.14b). These data suggest that temporal responses of 3b cells, at least as referenced to similarity to afferent responses, is not modified by multi-digit tactile training.

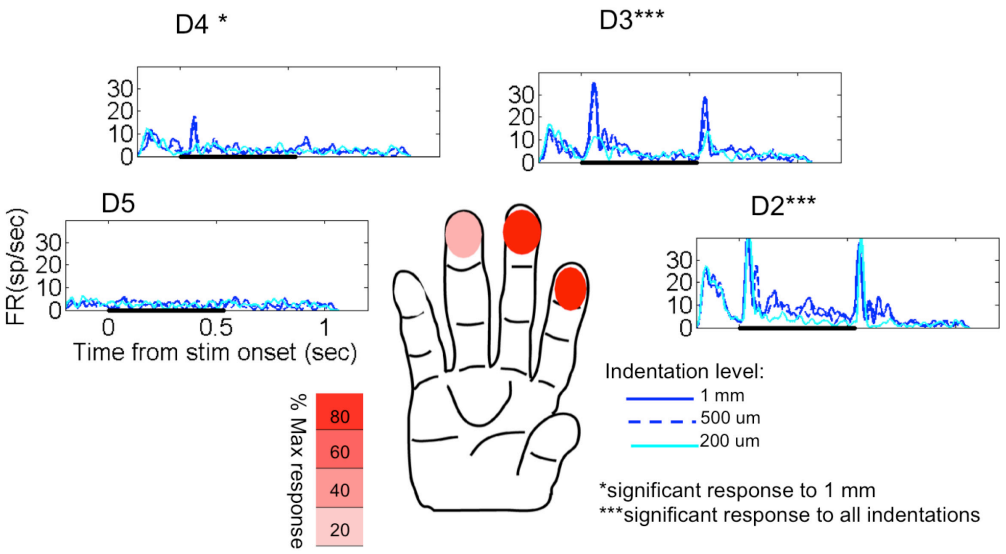


Figure 4.7. Example cell from the naïve hemisphere with responses at various indentation depths. Insets show instantaneous FR profile to bar stimuli at all orientations at a particular indentation level. Solid blue lines: 1mm, dashed blue: 500 μm, cyan lines: 200 μm indentation. Color of digits on hand inset indicates average digit response compared to hotspot at 1mm indentation depth. Cell was significantly responsive on digits 2, 3, and 4 at the 1mm and 500 μm indentation depth, on digits 2 and 3 at the 200 μm depth.

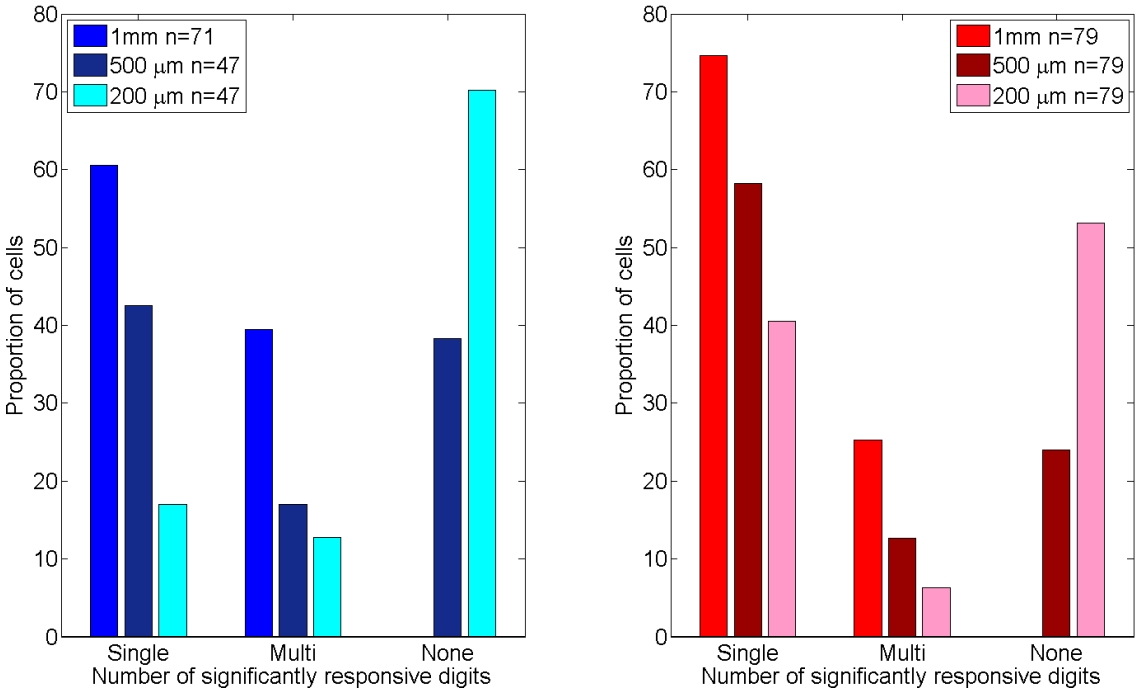


Figure 4.8. RF size at various indentations. Left: cells from the trained hemisphere of 43V, Right: the naïve hemisphere of the same animal. Only cells with excited hotspots and a significant response on at least one digit at the 1mm depth are included. Note that in the trained hemisphere, only a proportion of cells (47/71) were tested at all indentation depths due to time constraints and recording stability.

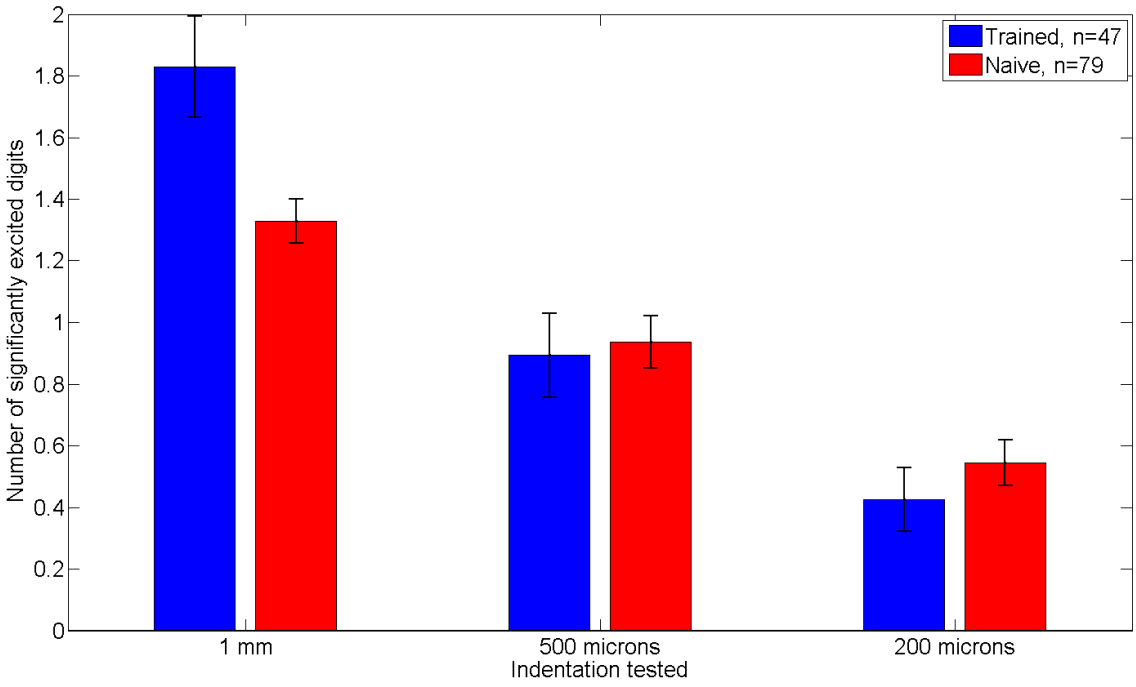


Figure 4.9. Average number of responsive digits with training across various indentations. Cells with excited responses on at least one tested digit at the 1mm indentation were included. Number of significantly responsive digits could range from 0 to 4 at 500 and 200μm indentations.

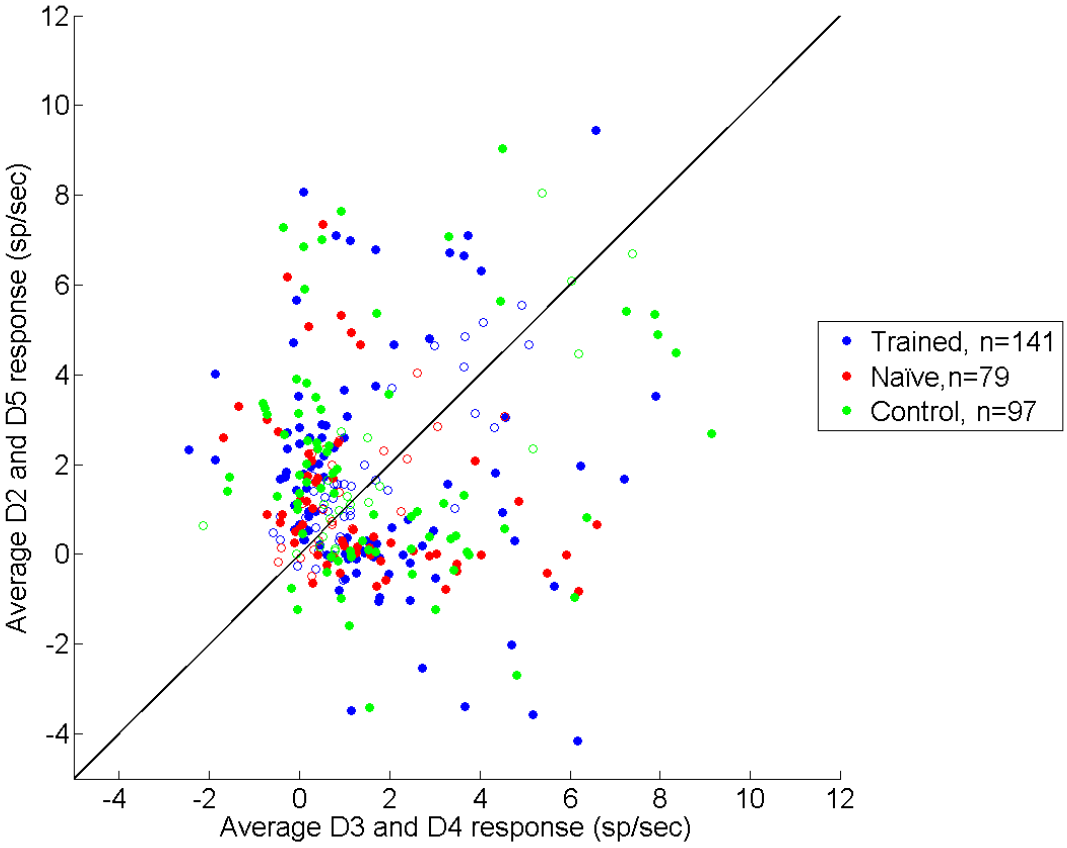
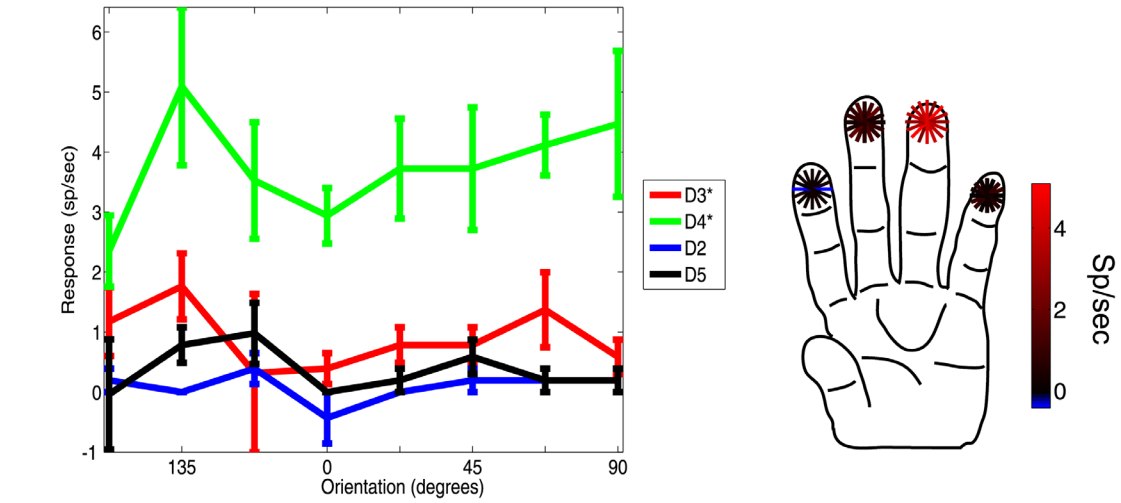


Figure 4.10. Training does not enhance responses of cells to stimuli on the trained digits. Black line is unity; cells in the trained hemisphere do not lie preferentially above unity. Filled circles: significant (Wilcoxon rank sum, $p<0.05$) difference between average responses of D3 and D4 versus average responses on D2 and D5.

a. Tuning curves for cell in trained hemisphere (4.2a)



b.Tuning curves for cell in naïve hemisphere (4.2b)

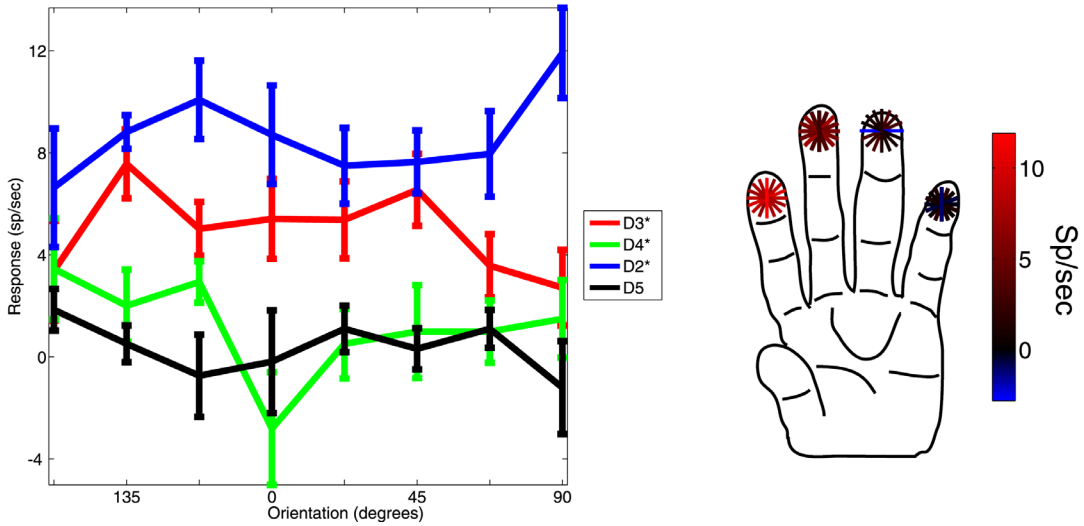


Figure 4.11. Tuning curves on various digits for example cells in Figure 4.2. These example cells were not significantly tuned on any tested digit. The zero degree orientation is oriented horizontally across the digit, the 90-degree orientation parallel to the long axis of the finger.

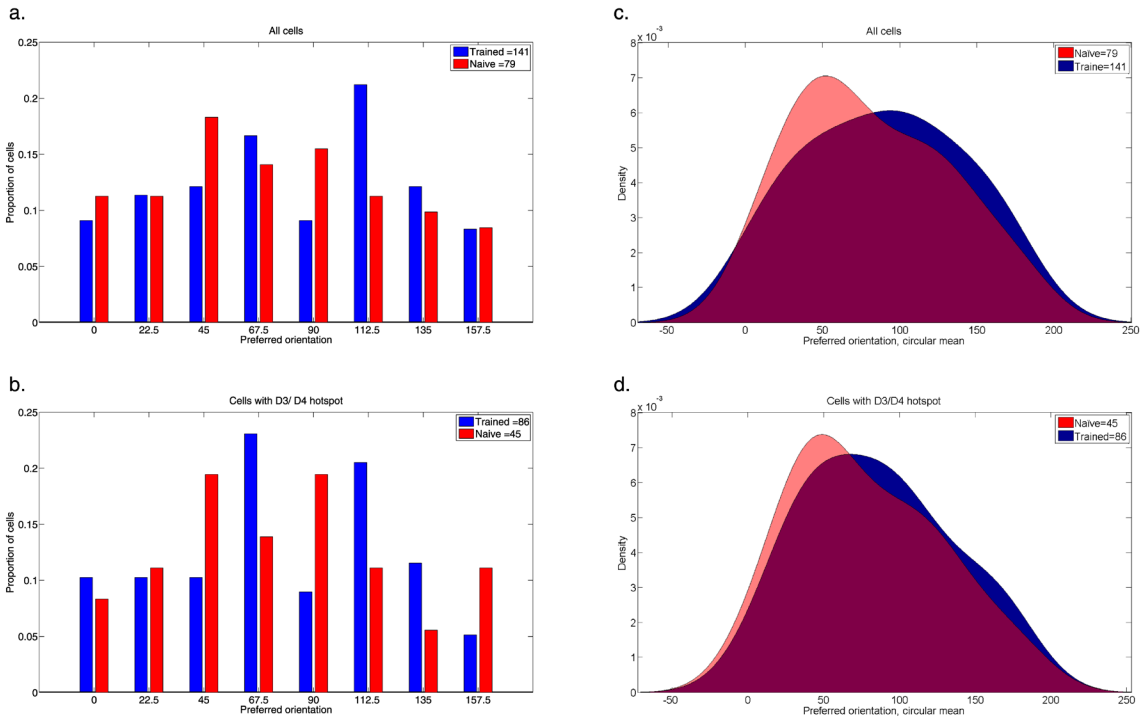


Figure 4.12. Training does not alter distribution of preferred orientations. (A) Histogram of population for preferred orientation only at tested orientations. **(B)** Probability density plot for average preferred orientation calculated as vector average of responses. **(C-D)** Preferred orientations for cells with excited hotspot on D3 or D4.

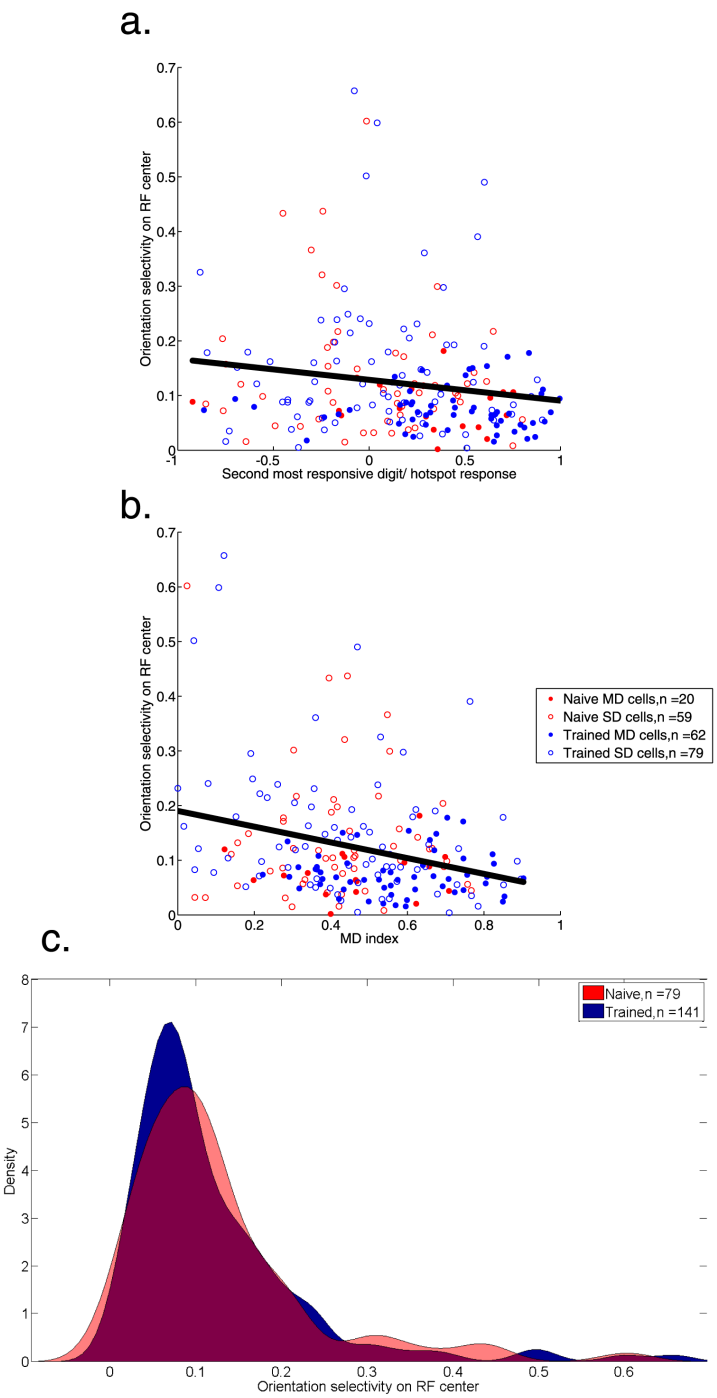


Figure 4.13. Orientation selectivity of 3b cells is not altered with training. (A) Relationship between ratio of second most responsive digit to hotspot response and orientation selectivity (measured on hotspot) ($p=0.02$, $R^2=0.03$). **(B)** Relationship between MD index and orientation selectivity. Black line, fitted curve ($p<0.001$, $R^2=0.08$) **(C)** Probability density function of orientation selectivity for cells in trained (blue) and naïve (red) hemisphere.

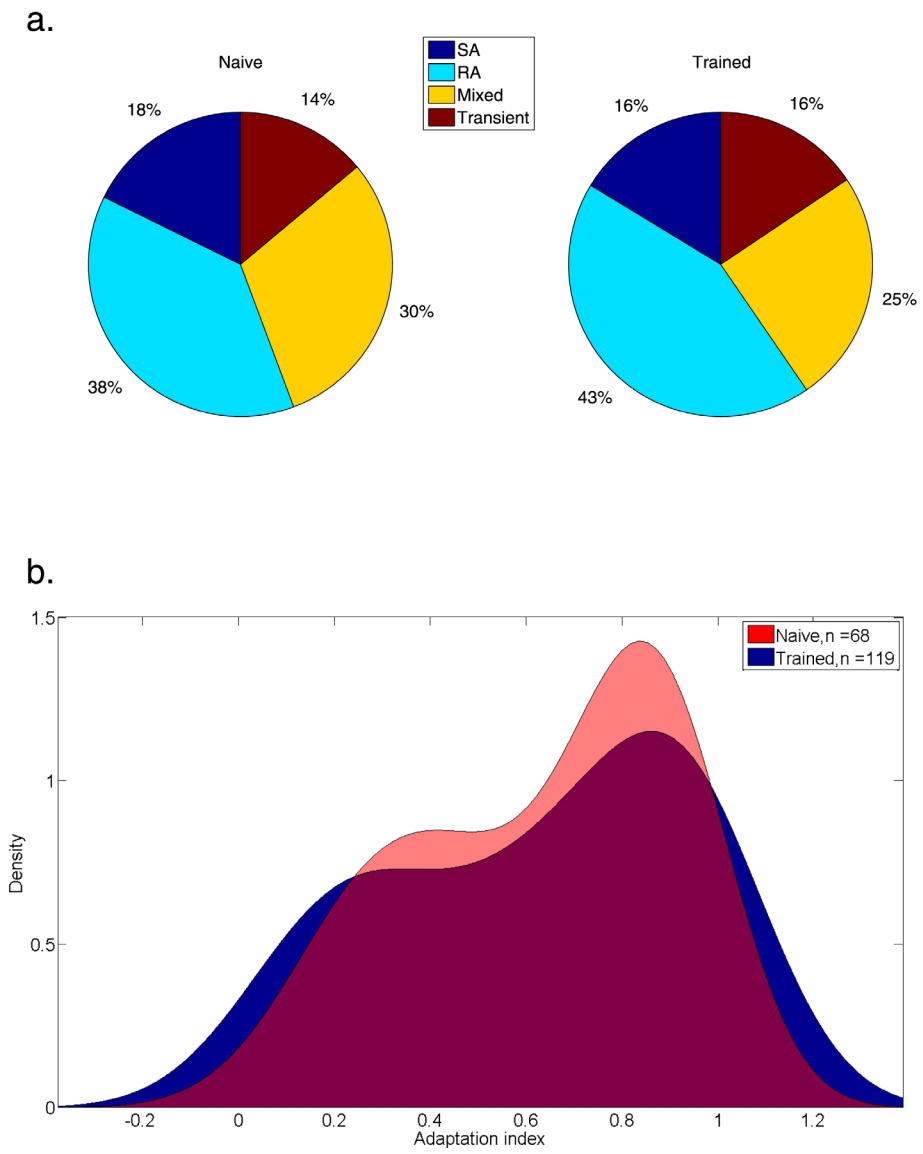


Figure 4.14. Cells' submodality specificity and adaptation indices are not altered with training. (A) Proportion of cells in the trained and naïve hemispheres exhibiting a significant sustained but no significant off response (blue-"SA-like"), a significant off but not sustained response (cyan-"RA-like"), both significant off and sustained response (yellow- "Mixed") and neither a sustained nor offset response (red-"Transient"). **(B)** Probability density function of adaptation index for cells in the trained (blue) and naïve (red) hemispheres.

4.3. Discussion.

Consistent with the results of Wang et al (1995), we found that after training rhesus macaques on a multi-digit task, we observed a higher proportion of 3b cells significantly responsive across several digits compared to an animal that had not experienced any tactile training (Figure 4.3). This result held when one considered cells with typical excitatory responses to the bar stimuli. One will note that the cells with significant inhibited responses (compared to spontaneous rate) to the stimuli were excluded from most of the analysis in this chapter; these cells are discussed in the following chapter. We observed a higher proportion of multi-digit responses in naïve 3b than previously described by other authors (25% versus 7% by Iwamura and colleagues, 1983), though we still find that excitatory RFs in 3b are often confined to single digits, particularly when tested at small indentations.

While we planned to use the control hemisphere as an untrained hemisphere and combine these data with those of the naïve hemisphere, we found a higher proportion of cells with multi-digit RFs in this hemisphere following training on the ipsilateral hand. As the animal could not immediately perform the multi-digit task on his untrained/ control hand, this result suggests that expansion of RFs in 3b is not sufficient for performing the one-back distal task. However, it is possible that RF expansion could correlate with faster learning of the multi-digit task if the animal was trained on the opposite hand. To prevent transfer effects across hemispheres from affecting our conclusions, we chose to compare responses of cells from the trained hemispheres to the naïve hemisphere. Our quantification of responses to four tested distal digits further supported the conclusion that cells in the trained hemisphere had more “multi-digit”-like

responses compared to cells in the naïve hemisphere. Both the relative responses of another digit compared to the hotspot digit, as well as the overall similarity of responses across digits (MD index) were significantly greater for cells recorded in the trained hemisphere compared to the naïve hemisphere (Figure 4.4 and Figure 4.5). While these measures positively correlate with spontaneous rate (Figure 4.6), RF expansion does not seem to be an epiphenomenon due to overall increased spontaneous rate of cells in the trained hemisphere, as cells in the trained hemisphere still had more similar responses across digits when we controlled for spontaneous rate. We acknowledge that we have no knowledge of the cortical layer from which these cells originated; previous authors have found that RF size is larger (though still described as confined to a single digit pad) in supragranular and infragranualar than granular layers (Sur et al., 1985). However, because we randomly sampled cells from all electrodes that were oriented perpendicular to the pia surface and parallel to the central gyrus throughout recording, we believe our results are unlikely to be explained by consistent recording in certain layers in the trained animals and other layers in the naïve animal.

We found that RF size decreased with indentation depth, and that the greatest effect of training on RF size could be observed at the largest tested indentation depth (Figure 4.8 and 4.9). We had originally hypothesized that multi-digit expansion occurs as weak inter-digit connections through 3b are strengthened, and therefore, the effect of training may be more apparent at the smaller tested indentations (that is, stimuli at large indentations could activate these weak inter-digit connections in the naïve animal). However, many of the cells tested only responded at the largest indentations, particularly

in the trained hemisphere. This is in contrast to Wang and colleagues (1995), who observed multi-digit responses of cells tested at 50 μm in the trained animal.

We wished to expand further upon the results of Wang and colleagues (1995) by exploring whether features of the stimuli used during months of training determined changes in RF properties. We first asked if the location of the stimuli would specify RF plasticity and if cells in the trained hemispheres would have greater responses across the trained digits, but did not find this to be the case (Figure 4.10). We did not find that training changed the proportion of cells representing the horizontal orientation or orientation selectivity (Figure 4.12 and 4.13). We therefore conclude that while this training may expand responses to multiple digits, it does not alter orientation-tuning properties, at least as measured on single digit pads. It is possible that this training alters responses to oriented bar stimuli presented over several digits when the stimuli are presented simultaneously (i.e. a curve that spans several digits); these data only examines responses to bars presented on a single pad at a time. Finally, we did not find that training altered the temporal profile of responses, as measured outside the multi-digit task (Figure 4.14). Chapter 6 will explore the temporal pattern of responses during performance of the multi-digit task. As previous authors had observed (Pei et al., 2009), we found that many 3b cells exhibited both transient responses to stimuli onset and offset, similar to rapidly adapting peripheral afferents, and sustained responses to stimuli, similar to slowly adapting afferents. We additionally find cells with significant but only initially transient responses to the bar stimuli. We acknowledge that the stimuli used were likely not ideal in these cases, as other authors have observed transient responses to non-preferred stimuli compared to sustained responses to preferred stimuli (Wang et al., 2005).

Overall, these data support the finding that training animals over several months in a task that involves multi-digit stimuli expands the RF size of cells in 3b. However, this process appears to occur over both hemispheres in the trained animal. Additionally, we find that this multi-digit training task did not alter stimulus feature selectivity. It suggests that RF expansion, by itself, is not sufficient to predict task proficiency. The relevance of these conclusions with those of Chapter 2, human psychophysics, is discussed in Chapter 7.

CHAPTER 5. RESPONSES AND FEATURE SELECTIVITY ACROSS DIGITS IN PRIMARY SOMATOSENSORY CORTEX, AREA 3B.

While the previous chapter explored how training modifies RF properties in area 3b, our data revealed more general principles of 3b processing, particularly cells' responses across several digits. This study, to our knowledge, is the first of its kind to record from single units in 3b and map RFs across digits with oriented bar stimuli. Previous studies that have quantitatively described RF size, shape, and stimulus selectivity in 3b have defined these properties on a single finger pad, typically chosen by the experimenter after hand-held probes established a cells' hotspot digit (e.g. DiCarlo et al., 1998; DiCarlo and Johnson, 2000; Bensmaia et al., 2008; Pei et al., 2009, 2011). A few studies have presented stimuli which spans several digits and described inhibitory and nonlinear interactions among digits in 3b (Chen et al., 2003; Reed et al., 2010; Thakur et al., 2012). One study had observed, as described in the previous chapter, that 3b classical RFs in a naïve animal can often extended across digits (Lipton et al., 2010), though this study used large probe-like stimuli encompassing the entire digit and did not record from single neurons. Therefore, our study was unique in its ability to characterize 3b processing and feature (i.e. orientation) selectivity over several digits at the single neuron level.

In our analysis we observed that a small proportion (~20%) of cells had overall inhibited responses to the bar stimuli relative to baseline or spontaneous rate. We

acknowledge that we have no knowledge of the molecular or pharmacological principles of our cells. The balance of excitation and inhibition appears necessary for cells' feature selectivity (DiCarlo et al., 1998; DiCarlo and Johnson, 2000), and it has been proposed that inhibitory drive often lags excitatory thalamocortical input and serves to enhance acuity (Shapley et al., 2003; Zhang et al., 2003; Sripati et al., 2006). We asked if these inhibited cells had different properties (e.g. size, orientation tuning, temporal response) than cells with typical excitatory RFs.

Neural responses to bar stimuli on all digits and finger pads has been recorded in SII cortex, where it has been observed that cells exhibit similar orientation preference across multiple adjacent tuned pads (Fitzgerald et al., 2006a). We therefore asked how stimulus selectivity was represented across digits in an earlier stage of cortical processing; hypothesizing that feature selectivity across digits is an emergent property of somatosensory cortices and is not present in 3b. We also examined the temporal properties and characterized submodality specificity (i.e. similarity to afferent type) across digits, hypothesizing that submodality specificity would not be maintained across digits. This is based on data that submodality specificity is not maintained within a single digit. That is, cells in SI cortex do not fall into discrete rapidly adapting or slowly adapting type categories (Pei et al., 2009; Carter et al., 2014). Such data could add to our understanding how feature selectivity across digits is represented throughout the somatosensory hierarchy.

5.1. Specific methods.

5.1.1. Latency.

For each cell, we calculated the latency of the response on a digit with respect to the onset of the tactile bar stimulus (averaged over all orientations). This was defined as the steepest change during the first 40 msec window where a cell's response was significantly different than baseline (Wilcoxon rank sum $p<0.01$, two consecutive 20msec bins in the same direction relative to baseline, see Chapter 3.8). This measure was therefore only calculated on digits with a significant response. We feel this calculation allowed for a precise determinant of latency that could be flexible given the varying temporal responses of somatosensory neurons but not be biased by transient temporal fluctuations in the neural response.

All other receptive field properties were quantified in the same manner as described in Chapter 3 and Chapter 4.1, specific methods. By using bar stimuli on each digit, we were able to quantify orientation selectivity and the temporal profile of responses on each digit. See Chapter 3 for more detailed descriptions of the experimental design and data analysis.

5.2. Results.

5.2.1. RF size for cells with excitatory and inhibitory responses to bar stimuli.

We observed cells with typical significant excitatory responses to the indented bar stimuli, as well as those with significant inhibitory responses compared to baseline firing rate. Several example cells are depicted in Figure 5.1. These include a cell with a

significant response on only one tested digit (Figure 5.1a) and ones with significant responses on several tested digits (Figure 5.1b-c). We classified cells as inhibited or excited based on the response of their hotspot digit; that is, the digit with the absolute greatest response relative to baseline (see Chapter 3.9, response averaged across all orientations). We examined 370 cells with a significant response on at least one tested digit; 53 of these were classified as inhibited and 317 as excited cells. 35 of these inhibited cells were from the trained hemispheres, 12 from the naïve hemisphere, and 6 from the control hemisphere. The proportion of inhibited cells was not statistically different in the trained compared to naïve hemisphere (χ^2 (1,N=267)=1.85, p=0.17), though there were a higher proportion of inhibited cells in the trained compared to the control hemisphere (χ^2 (1, N=279)=10.25, p=0.001). The two trained hemispheres did not exhibit statistically different proportions of inhibited cells (χ^2 (1, N=176)=2.66, p=0.10). Multi digit cells with inhibited responses on the hotspot digit (e.g. Figure 5.1c) were equally likely to exhibit inhibited or excited responses on the other responsive digits, while cells with an excitatory hotspot were slightly more likely to have homogeneous (all excited) responses across digits (Figure 5.2).

We noted that across the population, inhibited cells were more likely to have significant responses across multiple digits (Figure 5.3a, χ^2(1, N=370) =10.02, p=0.002). Among cells with an inhibited response on the hotspot digit, 74% had significant responses across several digits, while 50% of excited cells were classified as multi-digit (significant responses on other digits could be excited or inhibited compare to baseline). We also found that responses across digits were more similar in inhibited than excited cells. Figure 5.3b is a scatterplot of the relative responses of adjacent digits relative the

hotspot digit response. One will notice that inhibited cells (blue) are located more at the extremes of this plot, where other digits responding as strongly as the hotspot digit. We compared the ratio of second most responsive digit to the hotspot response on inhibited and excited cells. We found (Figure 5.3c) that these distributions were significant different from one another (Kolmogorov-Smirnov test, KS test statistic=0.24, p=0.006), though the means were not (Mann-Whitney U test, Z=0.24, p=0.81). We took the absolute value of the second most responsive digits' responsive relative to the hotspot for a more accurate measure of strength across these two digits without regard for the sign of the response (Figure 5.3d). We found this measure was significantly higher in inhibited cells (0.63 versus 0.41, inhibited and excited respectively, Mann Whitney U- test, Z= 5.50, p<0.001, Cohen's d=0.29). We also calculated a multi-digit (MD) index for each cell to compare similarity of responses across the four tested digits. See Chapter 4.1 for a full description of this calculation; values could range from 1, for a cell with exactly equal responses on all four digits, to 0, for a cell responding singularly on one digit. This measure was significantly higher in inhibited cells than in excited cells (0.62 versus 0.47, inhibited and excited respectively, Mann Whitney U-test, Z=5.01, p<0.001, Cohen's d=0.26, Figure 5.4b).

5.2.2. Baseline/ spontaneous rate of inhibited and excited cells.

Perhaps unsurprisingly, we observed that inhibited cells had a significantly higher baseline response than excited cells (11.85 versus 3.77 spikes/sec, inhibited and excited, Mann Whitney U-test, Z=9.01, p<0.001, Cohen's d=0.47, Figure 5.5a). We asked if measures of receptive field size positively correlated with baseline rate, and found this to

be the case, both when quantified over the two most responsive digits (Figure 5.5b, R^2 = 0.13, $p<0.001$) and over all digits (Figure 5.5c, MD index versus baseline, R^2= 0.13, $p<0.001$). We therefore asked if inhibited cells had larger RFs when we controlled for spontaneous rate, performing ANCOVAs with baseline as a covariate and sign of response relative to baseline (inhibited or excited) as the between-subject factor. We found that cells with inhibited RFs still had more similar responses across the two most responsive digits when we controlled for spontaneous rate (adjusted means, ratio of second most responsive digit to hotspot response, absolute value: 0.54 vs. 0.49, inhibited versus excited, $F(1,369)=6.55$, $p=0.01$, $\eta_P^2=0.02$) but enhanced responses across all digits was no longer significant when we controlled for spontaneous rate ($F(1,369)=3.19$, $p=0.08$). It therefore seems that inhibited cells' increased likelihood of responding across digits is at least partially due to enhanced spontaneous rate of these cell types.

5.2.3. Orientation selectivity of inhibited and excited cells.

We found that measures of RF size correlated negatively with orientation selectivity (OS, measured as the circular variance of the average response to eight equally spaced oriented bars). As the two most responsive digits became more similar, and as MD index increased, OS decreased on the cells' hotspot (Figure 5.6 a-b). In addition, OS decreased with increasing baseline response (Figure 5.6c).

Orientation selectivity on the hotspot digit was lower for inhibited than excited cells (0.07 versus 0.12, Figure 5.6d, Mann-Whitney U test, $Z=-3.83$, $p<0.001$, Cohen's $d=0.23$), and excited cells were more likely to be significantly tuned on their hotspot digit (43% of excited cells were tuned versus 15% of inhibited cells, χ^2 (1, N=339) =13.56,

$p<0.001$). However, re-running our analysis, using baseline as a covariate, showed this difference was no longer significant when we controlled for the enhanced spontaneous rate of inhibited cells (ANCOVA between inhibited and excited cells, $F(1,338)=0.08$, $p=0.78$). These results are likely due to the fact that the calculation for orientation selectivity (Ringach et al., 2002b) uses the absolute response of the cell and does not subtract baseline response.

5.2.4. Temporal response of inhibited versus excited cells.

We next asked if the temporal properties of responses were different in inhibited versus excited cells. First we examined if inhibited cells had stronger sustained or off responses to stimuli. We then compared the latency of responses for inhibited and excited cells, hypothesizing that these inhibited cells would have slower latencies, as lagging inhibitory drive is a defining characteristic of SI responses (DiCarlo et al., 1998; DiCarlo and Johnson, 2000; Sripati et al., 2006).

We found that the distribution of cells falling into our four submodality-specific categories (SA-like, RA-like, Mixed, and Transient) was different for inhibited and excited cells (Figure 5.7a, χ^2 $(3,N=370)$ $=52.81$, $p<0.001$). Post-hoc chi squared tests determined that inhibited cells were more likely to be categorized as "transient" or "SA-like" and less likely to be "RA-like" than excited cells (Transient: χ^2 $(1, N=370)$ $=9.84$, SA-like, χ^2 $(1,N=370)$ $=29.95$, RA-like, χ^2 $(1,N=370)$ $=31.32$, Bonferroni corrected for four comparisons, $p<0.05$). We further tested the adaptation indices (AI) of cells with significant sustained and/or offset responses (38 inhibited cells, 279 excited cells), and found that these inhibited cells' AI was significantly lower than excitatory cells (Figure

5.7b, 0.16 vs. 0.63, Mann-Whitney U test, Z=-8.32, $p<0.001$, Cohen's d=0.47). Inhibited cells were therefore more likely to have either transient 'on' responses to the stimuli or sustained responses to indented bars, and were less likely to respond at the offset of stimuli. It is unclear if this difference points to a functional difference between these two types of cells; cortical cells with sustained responses are thought to carry more information about the form of an object, and in excitatory cells, orientation selective neurons are more likely to be SA-like (Bensmaia et al., 2008). As the last section described, inhibited cells were less tuned for orientation (though perhaps they carry more orientation information when their signal is compared to spontaneous rate).

We compared the latency of the response of cells on the hotspot digit (defined as the steepest rise in response when the cell responded significantly, see 5.1.1), and found that it was greater in inhibited than excited cells (Figure 5.8b, 110 versus 89 msec, Mann-Whitney U test, Z= 4.35, $p<0.001$, Cohen's d=0.22). That is, inhibited cells responded slower on the hotspot digit than excited cells. We did not find any relationship between RF size, measured as the similarity of response for the two most responsive digits, and latency (Figure 5.8a).

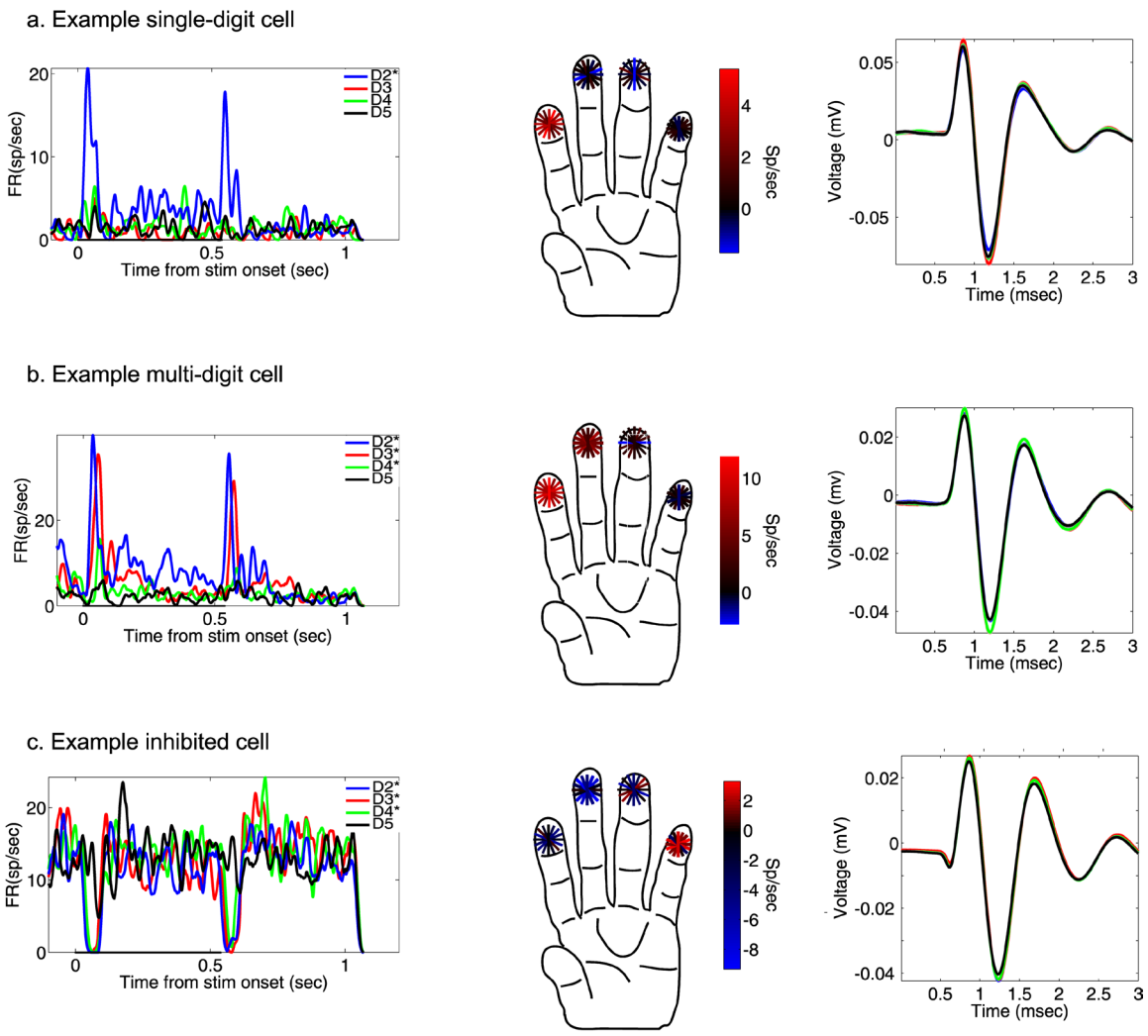


Figure 5.1. Example cells. (A) Single digit cell, only significantly (Wilcoxon rank sum, p <0.01, two consecutive 20msec bins) excited on digit 2. Middle: Responses to specific tested orientated bars. Right: Average action potential waveforms from protocols on the four tested distal digits showing no change in AP shape while testing various digits. **(B)** Example multi-digit cell, significantly responsive on digits 2, 3, and 4. **(C)** Example cell with an inhibited response to the bar stimulus.

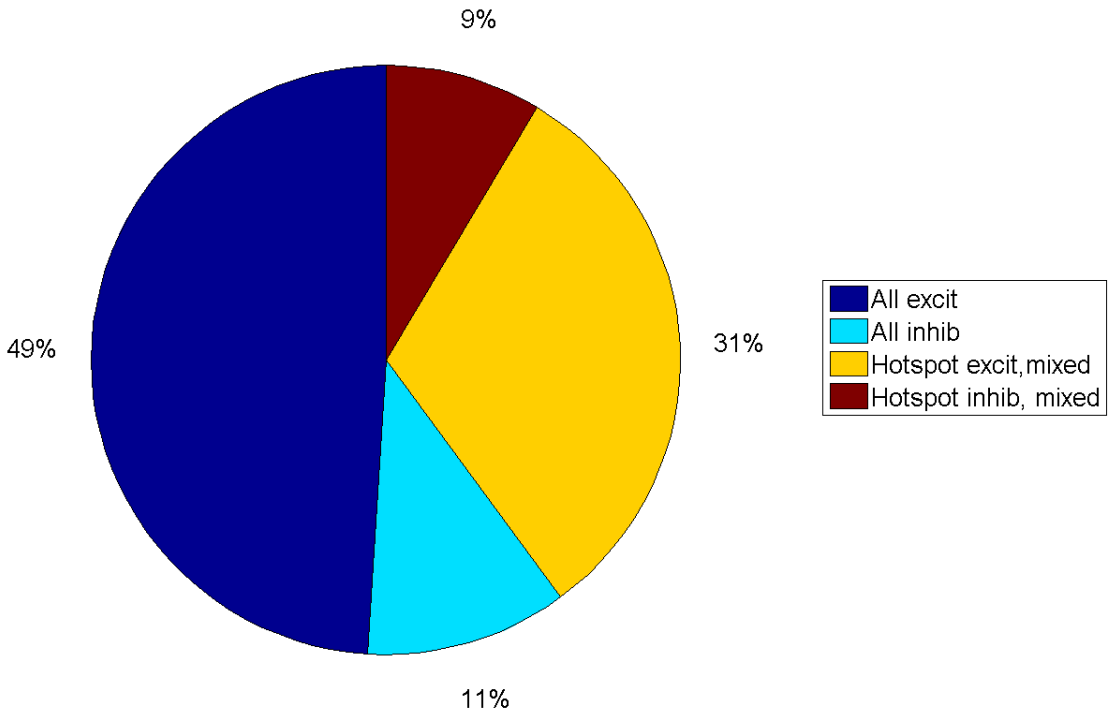


Figure 5.2. Distribution of sign of responses (positive or negative compared to baseline) across digits for multi-digit cells (N=198). Cells with inhibited responses are more likely to exhibit inhibited responses on other responsive digits.

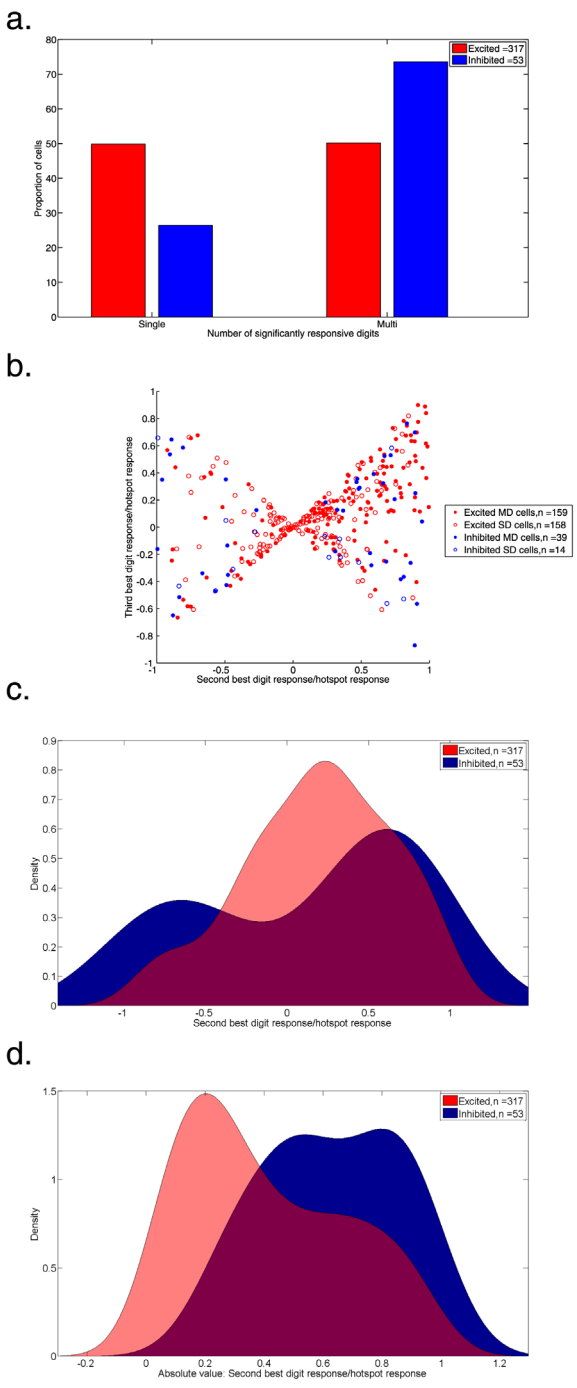


Figure 5.3. Inhibited cells have more equal responses across several digits. (A) Proportion of inhibited or excited cells with either a significant response on one or several tested digits. **(B)** Scatter plot of relative response to other digits compared to the hotspot response. Filled circles are cells with significant responses on several digits. **(C)** Density plot of second most responsive digit response relative to hotspot. **(D)** Density plot of the absolute value of second most responsive digit response relative to hotspot.

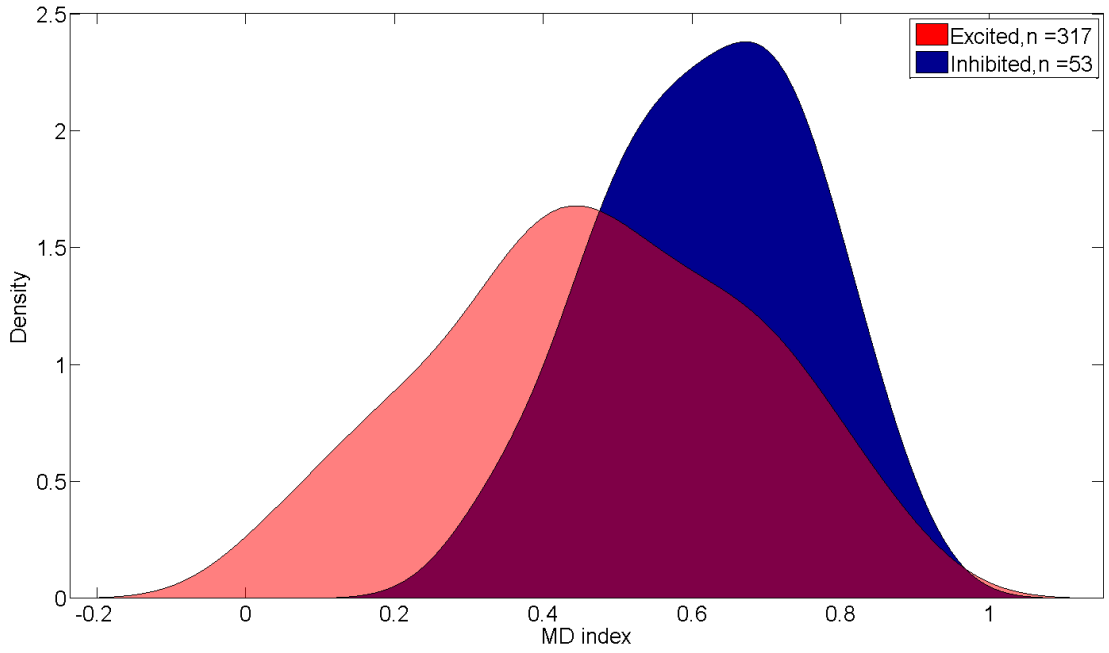


Figure 5.4. Inhibited cells have more equal responses across all four tested digits. Density plot of MD index for excited (red) vs. inhibited (blue) cells.

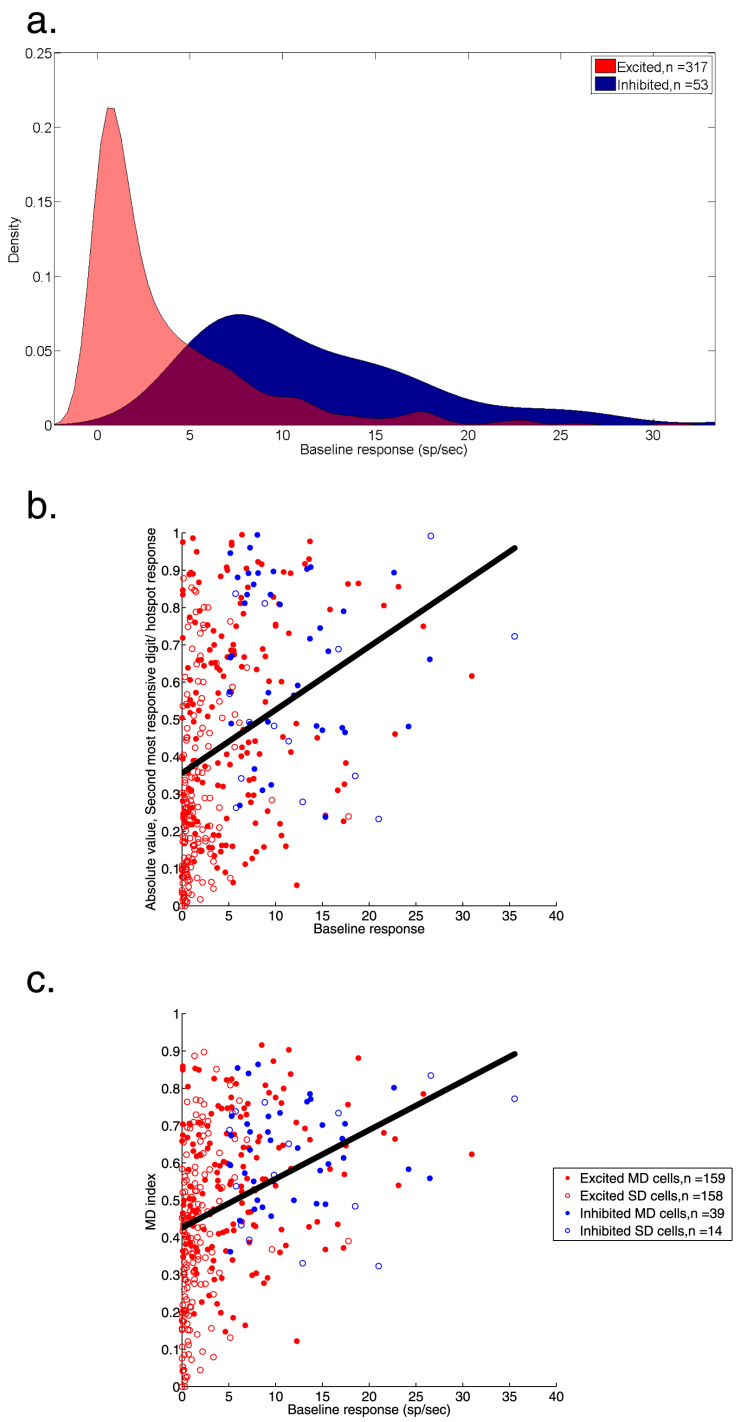


Figure 5.5. Inhibited cells have higher baseline responses that correlate to larger RF size. (A) Density plot of baseline response for inhibited (blue) versus excited (red) cells. **(B)** Positive relationship between baseline response and relative response of second most responsive digit to hotspot. **(C)** Positive relationship between baseline and MD index.

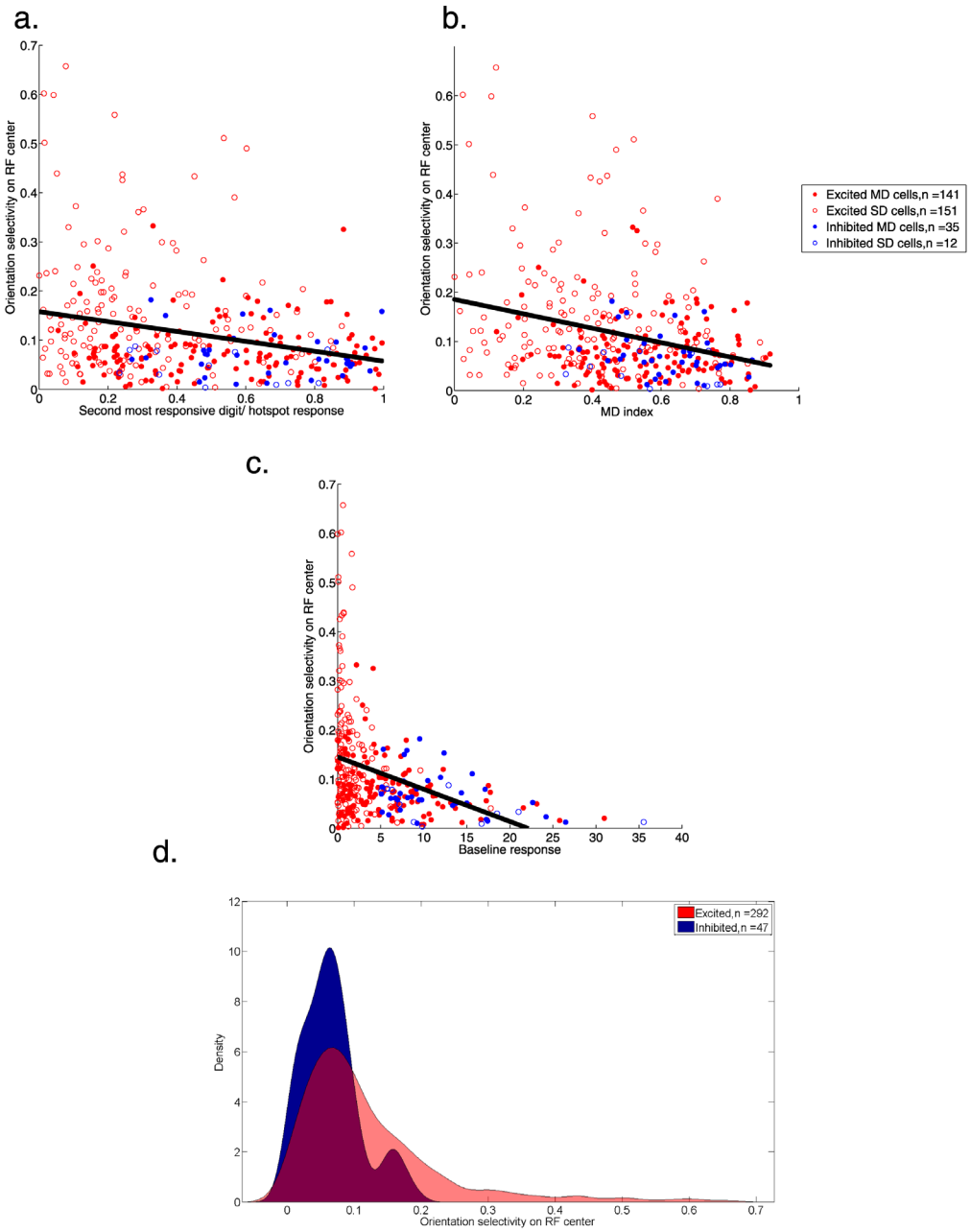


Figure 5.6. Orientation selectivity (OS) correlates with measures of RF size and spontaneous rate and is lower in inhibited cells. (A) Scatterplot of cells' relative response to the two most responsive digits versus OS on the hotspot digit. Blue cells had an inhibited response on the hotspot digit; filled circles were multi-digit cells. Black line; linear fitted curve: $p<0.001$, $R^2= 0.07$ **(B)** Negative linear relationship between MD index and OS (black fitted line, $p<0.001$, $R^2= 0.08$. **(C)** Negative relationship between baseline/spontaneous FR and OS (black line: $p<0.001$, $R^2= 0.12$). **(D)** Probability density plot for OS in inhibited and excited cells.

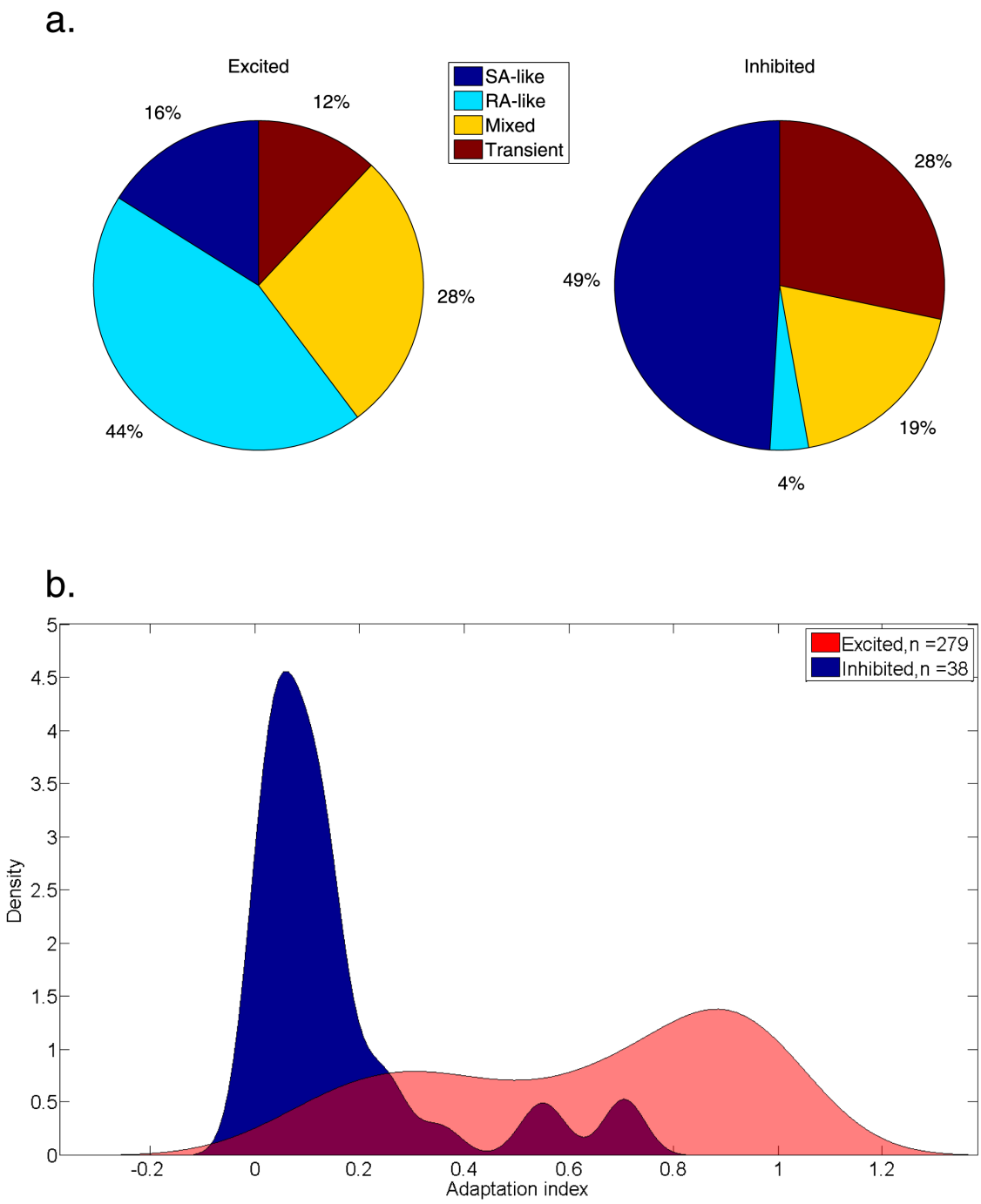


Figure 5.7. Increased sustained and decreased off responses in inhibited cells. (A) Distribution of submodality preference on the hotspot digit for excited and inhibited cells. **(B)** Density plot of adaptation indices on the hotspot digit for cells with a significant sustain and/or offset response.

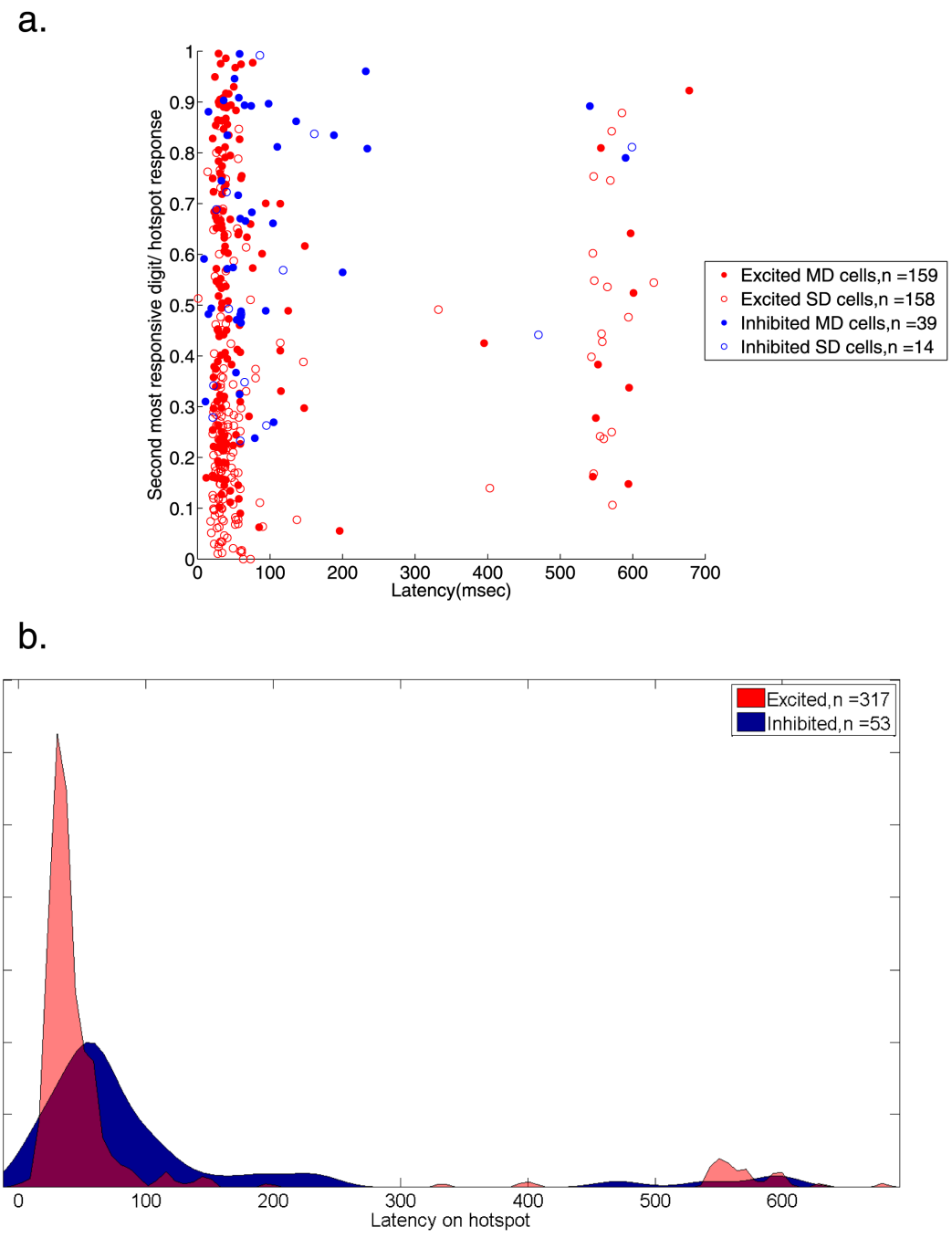


Figure 5.8. Increased latency of response on the hotspot digit for inhibited cells. **(A)** There was no relationship observed between the relative responses across two digits and the latency on the hotspot digit. **(B)** Probability density plot of latency for inhibited and excited cells; inhibited cells have significantly longer latencies on the hotspot digit.

5.2.5. Temporal response properties across digits for cells in 3b.

Although it has been established that the precise temporal properties of peripheral afferents is not maintained for individual cells on a single digit in SI or SII cortices (Pei et al., 2009; Carter et al., 2014), we asked if any aspects of the temporal response of cells were conserved across multiple digits for cells in SI cortex. First, we asked if multi-digit cells had similar submodality preference across responsive digits. We found that, among multi-digit cells, most did not have the exact same submodality preference across the responsive digits (Figure 5.9). However, when we examined cells that had significant responses on at least two digits and significant sustained and/or off responses on both digits (N=118), we observed that the adaptation indices were similar across the two most responsive digits (Figure 5.10 black line; R^2= 0.43, p <0.001). This effect was likely driven by cells with adaptation indices close to one (RA-like) across two digits (upper right quadrant, Figure 5.10). We conclude that for those cells with clear input from slowly adapting or rapidly adapting afferents, submodality (particularly for rapidly adapting peripheral input) may be crudely maintained across two digits, though it does not appear to be maintained across the entire hand.

We also asked if cells with multi-digit receptive fields responded at a similar latency across digits (see example cell in Figure 5.1b where the non-hotspot digits responded slower than the hotspot digit). We hypothesized that if responses on the hotspot digit are due to thalamocortical ascending input and responses on non-hotspot digit are the result of corticocortical interactions versus divergent thalamocortical ascending input, the hotspot would have a faster response than adjacent digits. We observed diversity in the latency of responses across digits, though most lay around the

unity line (Figure 11). A Wilcoxon sign-rank test found that average difference between latencies across the hotspot and second most responsive digits was different from 0 (Z=-2.92, p=0.005). The median of the difference in latency between the hotspot and the second digit was 3 msec (mean, 30 msec, likely due to outliers).

5.2.6. Orientation tuning across digits for cells in 3b.

As it has been observed that cells in SII exhibit similar orientation preference across digits (Fitzgerald et al., 2006), we asked if this property is found to any degree in SI cortex, area 3b. This would add to information about how shape perception across digits is represented along the somatosensory hierarchy. We examined cells' with significant responses on at least two digits, and assessed their preferred orientation (calculated by the average vector response) on the two most responsive digits. Figure 5.12 is a scatterplot of the difference between preferred orientations across two digits; one will notice that cells do not lie preferentially around zero (indicating similar orientation preference on two digits). A Rayleigh test confirmed that this distribution was not statistically different from uniform (Z=0.34, p=0.71). We also did not find a relationship between similarity of tuning and orientation selectivity (that is, cells with sharper tuning do not have more similar tuning across digits; Figure 5.12, R^2= 0.002, p=0.60).

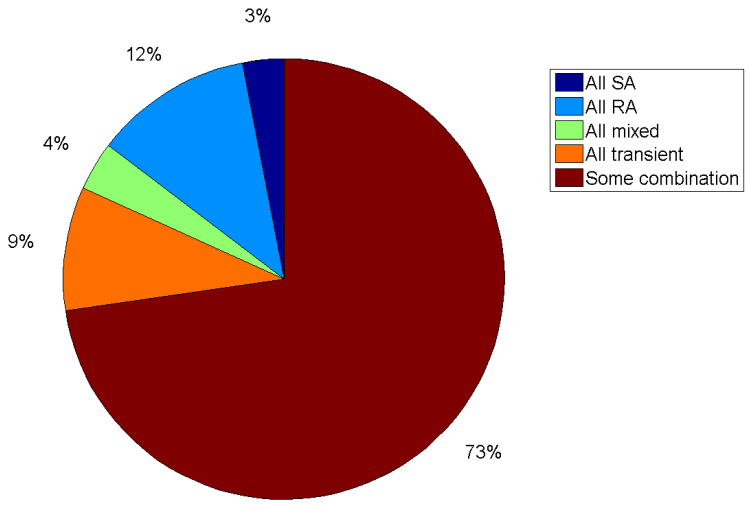


Figure 5.9. Distribution of similarity of submodality preference across significantly responsive digits for multi-digit cells (N=198). Cells were "SA-like" if all significantly responsive digits had only a significant sustain response, "RA-like" if all digits had a significant off response, "Mixed" if digits had both significant sustain and off responses, and "Transient" if they had neither a significant sustain off or sustain response. Most cells' with multi-digit RFs had various temporal responses across digits.

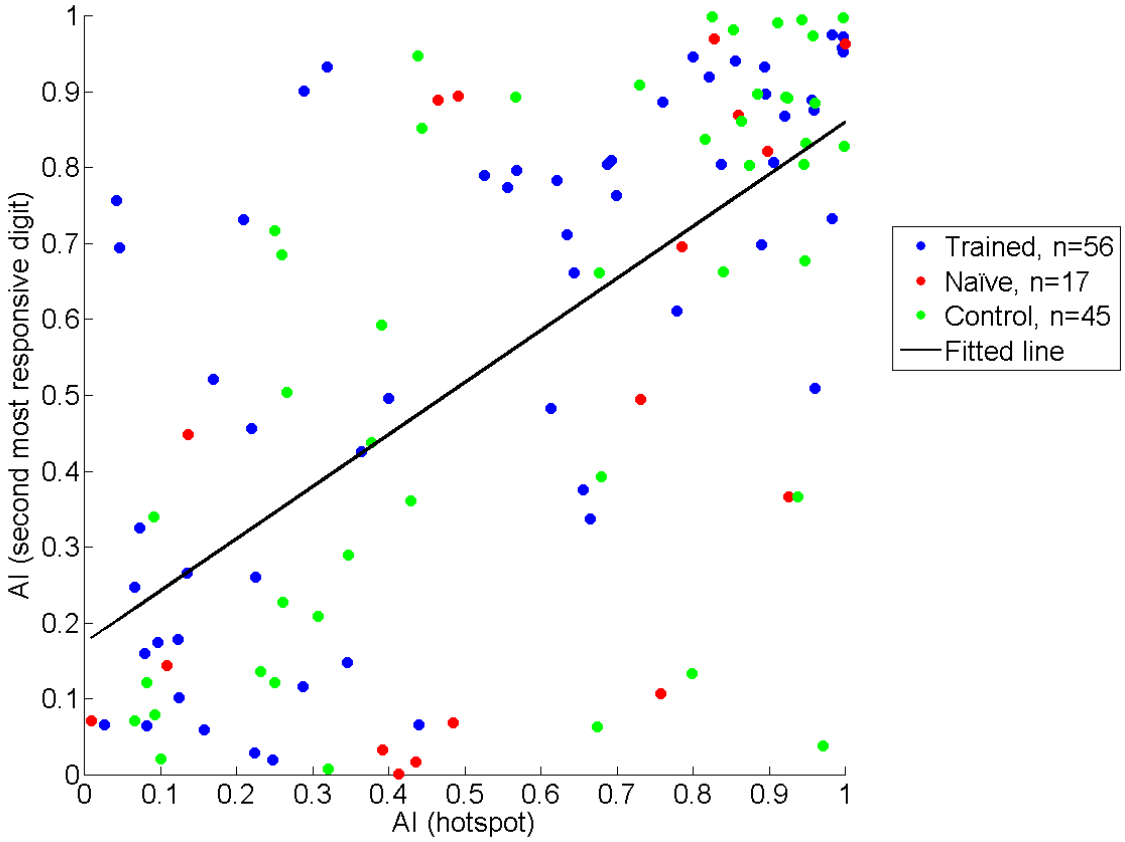


Figure 5.10. Adaptation indices on the two most responsive digits for multi-digit cells. Calculated only for multi-digit cells with significant sustain and/or off responses on two most responsive digits (N=118).

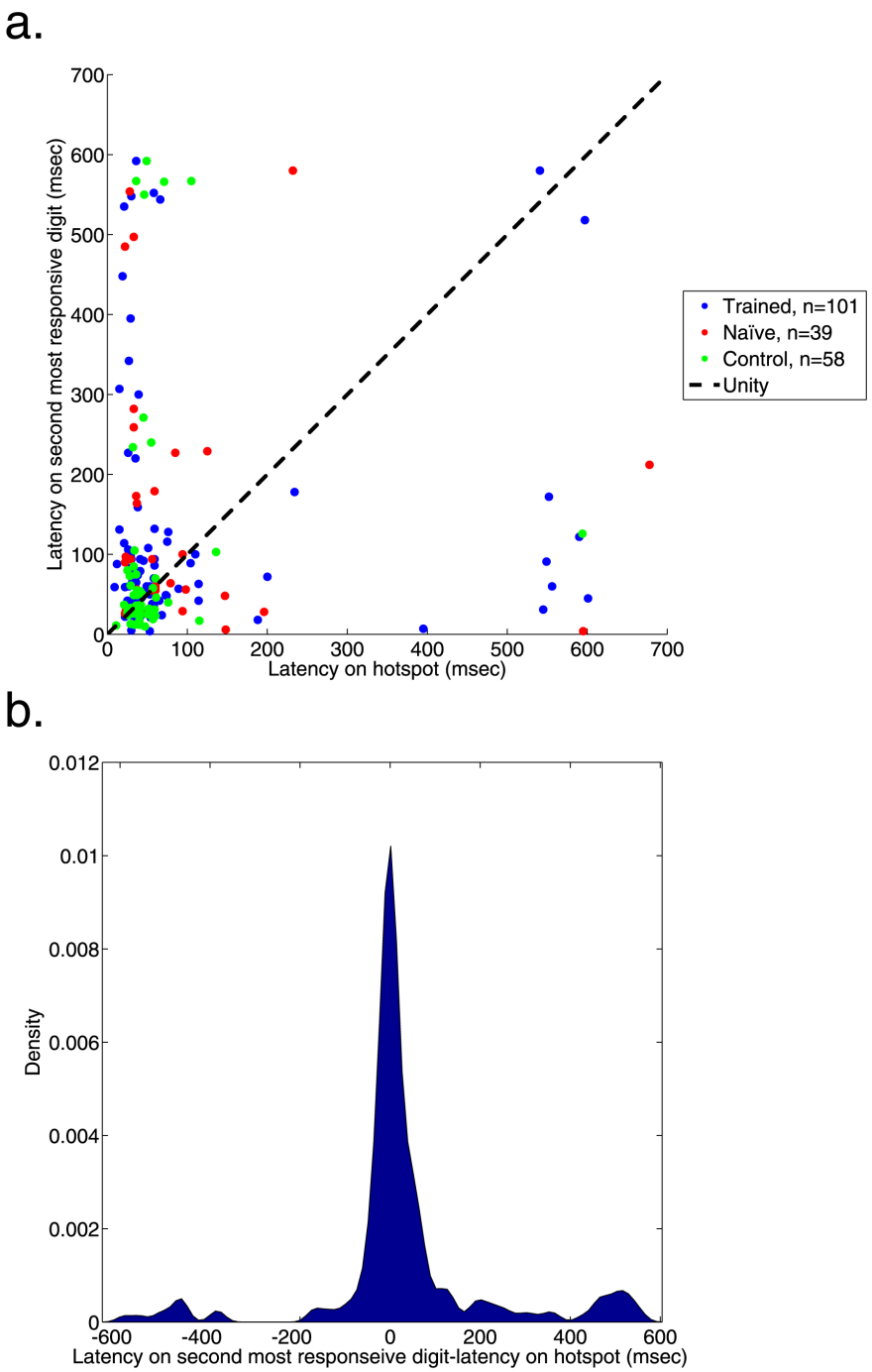


Figure 5.11. Latency of responses across digits in multi-digit cells. (A) Multi-digit cell latency on hotspot versus second most responsive digit, black dotted line indicates unity (exact same latency across two digits). Points above the unity indicate the hotspot had a faster response; points below, indicate the hotspot had slower latency. **(B)** Probability density of difference between latencies: positive= second digit responded slower, negative= hotspot responded slower.

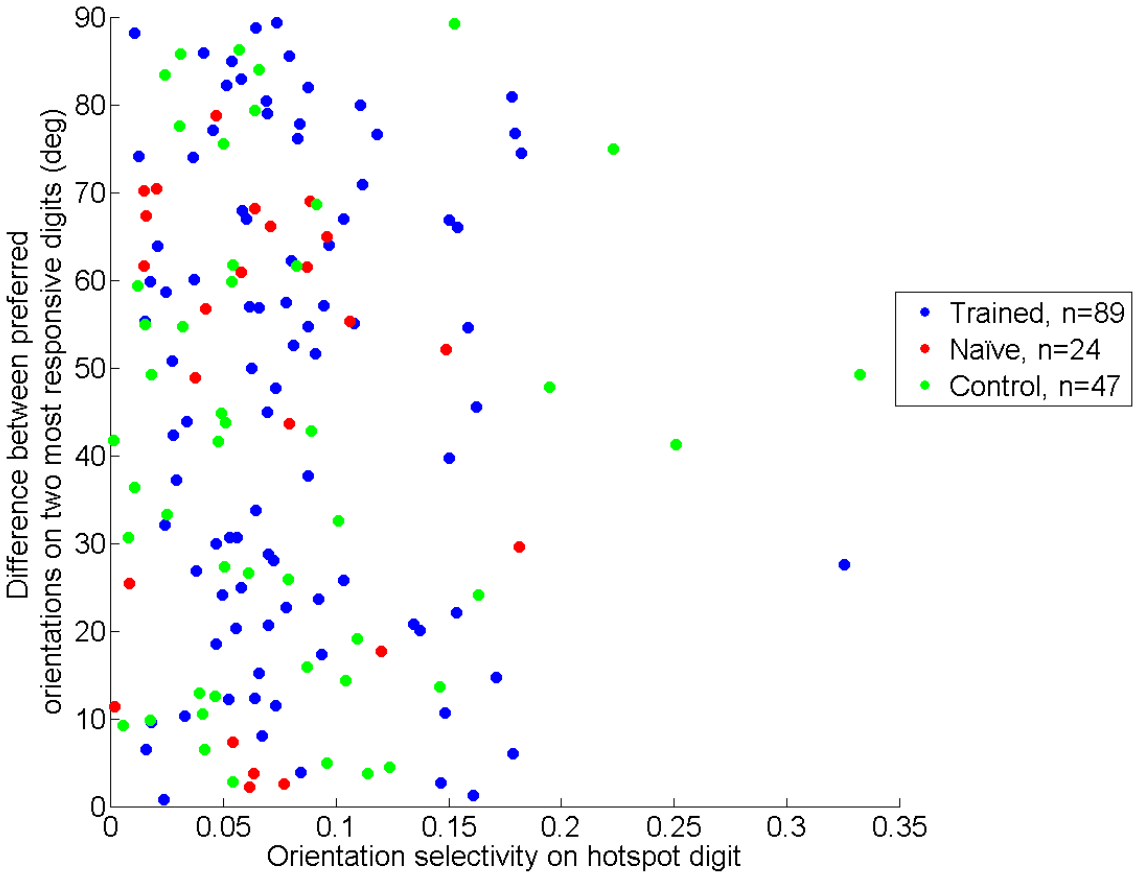


Figure 5.12. Multi-digit cells do not exhibit similar orientation preference across digits. Plotted are cells' orientation selectivity measured on the hotspot digit and the absolute difference between the preferred orientations on the two most responsive digits.

5.3. Discussion.

5.3.1. Mechanisms of inhibition and excitation across digits in 3b.

We found that 3b cells with overall inhibited responses to bar stimuli on the hotspot digit (that is, decreased firing rate compared to spontaneous, N=53) were more likely to have significant responses across multiple digits (Figure 5.3a). Such cells had stronger responses across multiple digits than cells with excited responses on the hotspot digit (Figure 5.3-5.4). Inhibited cells had higher spontaneous rates (Figure 5.5), which may at least partially explain why they were more likely to respond across multiple digits. Finally, we noted that inhibited cells had lower orientation selectivity on their hotspot digit (Figure 5.6), though this was likely due to increased spontaneous rate. Inhibited cells responded transiently or had sustained inhibition to the bar stimulus, often at longer latencies (Figure 5.7-5.8).

Purely inhibited somatosensory responses in 3b to single punctate hand probe stimuli have not been described to our knowledge(see Sur, 1980), though it has long been observed that 3b cells have inhibitory subfields revealed by stimulation using more complex or multi-point stimuli; for example, stimulation of two points on the same digit will reveal surround suppression (Mountcastle and Powell, 1959; Costanzo and Gardner, 1980; Gardner and Costanzo, 1980). DiCarlo and colleagues found that a majority of spatiotemporal RFs in 3b revealed by random dot stimuli scanned across a digit had inhibitory regions (DiCarlo et al., 1998; DiCarlo and Johnson, 2000). A large proportion of cells in these study had excitatory regions flanked by a larger distal and temporally lagging inhibitory region; lagging inhibition was also observed in non-classical RFs observed across digits in 3b (Thakur et al., 2012). We therefore acknowledge that our

"inhibited" cells could have been revealed due to misplacement of the bar stimuli in reference to the excitatory region of the RF (e.g. the excitatory region was located proximal to the bar stimulus and therefore the cell was not sufficiently stimulated on this region); it is also possible that such cells had excitatory dorsal RFs stimulated by the finger or hand holder. Regardless, our results still indicate that inhibitory drive to 3b cells is more likely to extend across several digits.

Although we have no knowledge of the pharmacological or molecular properties of the cells we have recorded from, we hypothesize that those cells with inhibited responses receive increased input from inhibitory cells than those cells with excitatory responses to tactile stimuli. There are many subclasses of inhibitory cells in sensory cortices with varying physiological and response properties (review, Markram et al., 2004). For example, one class, somatostatin-expressing GABAergic neurons, exhibit high spontaneous rates and decreased firing in response to whisker deflection in barrel cortex (Gentet et al., 2012) much like our inhibited cells; these cell types also respond slower than other inhibitory cell types in barrel cortex (Pala and Petersen, 2015).

Many models and data indicate that inhibitory drive is necessary to sharpen activity such that only a localized population will have an excited response to a particular feature (McLaughlin et al., 2000; Ringach et al., 2002a; Shapley et al., 2003; Zhang et al., 2003). In addition, non selective suppression can enhance feature selectivity of downstream cells (Xing et al., 2011). Inhibitory drive in 3b may ensure that most excitatory classical RFs are confined to a single digit, despite ascending divergent input across digits (Garraghty and Sur, 1990; Rausell et al., 1998). In SI cortex, it has been demonstrated that GABA antagonists enhance RF size and increase response latency

(Alloway et al., 1989); further suggesting that inhibitory drive often trails excitatory input and has less spatial acuity. If we hypothesize that our inhibited cells receive enhanced inhibitory drive, albeit from unknown sources, our findings support previous data describing inhibition within sensory cortices.

5.3.2. Feature specificity and temporal profile across digits in 3b.

We observe that feature selectivity and submodality specificity is not maintained across digits in area 3b. This is in contrast to SII cortex, particularly its central field, where cells have similar orientation tuning across several adjacent digits (Fitzgerald et al., 2006a). We do not observe similar orientation preference across digits in our data from 3b. This suggests similarity of tuning across digits, thought to allow for position-invariance and haptic shape perception (Hsiao et al., 2002; Thakur et al., 2006), is an emergent property in the somatosensory cortical hierarchy.

Submodality preference, defined by cortical cells' similarity to afferent responses (specifically slowly adapting and rapidly adapting peripheral afferents), is also not conserved across digits in 3b. Cells with significant responses across digits did not often exhibit the exact same submodality preference across digits. However, submodality similarity may be conserved on a smaller scale across two digits, particularly for cells with off responses. These results, however, are unsurprising as more recent data has suggested that cortical somatosensory responses should be defined by their functional role (e.g. texture, shape perception) which may not align perfectly with mechanoreceptor and afferent classes (review, Saal and Bensmaia, 2014).

Finally, we observed that the latency of responses was slightly offset on the non-hotspot digits, though the median time difference between the responses of an adjacent digit compared to the hotspot was only 3msec. Both divergent thalamocortical input (Garraghty and Sur, 1990; Rausell et al., 1998) and corticocortical interactions among digits (Négyessy et al., 2013; Wang et al., 2013) have been described anatomically and are thought to be a method of multi-digit interactions within 3b. We propose that the very small median difference in responses (3msec) across digits suggests that *most* non-hotspot digit responses come about due to ascending thalamocortical divergent connections as opposed to thalamocortical input into one digit's representation impacting another digit's representation via corticocortical connections.

CHAPTER 6. THE EFFECT OF ATTENTION ON RESPONSES IN PRIMARY SOMATOSENSORY CORTEX, AREA 3B.

The previous chapters have explored the receptive field (RF) properties across digits in 3b for animals trained on a multi-digit distal one-back task. We next sought to describe responses of 3b cells when the animal performed this tactile task, hoping to provide insight into the functionality of experience- dependent plasticity. In the original study that observed an increase in multi-digit RFs following multi-digit one-back training (Wang et al., 1995), the authors did not record responses during performance of the one-back task. We asked if 3b cells with larger RFs were enhanced when the animal performed the distal one-back task versus when its attention was directed to the visual modality and the same tactile stimuli were presented passively. Such data could illuminate, though not definitively say, whether RF expansion is simply an epiphenomenon due to continuous, synchronous input to several digits or if it is utilized in some manner for the task. We recorded responses of cells when the animal performed the distal one-back task or a visual discrimination task with the same tactile stimulation. RF characteristics of these cells were quantified in the protocols described in the previous two chapters.

It has been proposed that tactile attention has a smaller or even negligible effect on the responses in SI compared to SII cortex (Hsiao et al., 1993; Meftah et al., 2002) and targets cells with relevant feature selectivity only higher along the somatosensory

hierarchy (Hyvärinen et al., 1980; Chapman and Meftah, 2005). How tactile spatial attention acts on the single neuron level has been greatly unexplored, unlike in the visual system (e.g. Motter and Health, 1994; Connor et al., 1997; Luck et al., 1997; Mitchell et al., 2009). To our knowledge, the only study which explored tactile spatial attention at the single neuron level diverted attention across hands and observed responses of cells in SI cortex were greater when the animal was cued to attend to the contralateral hand (Burton and Sinclair, 2000a). While our study is not a controlled study of tactile spatial attention, its results suggests that tactile attention can alter 3b responses in a manner that corresponds to 3b RF properties.

Additionally, we asked if tactile attention had any effect on the temporal firing patterns of cell pairs recorded simultaneously; it has been previously reported that cell pairs respond more similarly as animal learns to detect timing of stimuli to two digits (Blake et al., 2005). As discussed in Chapter 1.3, tactile attention can enhance synchronous firing of cell pairs in SII cortex (Steinmetz et al., 2000; Gomez-Ramirez et al., 2014); furthermore, it can enhance synchronous firing of cell pairs whose feature selectivity matches an attended tactile feature (Gomez-Ramirez et al., 2014). These increases in synchrony are beyond that expected from the increases in synchrony that will happen by chance due to increases in firing rate. We therefore asked the temporal correlation patterns of neural populations (i.e. pairs of cells) are altered by attention in SI cortex. We hypothesized cells pairs with similar RF properties will exhibit enhanced synchrony with tactile attention. Such results could offer a method by which tactile attention results in the expansion of cell's RFs via recruitment of Hebbian mechanisms.

6.1. Specific Methods.

6.1.1. Experimental design.

After isolating 3b cells on up to four separate electrodes (Chapter 3.5), we asked the animal to perform the distal one-back task (see Chapter 3.3 for full description), or a visual contrast discrimination task (see Chapter 3.2 for full description) while the exact same multi-digit stimuli was presented passively. We quantified cells' RF properties in the single digit rotating bar paradigm (Figure 3.2, see Chapter 3.6 for full description of this protocol) before or after quantifying neural responses in these attention protocols. See Figure 6.1 for an overview of the experimental design. The tactile stimuli for both attention conditions consisted of 400 μm, 100 msec indentations of two horizontal bar stimuli that spanned the proximal and distal pads of D3 and D4, with an intertrial interval of 2300 msec and interstimulus interval of 500 msec (further details see Chapter 3.3). There were four tactile trial types used in both conditions, randomly interleaved (Figure 6.1c). The visual discrimination task proceeded asynchronously with multi-digit stimulation, though a "trial" was always defined by the tactile stimuli in the same manner as in the tactile one-back task. That is, "trial" onset was the first tactile stimulus after the 2300 intertrial interval window and concluded with two consecutive distal stimuli (Figure 6.1c). The animal performed the visual and tactile tasks in alternating blocks, with 40 tactile trials on each task. When the animal was attending to the visual modality, it performed enough visual discrimination trials to encompass 40 tactile sequences. We alternated at least twice between the two attention protocols.

The following results include responses only during trials where the animal responded correctly. In the tactile task, this required maintenance of eye position on the

central cue while the tactile sequence was presented and a saccade to the response cue within 500 msec of the second consecutive distal stimulus. In the visual task, we discarded tactile trials where the animal had performed incorrectly- that is, incorrectly performed contrast discrimination on the visual stimuli- within 1.5 sec of the trial to ensure that attention was properly maintained to the correct modality throughout. We acknowledge that the animal may have attended to the tactile stimuli between initiations of the visual trials, though as the visual task proceeded asynchronously and had a different time course than tactile trials, this would not lead to a consistent attention effect during one part of the tactile trial. Only cells where we had recorded at least 20 correct trials in each attention condition were considered. Both animals performed consistently over 70% correct on both tasks. Though it would be of great interest to correlate changes in neural responses with animal behavior, incorrect tactile trials included aborted trials, where the tactile stimulus was stopped following eye movement, and a there were only a very small number of trials where the animal maintained fixation throughout the entire trial and did not respond to the target stimulus. We are therefore unfortunately unable to correspond changes in neural responses to animal behavior due to these constraints.

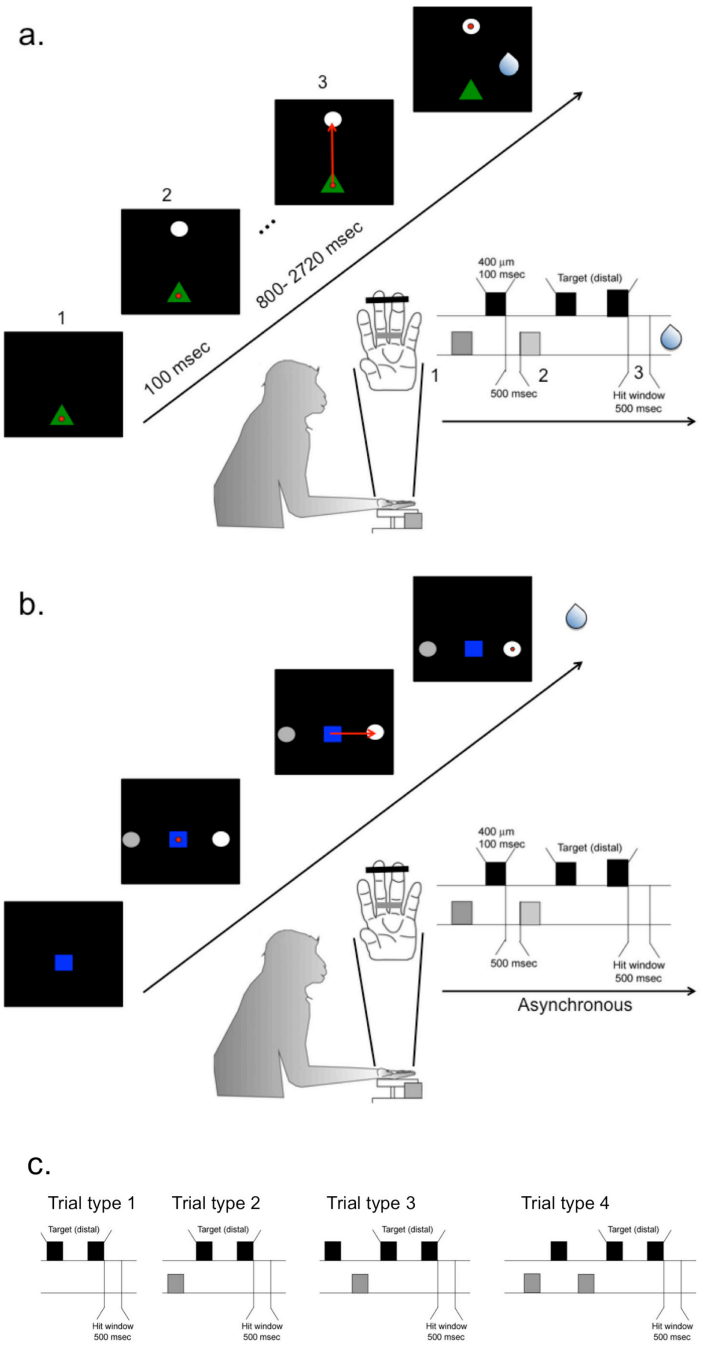


Figure 6.1. Experimental design for examining the effect of attentional state on somatosensory responses. RF properties were quantified in the protocol described in Figure 3.2. **(A)** Distal one-back task, where the animal was required to maintain fixation on a central cue until two consecutive distal tactile stimuli were presented. **(B)** Visual discrimination task, where the animal was required to ignore the tactile stimuli, presented asynchronously, and saccade to the brighter of two illuminated visual response cues**. (C)** Tactile sequences/trials presented to the animal in both conditions.

6.1.2. Ensuring single unit isolation.

As described in Chapter 3.7, we utilized several methods to ensure the same cell was held throughout attention protocols. Besides PCA analysis and elimination of aberrant action potential waveforms, we sorted baseline firing (measured 1.5 sec prior to trial onset) collapsed across an attention condition (using correct trials only), and fit this with a power function. Trials at the tail ends were removed until a non-significant ($p>0.05$) fit was produced.

6.1.3. Attention modulation index.

For every cell we calculated an attention modulation index using a cells' firing rate in the two attention conditions:

$$AMI = \frac{Attend\ tactile\ - attend\ visual}{Attend\ tactile + attend\ visual}$$

We calculated this index at similar time intervals within the trial. This ensured that there was no interaction between the number of correct trials per trial type and attention modulation. For example, there are more distal stimuli present in trial types 3 and 4 than in trial types 1 and 2; therefore one may observe increased modulation simply because the animal responded correctly during greater proportion of longer trials in the tactile condition and a greater proportion of distal stimuli were included in one attention condition. We broke each trial into the baseline period (1.5 msec prior to the trial onset), the periods where the D3-D4 distal bar stimulus was on the finger (including a 40 msec offset window), periods where the D3-D4 proximal bar stimulus was on the finger (with a 40 msec offset), and the periods of time following the distal or proximal stimuli (40msec after the stimulus to the beginning of the next stimulus, 460msec later). We excluded the

time period after the last distal stimulus, to ensure no confound with the response or reward period.

6.1.4. Measuring spike synchrony among neural pairs.

We quantified spike synchrony as described in (Gomez-Ramirez et al., 2014). We used a spike-synchrony counting method (SSCM) that computed the number of times a neural pair spiked within a window of ±2 msec.

$$SSCM(t) = \frac{1}{M}\sum_{i=1}^{M} W \qquad , \ for\, t = 1 \ to \ N$$

$$W = \sum_{j=t-\tau}^{t+\tau} \left(X_{it} \times Y_{ij} \right) \qquad , \{W > 0 = 1\}$$

Where M = the number of trials (intervals), N = the number of bins in each spike train (1msec bins), X and Y represent the spike trains (composed of binary values) for each neuron in the neural pair, and τ is the time lag, which was set to 2 msec. The variable 'i' indicates the trial number, while 'j' indicates the time bin for the second neuron composing the neural pair. Summing across 't' results in the same value as integrating the area under the cross-correlogram (CCG) across τ, but the SSCM procedure has the advantage in that it maintains temporal structure of spike-synchrony, thus allowing us to assess attention effects across time, instead of using the mean coincident spikes across the entire spike-train. The SSCM is analogous to an instantaneous CCG at a 2msec window.

Increases in firing rate will increase average spike synchrony simply due to chance as the number of spikes occurring in each cell increases (Brody, 1998). We therefore corrected the SSCM in each attention condition for effects due to spike-rate modulations using a jitter method devised by (Amarasingham et al., 2012) and employed

in (Gomez-Ramirez et al., 2014). We divided each neuron's spike train into bins of 50msec (as suggested by Smith and Kohn, 2008), starting with the stimulus onset. For each spike in a trial, a new spike time was chosen randomly from all possible times in the same jitter bin. This method was repeated 5000 times to derive a surrogate spike-synchrony distribution for each attention condition. The average surrogate data was then subtracted from the raw spike-synchrony. This method maintains average firing rate fluctuations in 50msec periods, but disrupts the precise timing of spikes within this window.

6.2. Results.

6.2.1. Animal performance.

We assessed animals' performance on the one-back distal tactile task over 21 sessions (40 trials each) for 43V and 24 sessions for MR4358M. As discussed in Chapter 4.2.1, we found that while both animals learned the one-back task and were able to perform it at a high level, MR4358M's accuracy was better, while 43V responded faster (Figure 4.1). We further examined this result by examining animals' performance on the various trial types.

We performed mixed-factor ANOVAs on animals' reaction time and performance, with the within-subject factor of trial type (4 types, see Figure 6.1c) and a between subject factor of animal. There was a main effect of trial type in both measures (percent correct, $F(3,129)= 41.26$, $p<0.001$, reaction time, $F(3,129)= 6.97$, $p=0.001$) whereby the animals performed significantly worse but responded faster on the longer trial types (Figure 6.2a-b). This was likely due to difficulty maintaining fixation and

anticipation of upcoming target stimuli during longer trial types. We observed a significant interaction between animal and performance on the various trial types ($F(3,129)=4.38$, $p=0.006$), whereby 43V performed worse than MR4358M on the longer trial types. No other significant effects were observed.

We realize the animal could use several strategies to perform the one back distal task, including ignoring the proximal stimuli completely and simply attending to the time between distal stimuli (i.e. responding when there is a shorter time interval between the two distal stimuli). In one animal (MR4358M), we tested if the animal used the timing of stimuli by introducing "catch" trials where the time between the last two distal stimuli was doubled (Figure 6.2, bottom panel). These tests were performed at the completion of training and recording; 3b responses were not recorded at the time. We found that on the first day we introduced trials with altered timing (20% of trials), the animal often responded incorrectly to these trials (values closer to 1, Figure 6.2). However, in only a few days, the animal was performing almost equally well on the catch trials (values closer to 0) as during regular timed trials. This suggests that the animal was using, to some degree, the timing of distal stimuli to perform the task, though he could quickly develop a spatial strategy that ensured high performance even when the timing between stimuli was altered.

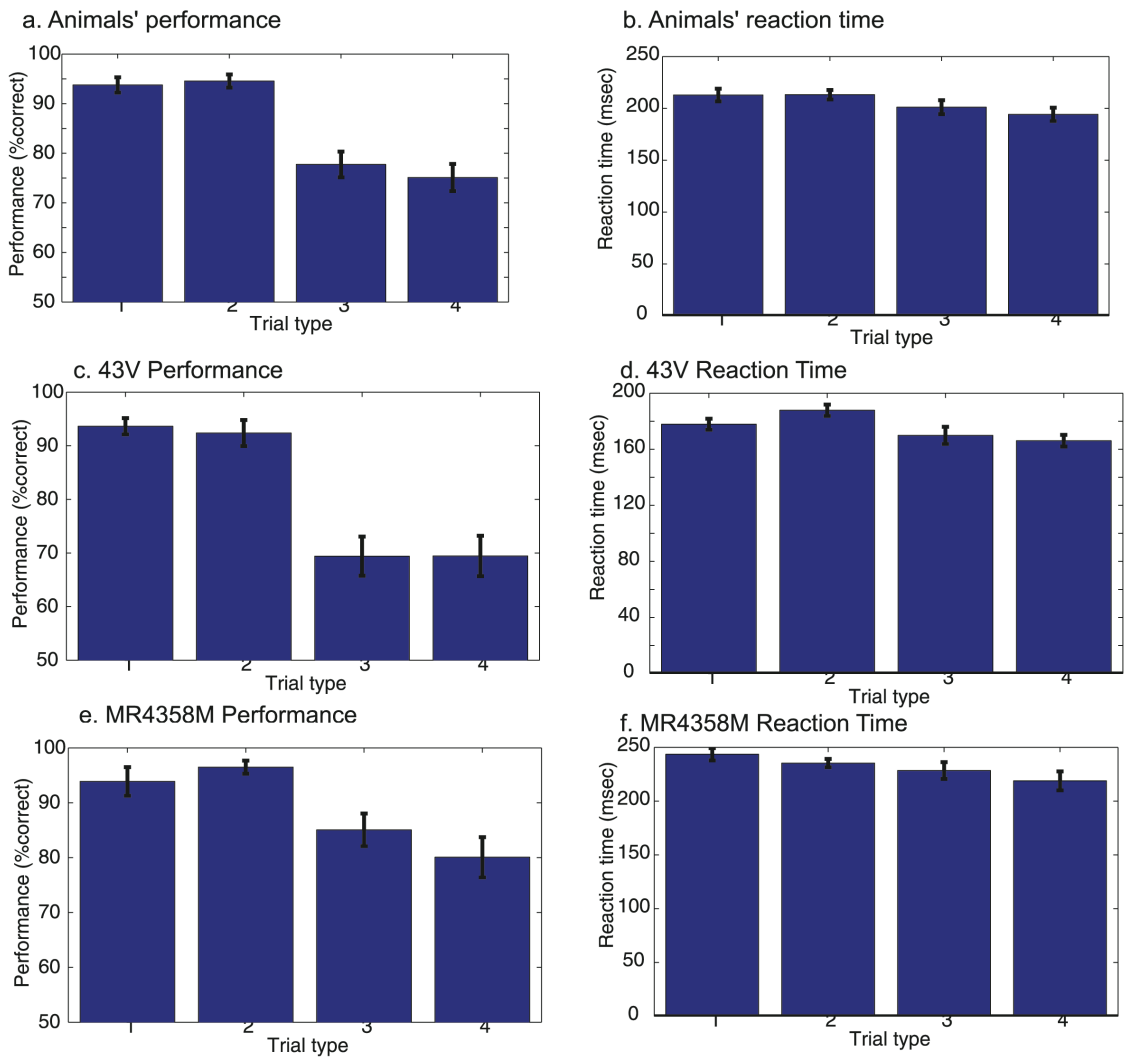


Figure 6.2. Animal performance on various trial types. (A) Average percent correct of both animals (see Figure 6.1c for description of trial types). Performance measured as percent correct over ~20 blocks (40 trials each). **(B)** Reaction time on correct trials of both animals, measured after stimulus offset. **(C-E)** Individual animal performance and reaction time for various trial types.

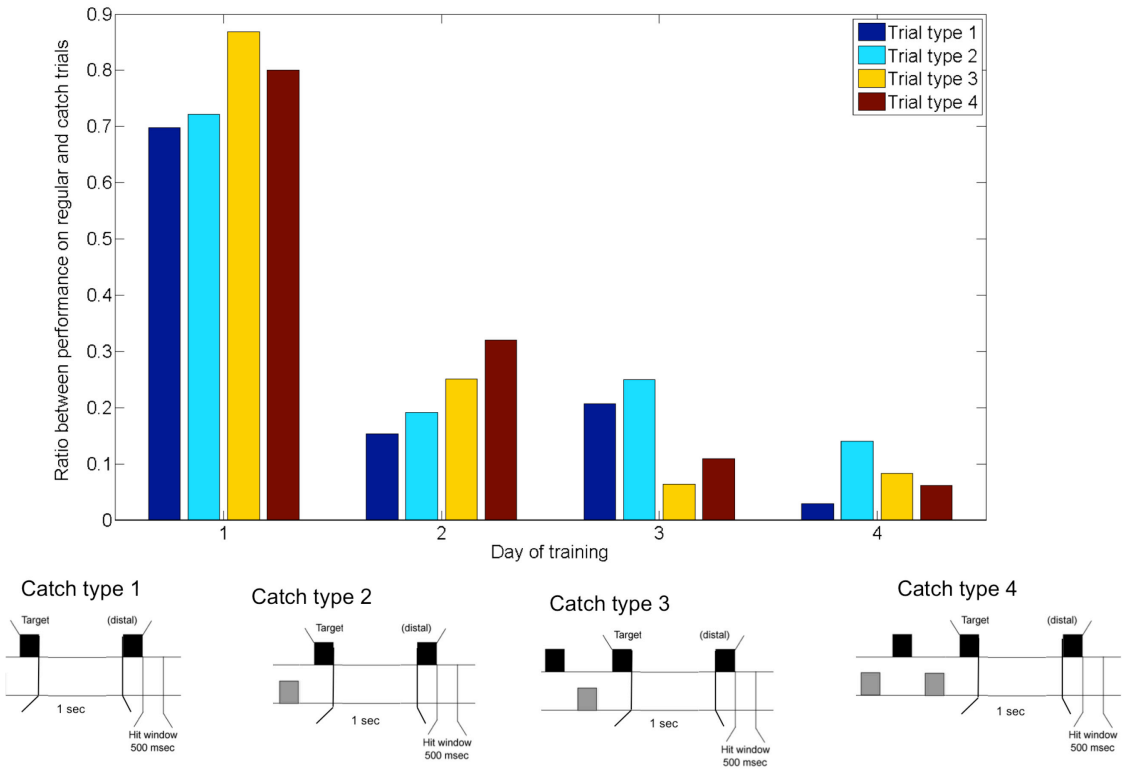


Figure 6.3. Performance of one animal (MR4358M) on catch trials indicates temporal pattern of stimuli was used to perform the distal one-back task, but the animal had the ability to learn a new strategy. We assessed the animal's performance when 20% of trials had increased time between the last two distal stimuli (1000 msec on catch trials vs. 500 msec for regular trials). The animal performed ~500 trials/ day. Ratio of performance on regular trials compared to catch trials: one means the animal responsed incorrectly on all catch trials; zero indicates equal performance on catch and regular trials.

6.2.2. Effect of attention on 3b responses.

We recorded from 176 cells that had a significant response on at least one digit as quantified in the oriented bar protocol (Figure 3.2) and was tested fully in both attention conditions (Figure 6.1). We observed cells in both animals that had divergent responses to the exact same tactile stimuli depending on the task the animal was performing. We will refer to this as an effect of "attention", though behavioral relevance of stimuli, reward contingency, eye position and visual stimuli are also changing between these two tasks. Some cells' firing rate was clearly enhanced when the animal performed the distal one-back task, while others were inhibited when the animal performed the tactile task. A cell enhanced by "tactile attention" is shown in Figure 6.4. One will notice that this cell's response was greater during the proximal (grey bars) stimuli and between stimuli (Figure 6.4b) as the animal presumably attended to the tactile stimuli. We observed that this cell had significant responses to the single-digit oriented bar stimuli on digits 3 and 4 (Figure 6.4a). We are fairly confident that this same cell was held throughout protocols, as the action potential waveform shape did not change across protocols (Figure 6.4a, right panel).

However, we also observed cells with opposite attention effects. An example of a cell with decreased responses during the tactile task compared to the visual task is shown in Figure 6.5. This cell had a RF confined to D2, as defined in the single digit bar RF characterization protocol (6.5a). The cell's response decreased both between and during the trial (Figure 6.5b, blue traces) compare to the responses during the visual discrimination task while the multi-digit stimuli passively indented digits 3 and 4 (Figure 6.5, red traces).

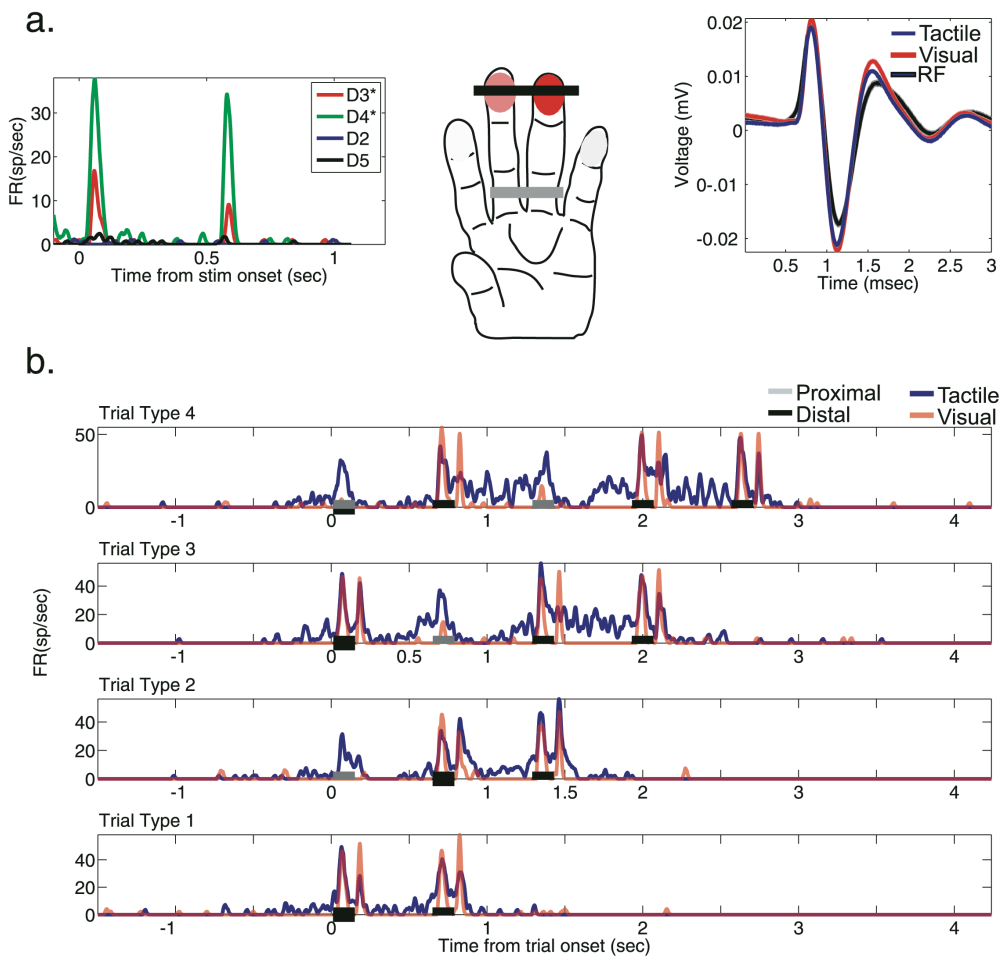


Figure 6.4. Example cell with enhanced responses during the distal one-back task. (A) Left: Response of the cell during RF characterization (Figure 3.2) on distal finger pads. Colors indicate average response to oriented bar on a specific digit. Middle panel: Relative response of digits compared to hotspot response; the tactile stimuli used in the attention protocols are overlaid on the cell's RF. Right panel: action potential waveform shape recorded in RF characterization (black), visual discrimination (red), and distal one-back task (blue). **(B)** Response of cell during attention protocols. Blue: Response of cell while animal performed distal one back task (Figure 6.1a), Red: response while animal performed visual discrimination task (Figure 6.1b). Note that the animal experienced all trial types randomly and the tactile and visual tasks were presented in alternating blocks of trials. The animal's hand and fingers were kept immobile through all paradigms.

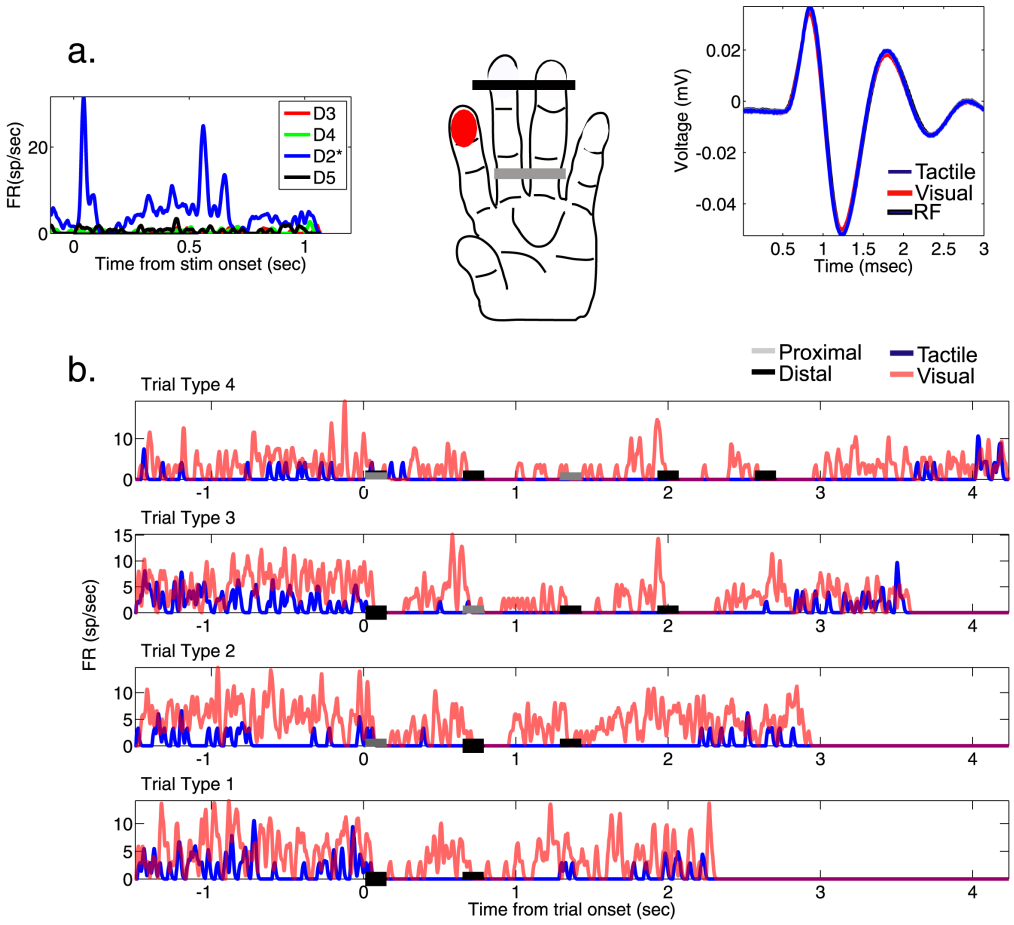


Figure 6.5. Example cell with decreased responses during the distal one-back task. (A) Left: Response of cell during RF characterization on distal finger pads. Cell was only significantly responsive to the single digit oriented bar stimuli on D2. **(B)** Response of cell during the distal one back task (blue) where the animal had to indicate when it felt two consecutive stimuli on the distal pads of D3 and D4 or during the visual task when the same horizontal bar stimuli was presented across D3 and D4 (red).

We asked if tactile attention acts as a spatial spotlight in 3b, enhancing responses of cells with RFs that overlap the attended digits and suppressing those cells with RFs not including the attended digits. We chose to examine cells with excitatory RFs including the stimulated/relevant digits, digits 3 and 4 (N=90), or with an RF that excluding the stimulated digits and had an excitatory response on unstimulated digits 2 or 5 (N=35). Cells with significant inhibitory responses were not included, as it is unclear how tactile attention would act upon such cells (N=51). We acknowledge that our task does not completely elucidate how tactile spatial attention acts in 3b, as the stimuli was always presented to digits 3 and 4 and the animal's attention was never switched to other digits nor did we present competing stimuli on the untrained digits (digits 2 and 5).

We observed that across the population, cells with excitatory RFs including the stimulated digits were more likely to be enhanced during the tactile as opposed to visual task (Figure 6.6.a), while those with RFs not including digits 3 and 4 were generally suppressed or their firing rate unchanged (Figure 6.6.b). We divided each trial into parts depending on presence (during or between stimuli) and location (proximal or distal) of the D3 and D4 multi-digit stimuli; we also examined how responses were altered during the baseline period prior to trial onset (1.5 seconds).

To determine how attentional state directly impacted cells' firing rate at various time intervals, we ran a 2x 2 x 2 x 2 mixed factor ANOVA, with a between group factor of RF type (including trained digits, N=90, excluding trained digits, N=35), and within group factors of Attention (animal performing tactile or visual task), Tactile Stimulus Location (proximal or distal), and Stimulus Presence (during or following stimuli). For brevity, we report significant effects that include the factor of attention (i.e. it

unsurprising that there is a main effect of stimulus location, as the cells had RFs on the distal pads and often responded stronger to those stimuli). We found a significant interaction between RF type and attention ($F(1,123)=6.73$, $p=0.01$, $\eta_P^2=0.052$). Cells with RFs that included the attended digits had an increased firing rate when tactile attention was deployed (6.26 sp/sec during the visual task, 7.66 during the tactile task) while cells with RFs that did not include the attended digits were slightly depressed (1.43 sp/sec during the visual task, 1.15 during the tactile task).

We tested if attention altered overall spontaneous rate or baseline responses (measured between trials) as the animal performed either the tactile or visual task, running a mixed factor ANOVA with RF type as a between group factor and Attention (visual or tactile) as a between subject factor. While we observed that cells with RFs that spanned the stimulated digits had slightly higher spontaneous rates in the tactile task (4.36 vs. 4.61 sp/sec, visual versus tactile) and those with RFs on the unstimulated digits had lower firing rates between trials when tactile attention was deployed (1.72 sp/sec versus 1.29, visual versus tactile). However, this interaction was not significant ($F(1,123)=3.05$, $p=0.08$), and no other significant effects were observed.

We then calculated attention modulation indices (AMI) for each cell (Chapter 6.1.3), which, unlike the previous analysis, normalizes the effect of attention with respect to average response across the two attention conditions (Luck et al., 1997). AMI can vary between -1, suppressed in the tactile task, to +1, enhanced in tactile task. We performed Mann Whitney U-tests, comparing the AMI distribution for cells falling into either RF type category (including or excluding trained digits) in various time intervals in the trial. These distributions were significantly different in the baseline period prior to each trial

(AMI means: 0.03 versus -0.08, RFs including and excluding D3 and/or D4 respectively, $Z=-2.09$, $p=0.04$, Cohen's $d=0.19$), during the distal tactile stimuli (0.03 versus -0.18, $Z=-2.66$, $p=0.008$, Cohen's $d= 0.24$), following the distal stimuli (0.10 versus -0.16, $Z=-2.79$, $p=0.005$, Cohen's $d= 0.25$), and following the proximal stimuli (0.09 versus -0.14, $Z=-2.71$, $p=0.006$, Cohen's $d=0.24$). We followed these results by asking which distributions were significantly different from zero, performing post-hoc Wilcoxon sign-rank tests. We found that the median AMI for cells with RFs including digits 3 and 4 was significantly greater than zero in the time period following distal stimuli ($Z=-2.78$, $p<0.05$, Bonferroni corrected for ten comparisons). This suggests that cells with varying RF types were differentially affected by attention throughout the trial, and cells with RFs including the stimulated digits were particularly enhanced in the period of time following distal stimuli. We cannot definitively conclude that cells with RFs covering the unattended digits (D2 and D5) are specifically suppressed by tactile attention, though this was likely because there were not irrelevant stimuli present on these digits during the attention protocols.

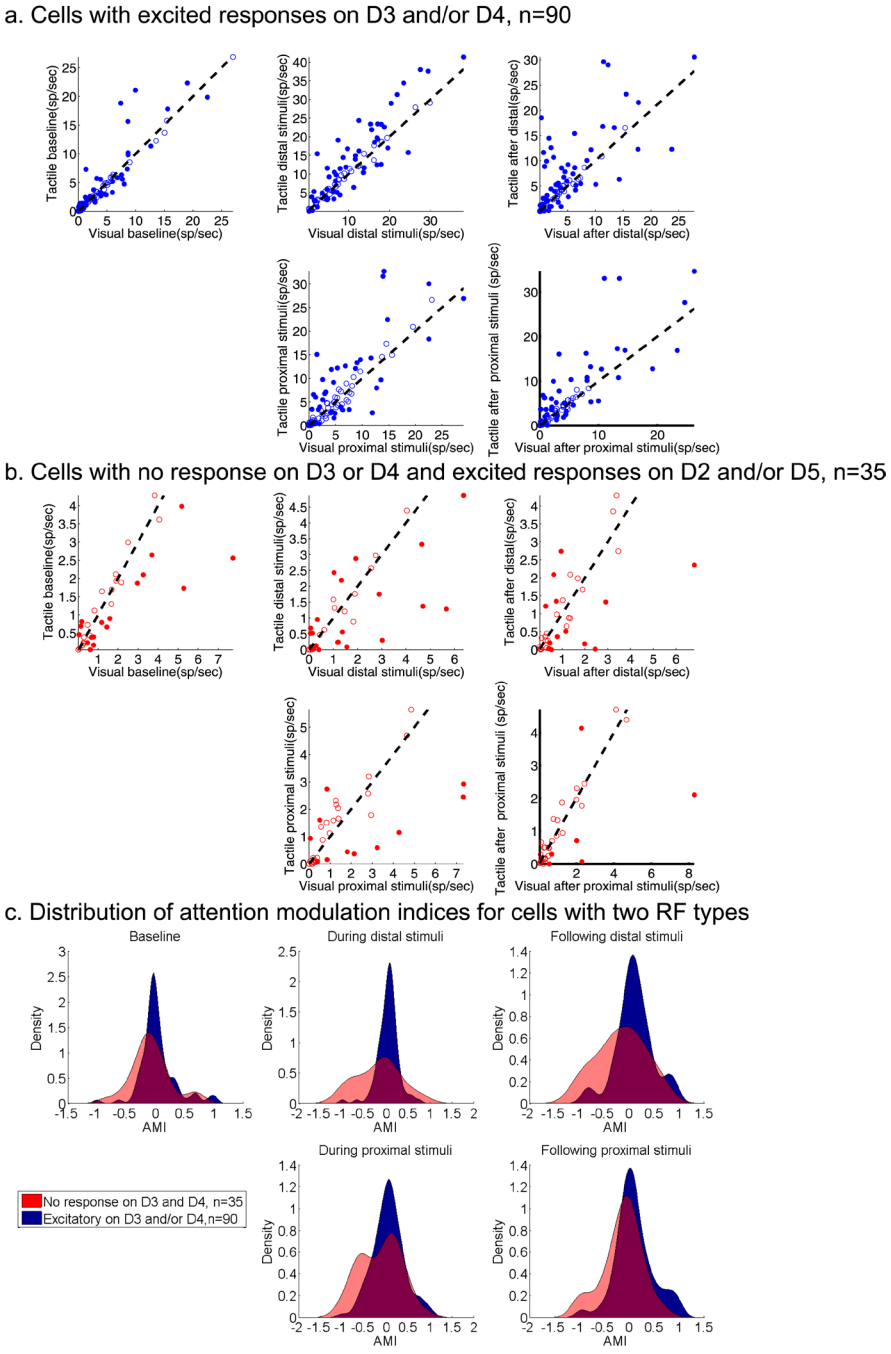


Figure 6.6. Responses and modulation indices for cells with varying RF properties throughout attention protocols. (A) Firing rate of cells with excitatory RFs containing D3 and D4 (e.g. Figure 6.4) during various time points in the tactile trial when the animal was performing the visual task (abscissa) or attending to the tactile stimuli (ordinate) **(B)** Firing rates of cells with excitatory RFs covering D2 or D5 and no response on D3 or D4 (e.g. Figure 6.5) depending on attention and time interval in trial **(C)** Distributions of attention modulation indices (AMIs) throughout various time intervals for cells with these two RF types.

6.2.3. Attention and RF size.

We followed this result by asking if cells with multi-digit RFs (such as the example cell in Figure 6.4) including *both* trained digits showed a greater effect of tactile attention than those with RFs including *only one* stimulated digit. Such data would elucidate if cells with RFs spanning the entire bar on the distal pads were preferentially enhanced (though note that the task can be performed by only attending to one digit), and would suggest a function for the development of multi-digit RFs in 3b following training on this task. We chose to examine the time period with the largest attentional enhancement as described in Chapter 6.2.2: that is, the period following distal stimuli. We found that the distribution of AMIs for cells with excitatory RFs covering both D3 and D4 (N=35, mean=0.20) was significantly greater than for cells with RFs that only included one digit (N=55, mean=0.04) (Figure 6.7a, Mann Whitney U- test, Z= 2.00, p=0.04, Cohen's d= 0.21). Post hoc Wilcoxon rank sum tests determined that the distribution of AMI for cells with RFs on both digits was significantly greater than zero (Z=-3.11, $p<0.05$, Bonferroni corrected for two comparisons).

We also asked if the degree of similarity between responses across digits correlated with attentional effects, hypothesizing that cells that responded very similarly across D3 and D4 as determined in RF characterization protocol would show stronger enhancements of tactile attention. However, we did not find this to be the case (Figure 6.7b, $R^2=0.0002$, p=0.89). We therefore conclude that while tactile attention may preferentially target cells receiving input from both attended digits, particularly following distal stimuli, this is not a graded enhancement depending on the strength of input from the two digits.

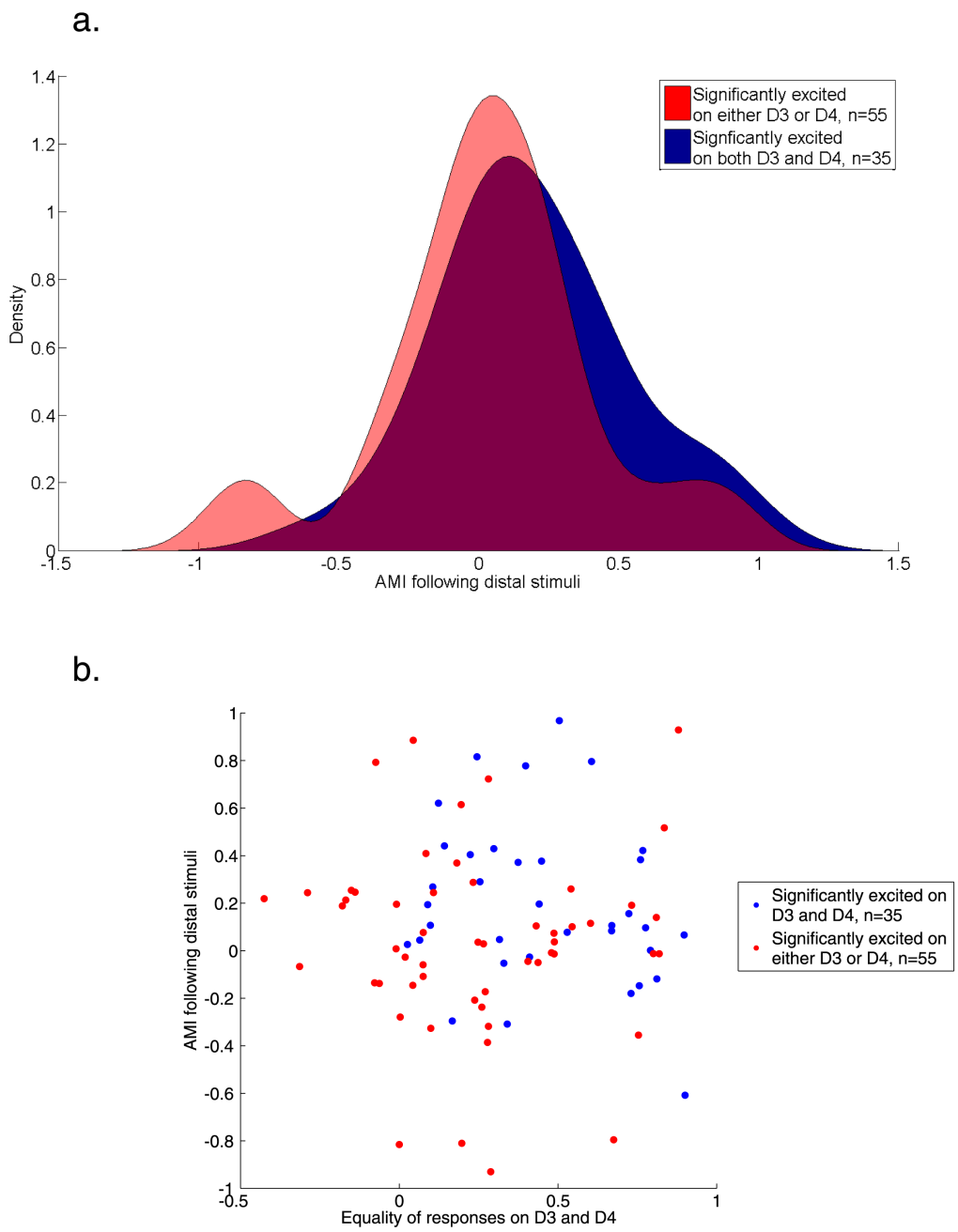


Figure 6.7. Attention modulation and RF size. (A) Distribution of attention modulation indices calculated in the time intervals following distal stimuli for cells with RFs covering *both* D3 and D4 (N=29, blue) and with their RF covering *only one* digit (N=50, red). **(B)** Relationship between similarity of responses across D3 and D4 (as determined in the RF characterization protocol) and AMI following distal stimuli.

6.2.4. Attention and temporal correlation among neural pairs.

A key follow-up question is if tactile attention not only increases the firing rate of cells whose RFs match the attended digits, but if it also selectively increases synchronous firing of cell pairs representing the attended digits. Such a mechanism could link attention and experience-dependent plasticity mechanisms; increases in synchronous firing between cell pairs by attention would presumably strengthen connections among cells with ascending input from individual digits via Hebbian mechanisms and expand cells' RFs. We therefore asked if we observe increases in synchrony in a trained animal performing the one-back distal task, examining cell pairs that had shown the largest enhancement in firing rate. An example pair's raster is shown in Figure 6.8; both of these cells had significant excitatory RFs covering the stimulated digits as measured in the RF characterization protocol. We then examined cell pairs and time intervals with the largest enhancements in firing rate as described in Chapter 6.2.2, that is, cell pairs with RFs including one or both of the trained digits (N=34 pairs; there were not enough pairs of cells including both trained digits or just one digit), and examined the time interval between distal tactile stimuli. We compared synchronous firing, corrected for firing rate changes, in these time intervals while the animal performed the distal one-back task or the visual task, and did not find any differences in spike synchrony (Figure 6.9, Mann-Whitney U test, $Z=0.006$, $p=0.99$). In fact, in many cases, spike synchrony was less than that expected by changes due to firing rate fluctuations. This result suggests that spike synchrony is not enhanced by tactile attention for cells with RFs covering the attended digits.

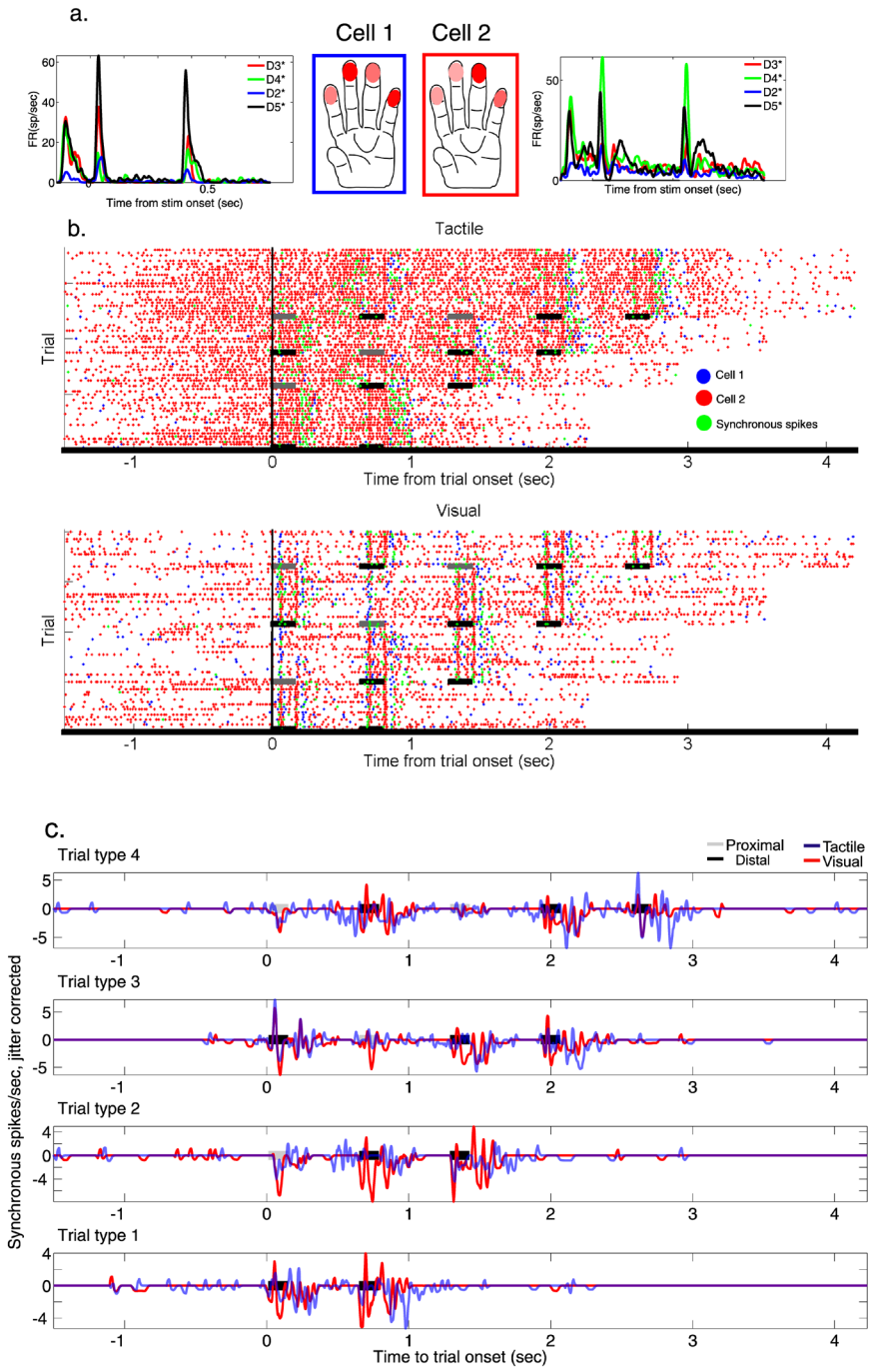


Figure 6.8. A cell pair recorded simultaneously during varying attentional states. **(A)** Cell RFs as measured by the single-digit oriented bar protocol. **(B)** Raster plots when the animal performed the distal one-back task (top) or visual task (bottom). Black bars: multi-digit (D3 and D4) distal stimuli, Grey bars: proximal multi-digit stimuli. Blue dots indicate spikes by cell 1, red dots spikes by cell 2, and green dots indicate synchronously occurring spikes (±2 msec). **(C)** Spike synchrony corrected for firing rate modulations (50msec bins) in two attention conditions.

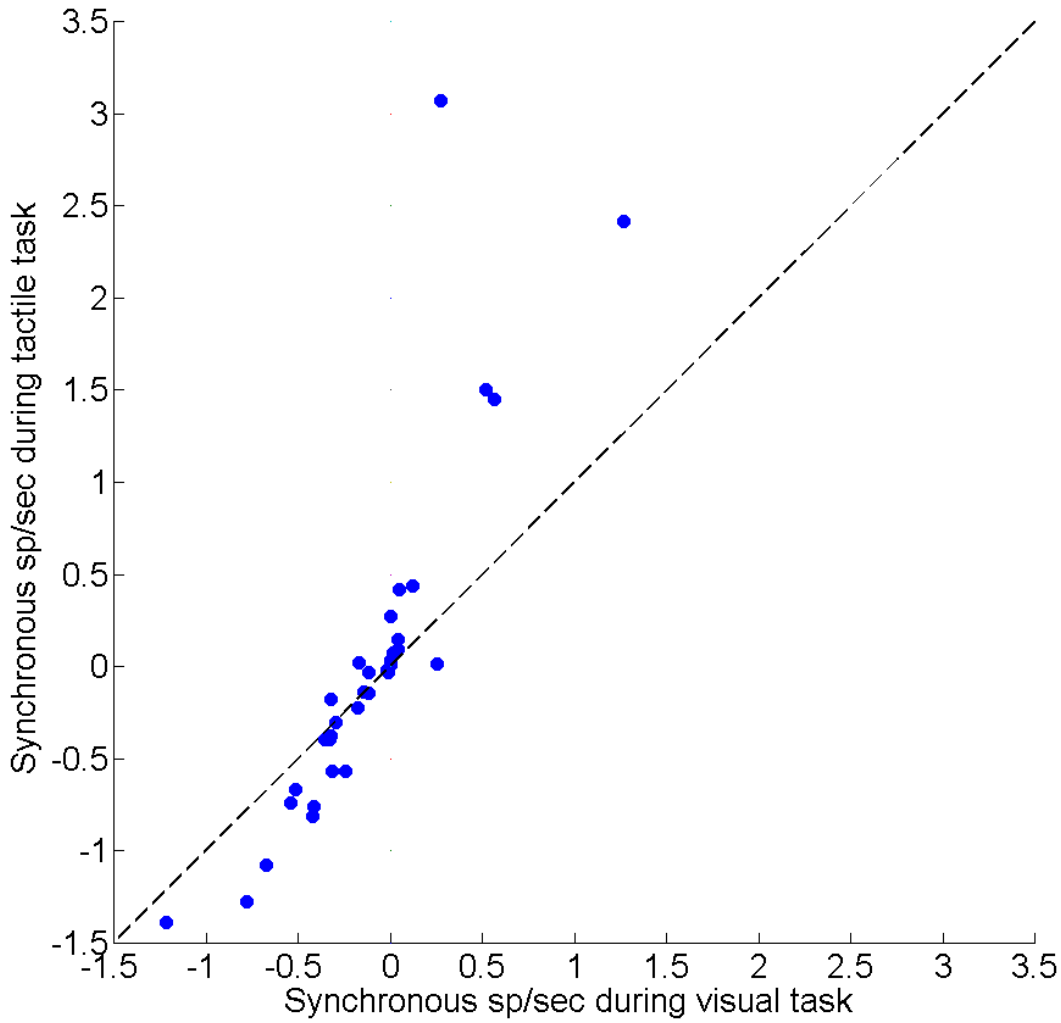


Figure 6.9. Lack of consistent increase in spike synchrony with tactile attention. Spike synchrony for cell pairs with significant excitatory RFs on D3 and/or D4 (N=34), averaged over the time periods in the attention protocols immediately following distal tactile stimuli. Negative values indicate less spike synchrony was observed than expected due to modulations in firing rate (50msec bins).

6.2.5. The effect of stimulus history on 3b responses.

Finally, we asked if effects of tactile attention are altered depending on stimulus history and trial type. Because of the rules of the task, as a trial progresses the animal could anticipate upcoming target stimuli, particularly in the longer trial types (Figure 6.1c). We hypothesized this would be manifested in the neural responses and one would observe increased modulation later in longer trials, as more information has become available that would indicate an upcoming target. We quantified average response during the time between the consecutive distal stimuli (target stimuli in the tactile task, black boxes in Figure 6.10a). At this time interval, immediate stimulus history is the same across trial types (a distal stimulus), but stimulus history across a longer time period is different (e.g. in trial type one, a proximal stimulus had been presented, in trial type 3, a distal and proximal stimulus had been presented). We examined cells with RFs covering both D3 and D4 (N=35). The average modulation index was greater between the target stimuli in longer trials (Figure 6.10b, 0.15, 0.19, 0.24, 0.23 for trials 1-4, respectively), and there was a significant effect of trial type as reported by repeated measures ANOVA on these data ($F(3,102)=4.55$, $p=0.012$, Greenhouse-Geisser corrected, $\eta_P^2= 0.12$). This was captured by a linear decreasing function as assessed by polynomial contrasts ($F(1,34)=6.36$, $p=0.017$, $\eta_P^2= 0.16$), indicating that AMI increased in the period between the last two stimuli with longer trials types. However, it is unclear if this increased modulation predicts animal behavior on a trial-by-trial basis.

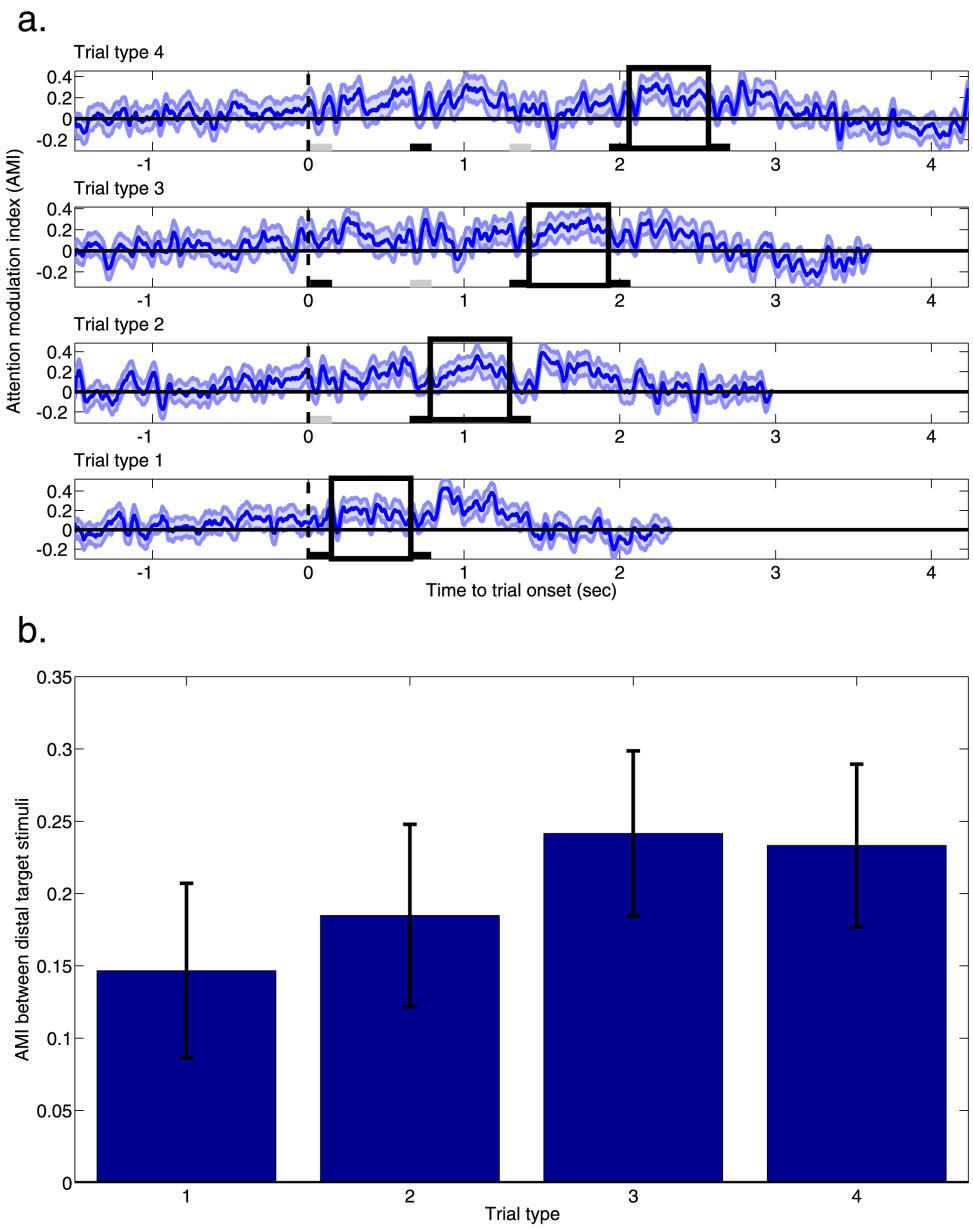


Figure 6.10. Enhancement of responses in longer trial types with tactile attention **(A)** Attention modulation index over time on the various trial types for cells with RFs covering both D3 and D4 (N=35). **(B)** Average AMI between distal target stimuli (black squares in (A))

6.3. Discussion.

There have been varying reports on the effect of attentional state on responses in SI cortex. While functional imaging and ERP studies of humans have suggested that SI activity is modified by tactile spatial and cross modal attention (review see Burton and Sinclair, 2000), effects at the single neuron level have been relatively unexplored. This may be because it has been suggested that effects of tactile attention are only manifested higher along the cortical hierarchy (e.g. in SII) (Chapman and Meftah, 2005). While we did not record in other cortical regions, we did observe many cells in area 3b, thought to be one of the first cortical processing regions for non-painful cutaneous input, with very different responses depending on the attentional state of the animal (Figure 6.4-6.5). We observed cells with enhanced responses to tactile stimuli, as well as increased firing between tactile stimuli when the animal was required to discriminate on the pattern of stimulation across two digits and detect two consecutive distal stimuli, versus when presented with the same tactile stimuli and attention was directed to the visual modality. As the animals' hands and digits were kept immobile throughout all protocols and the tactile stimulator unchanged, we are confident that these results are not due to changes in afferent tactile input. It could be that these results are due to motor preparation (for example, eye movement or motor tone of the hand or digit). However, we observe specifically that cells with RFs covering the stimulated digits are most enhanced by tactile attention and between distal stimuli; therefore it is unlikely that an overall change in cognitive state (e.g. arousal or eye movement) may explain our results. Indeed, we observe that cells with RFs including both stimulated digits are enhanced during the task versus those whose excitatory classical RF does not include the stimulated digits. The

relevance of this finding for animal performance is unknown as the animal could hypothetically perform the task by attending to only one digit. A future study could intersperse irrelevant single digit stimuli (the "catch" trials used in our human subjects in Chapter 2) to ensure attention to both digits. We hypothesize one would observe larger enhancement of multi-digit cells and a greater degree of RF expansion following training with the addition of such single-digit stimuli if tactile attention mechanisms are related to cortical plasticity mechanisms. We acknowledge that increased motor tone of the stimulated digits during the tactile task could explain our results. We also acknowledge that knowledge of the extra classical RF of our cells could elucidate more precise mechanisms of tactile attention. We see small decreases in responses of cells with RFs covering the unattended and unstimulated digits; future experiments would present nonrelevant stimuli on these digits to examine if active suppression on unattended digits alters the firing rate of these cells.

These changes are not accompanied by significant increases in synchronous firing of cell pairs with RFs covering one or both of the stimulated digits, though perhaps more specific RF types are enhanced by attention (for example, we did not record from sufficient cell pairs with RFs covering both digits or covering just one digit). In fact, may of our cell pairs showed synchronous firing below that expect by firing rate modulations. Others have noted no change or even decreases in precise spike synchrony for cell pairs in visual cortex with attention (Cohen and Maunsell, 2009; Martin and von der Heydt, 2015). In addition, several studies have noted attention decreases slow firing rate correlations among neural pairs (Cohen and Maunsell, 2009; Cohen and Kohn, 2011; Gomez-Ramirez et al., 2014). However, it may be that enhancements in synchrony would

only be observed as the animal learned the tactile task and not following many months of training when proficiency has been reached and presumably, RF expansion has plateaued.

Enhancement is greatest in cells with RFs covering *both* stimulated digits, suggesting, albeit it tentatively, that multi-digit expansion may be utilized in some manner for task performance. Indeed, more substantial evidence would observe a direct correlation between enhancement of these cells during the task and animal behavior, in a similar manner as (Hsiao et al., 1993; Cohen and Maunsell, 2009, 2011; Gomez-Ramirez et al., 2014 though one should consider that such a correlation may not indicate functionality, see Zénon and Krauzlis, 2012) . Unfortunately our task structure was not precise enough to perform such correlations, as aborted trials stopped the tactile stimulus; therefore animal performance and tactile stimuli were both changing. Additionally the animal performed with very high accuracy. We propose future experiments in Chapter 7.4 that could elucidate such questions. We observed that enhancement is greatest in the time period following distal stimuli, suggesting an effect of tactile working memory (see the Chapter 7.3 for further discussion of this result). Such sustained firing between stimuli could also signify timing of consecutive distal stimuli, which we hypothesize at least one of the animals likely used to perform the task (Figure 6.3). This enhancement also appears to be dependent on trial type and stimulus history beyond a few hundred milliseconds, though it is again unclear if this enhancement predicts animal behavior on a trial-by-trial basis.

CHAPTER 7. GENERAL DISCUSSION.

We now know through many decades of studies that the adult brain can change as a result of experience, and even primary sensory cortices can be altered depending on an organism's sensory experience. This has been well documented in the somatosensory system, where cortical representations of body regions receiving behaviorally relevant input may be expanded as a result of many weeks or months of training. Additionally, features of tactile inputs can alter properties of receptive fields (RFs) in somatosensory cortex. Crucially for this thesis, coincident input to several digits and discrimination of the temporal sequence of such multi-digit input can lead to the expression of cells in SI cortex, area 3b, with receptive fields covering the stimulated digits (Clark et al., 1988; Allard et al., 1991; Wang et al., 1995). However, it has not been determined (1) what are the RFs of 3b cells defined by well controlled stimuli presented to multiple digits, and in subjects trained to discriminate the temporal sequence of multi-digit tactile input (2) what are the tactile perceptual consequence of such training (3) what stimuli features confer RF changes and (4) if expanded RFs are targeted by tactile attention. We will summarize our experiments and findings with regards to these questions.

7.1. Perceptual consequences of multi-digit training on tactile perception and its cortical origin.

In Chapter 2, we observed relatively modest changes in tactile acuity as human subjects became proficient at the one-back multi-digit task. We had hypothesized that subjects, as they trained with the multi-digit stimuli, would show decrements in spatial acuity on a single digit and temporal acuity between the trained digits. We observed that

subjects had small location and orientation-specific decrements in intradigit spatial acuity, though their acuity was not impaired beyond its original tested capacity. Instead it appeared that training interfered with typical enhancements in spatial acuity that occur with exposure on the grating orientation task used to quantify spatial acuity. This was confirmed by observing that spatial acuity on the untrained hand was enhanced similarly in control subjects who only performed the acuity tests and were not trained on the multi-digit task. Contrary to our original hypothesis, subjects' temporal acuity improved across the trained digits, and this was specific to the trained hand.

We had hypothesized that such perceptual changes would correlate to changes in RF properties in area 3b, potentially in RF size, orientation tuning, or temporal response properties. However, in Chapter 4 we did not observe obvious changes in RF characteristics after multi-digit training to account for perceptual changes observed in Chapter 2. First, we observed that the percentage of 3b cells with multi-digit RFs increases equally in both the trained and untrained hemisphere of a trained animal; therefore we conclude 3b RF expansion is not sufficient to explain hand-specific perceptual changes following multi-digit training. Next, we find that the number of cells representing the horizontal orientation was unchanged in trained compared to naive hemispheres, there were not significant changes in orientation selectivity, and there were no changes in the temporal responses of cells to single-digit oriented bar stimuli following multi-digit tactile training.

At the same time, we acknowledge that we do not have knowledge of the precise somatosensory cortical properties that relate to tactile spatial acuity or temporal discrimination across digits, nor did we quantify cells' responses to the exact same tactile

stimuli as used in our measures of tactile acuity (e.g. grating domes used to measure spatial acuity or asynchronous multi-digit stimuli used to measure temporal acuity). This was because the primary goal of our neurophysiology study was to quantify RF size and orientation tuning properties on multiple digits in trained and untrained animals. While the innervation density of slowly adapting type I afferents accounts for the limits of tactile spatial acuity (Johnson and Phillips, 1981; Phillips and Johnson, 1981), only one study has found a correlation between SI responses and perception of tactile orientation; this study found that the most orientation selective neurons in area 3b could account for subjects' psychophysical tactile angular thresholds (Bensmaia et al., 2008). We also do not know the neural mechanisms serving tactile temporal judgments, though it has been suggested that parietal cortex is involved in such abilities (Aghdaee et al., 2014). To our knowledge, only one study has examined neural responses during a visual temporal order judgment task. In this study, the authors found LIP neurons with RFs encompassing two stimuli that responded stronger to the stimulus the animal reported as being presented first (Aghdaee et al., 2014). Though we had originally hypothesized that presumed expansion of RFs as a result of multi-digit training would make discrimination between digits more difficult, a previous study observed RF expansion in 3b for animals trained to detect the temporal window of stimuli presented asynchronously to two digits (Blake et al., 2005). Indeed, decoding mechanisms could use temporal information from larger receptive fields to determine the timing of stimuli on two digits (Foffani et al., 2008).

We also acknowledge that the expectation that the percentage of neurons sensitive to a particular feature or somatotopic location will correspond to perceptual thresholds of that feature or at that location is likely simplistic. One does not observe that individuals

with larger representations of uninjured body regions near an amputation exhibit enhanced tactile spatial acuity on that body part (Vega-Bermudez and Johnson, 2002). Instead, it is possible that complex response properties (Recanzone et al., 1992b), perhaps in only the most sensitive population of cortical cells (Bensmaia et al., 2008) and maintained in only a few cells (Yang et al., 2009), account for perceptual changes following plasticity. It is also likely that changes in neural mechanisms further along the somatosensory hierarchy relate to changes in tactile perception following experience-dependent plasticity. For example, training animals on a visual orientation discrimination task leads to experience-dependent plasticity in V4 as opposed to V1 or V2 (Ghose et al., 2002; Yang and Maunsell, 2004). As we observed only modest and orientation-specific decrements in tactile spatial acuity following multi-digit training, we may conclude that short term training with multi-digit stimuli does not lead to the perceptual decrements (e.g. decreased overall spatial acuity and temporal discrimination) observed following long term consistent and repetitive multi-digit input in patients with focal dystonia (Bara-Jimenez et al., 2000a, 2000b). Additionally, we did not observe clear relationships between subject-by-subject variability in performance on the tactile one-back task and changes in tactile acuity. This relationship would be presumably be observed if one predicts anatomical/neural reorganizations that enhance performance on a tactile task (i.e. the tactile one-back task) alter perceptual functions (i.e. tactile spatial acuity) that rely on the same cell populations undergoing plastic changes. It is clear more work is to be done to understand if and how perceptual learning on one sensory task alters related sensory abilities, and if so, the exact cortical changes which underlie this relationship.

7.2. Receptive fields across digits of 3b cells in trained and naïve animals

The results of Chapters 4 and 5 offer general principles about the malleability and general processing of tactile stimuli over many digits in area 3b of the SI cortex. Our data support others' findings that the RF size of cells in this region can change based on experience (Clark et al., 1988; Jenkins et al., 1990; Allard et al., 1991; Recanzone et al., 1992a; Wang et al., 1995). Unlike others, we have quantified this effect with well-controlled single-digit bar stimuli in the trained animal, in an untrained hemisphere following training, and in a completely naïve animal. This RF expansion appears non-specific, occurring across hemispheres in a trained animal and does not appear to target the trained digits. We note that we did not observe the large increase in multi-digit RFs (from less than 10% in a naïve animal to over 50% of cells in one trained animal) described by Wang and colleagues (1995); however, this may have been due to differences in task difficulty (our task only required identification of distal stimuli and only four types of sequences were used), training length (over a period of several months versus over a year), stimulator (the animal was not required to stereotypically grip the stimulator in each trial) and arousal during RF characterization (measured in an awake behaving animal versus under anesthesia). It may be that our use of a suprathreshold (1mm) indentation depth to characterize RFs precluded the identification of more multi-digit connections as a result of training; however, we did not observe a greater number of multi-digit cells in the trained hemispheres when tested at smaller indentation depths. We also did not observe significant modifications in orientation tuning or temporal response properties of 3b cells following training. This non-specificity suggests 3b experience-dependent plasticity does not conform to all features of the stimuli used during training.

This is perhaps unsurprising, as not all features (e.g. the orientation) needed to be discriminated upon for the animal to perform the task. However, why the orientation of the stimuli specifies changes in tactile spatial acuity, described in Chapter 2, is unknown.

Thought to be one of the first cortical regions processing innocuous light touch, 3b utilizes mechanisms balancing inhibition and excitation to exhibit orientation tuning to tactile stimuli on a single digit (DiCarlo et al., 1998; DiCarlo and Johnson, 2000). We argue that the viewpoint that 3b responses are always limited to a single digit (Sur, 1980; Sur et al., 1980; Iwamura et al., 1983) is outdated; we observe many cells, even in a completely naïve animal, with classical RFs extending over many digits. Previous authors have suggested this (Lipton et al., 2010; Reed et al., 2010; Thakur et al., 2012), though our study is the first to characterize single-unit responses to bar stimuli on individual digits. We find that suprathreshold stimuli (using 1mm indentations, still smaller than most everyday tactile experience) are more likely to expose multi-digit responses in 3b. Nonetheless our data maintain the view that 3b processing is *primarily* concerned with processing on single digit: we do not observe that feature selectivity extends across digits, and a majority (75%) of 3b neurons in the naïve animal have excitatory RFs confined to one digit. The average response of a cell to an adjacent digit in the naïve animal was 9% that of the hotspot digit. We also find that cells with inhibitory responses to tactile stimuli are more likely to have RFs extending across digits. Interestingly, this result is not maintained throughout the somatosensory hierarchy: in SII cortex, cells with excitatory responses extend across more digit pads (an average of six) than those with inhibitory responses to bar stimuli (an average of four) (Fitzgerald et al., 2006b). It is therefore still

unclear the function of larger inhibited RFs in 3b, or what are the pharmacological properties of these cells.

7.3. The effect of attention on the pattern of neural responses

We observe responses of 3b cells are altered depending on which task the animal is performing, and most interestingly, we observe a shift in the temporal response of cells with the addition of tactile attention. We see cells that exhibit enhanced responses following distal stimuli; these stimuli match cell's RF location (D3 and D4) and have the most behavioral relevance, as two distal stimuli indicate a target to the animal. Such responses may be a mechanism to determine the timing between stimuli, perhaps a "working memory" trace that is useful given the rules of the task. However, we acknowledge that the limitations of our task preclude us from this conclusion. To characterize if these sustained responses between stimuli reflect a "working memory" trace, one would also wish to record from cells with RFs covering the proximal pads. Would these show enhanced responses specifically following proximal stimuli? We did not do so as the anatomical location (area 1 or 3b) of such recordings would be ambiguous. It is also unclear if such responses predict animal behavior on the one-back task.

Mountcastle and colleagues first observed that in a tactile vibratory discrimination task, where an animal must compare one vibration to another presented after a delay, there is little evidence of sustained firing in the time period between stimuli in SI cortex (Mountcastle et al., 1990). This was followed by years of work by Romo and colleagues examining responses in other cortical regions during this same task. These studies describe sustained firing that corresponded to the frequency of vibration of the first

stimulus in cells in SII cortex (Romo et al., 2002), and in in prefrontal cortex (Romo et al., 1999). The overall firing rate of cells to vibrations in these higher-level areas was found to be a better predictor of animal discriminability and trial-to-trial performance than the temporal entrainment observed by cells in SI cortex (Salinas et al., 2000). Another study examining the effect of attention in a roughness discrimination task found attention effects during the instructional period, when a visual stimulus indicated what task to perform, only in SII, and not SI cortex (Meftah et al., 2002, 2009). However, others have observed sustained firing of SI cells in the delay period during a haptic matching or visual-haptic matching task (Zhou and Fuster, 1996, 1997). Additionally, experience can lead to the alteration of temporal firing patterns in visual cortices; V1 cells exhibit sustained firing following a visual stimulus which indicates a time interval after which reward will be delivered (Shuler and Bear, 2006), and attention modulation in V4 can vary depending on the timing rules of a visual task (Ghose and Maunsell, 2002).

Why may our task have elicited such strong effects on neural firing patterns not described by others (Mountcastle et al., 1990; Hsiao et al., 1993; Chapman and Meftah, 2005)? We acknowledge in Chapter 6 that changes in motor tone on the stimulated digits may partially explain our result. However, we also propose that our task could be performed simply using the timing and/or location of a tactile pattern; complex tactile features did not need to be extracted by higher-level areas, and therefore, we hypothesize that attention effects could be manifested and serve a functional purpose in a primary sensory area. In contrast, those studies that observed little attention effects in SI used tasks like roughness, vibratory, and letter discrimination, which likely require feature integration or extraction employed higher along the somatosensory hierarchy. Future

studies would be required to test this hypothesis, recording in several areas in animals trained on various tactile discrimination tasks requiring feature integration or detection.

7.4. Limitations and future directions.

Due to technical limitations, we only recorded from 3b after the animal was completely proficient at the one-back distal tactile task. It would be of great interest to follow single unit responses, particularly in several relevant cortical regions, throughout tactile training. Though we described in Chapter 1 that tactile attention alters the temporal correlation patterns among cells in somatosensory cortex, it has not been established as a mechanism for experience-dependent plasticity. One study observed increased correlations among cell pairs along with RF expansion concurrent with learning a multi-digit task (Blake et al., 2005), though the authors did not quantify synchrony precisely and did not explore if neural pairs' temporal patterns correlated with the degree of RF expansion observed outside the tactile task. We tested if spike synchrony is altered in SI with tactile attention at the end of training among cell pairs with similar RF properties. While we did not observe enhancement in synchrony at the end of training, perhaps this is a mechanism that is utilized during the learning process as RFs are expanded. Note that to test this would require the ability to stability record from many single units from an animal over a long time period. Future studies could quantify RF size precisely as tactile training progresses, correlating RF size with spike synchrony quantified with and without tactile attention. Random dot stimuli could provide precise calculations of the structure of inhibitory and excitatory subregions, and stimuli presented simultaneously over multiple digits could also reveal how multi-digit training alters non-classical RF properties and interdigit modulations of 3b cells. Additionally, it would be of great interest to examine

responses in prefrontal regions thought to control attentional signals to sensory cortices; how do responses in these regions change during the learning process? We propose future experiments should examine responses in the ventrolateral prefrontal region throughout tactile learning, as this region is anatomically connected with somatosensory cortices (Barbas and Mesulam, 1985; Preuss and Goldman-Rakic, 1989; Cipolloni and Pandya, 1999) and implicated in tactile working memory and feature selection tasks (Romo et al., 1999; Kostopoulos et al., 2007; Gomez-Ramirez et al., 2010).

As we sought to expand upon the work of others (Wang et al., 1995), and also because of the novelty of the distal one-back task, we kept the task as simple as possible. We only asked the animal to respond to two consecutive distal stimuli, amidst four sequence types, and therefore, it is possible that the animal ignored the proximal stimuli and performed the task based on the temporal pattern of the distal stimuli. A fixed interstimulus interval also meant that it was likely that the animal used the timing and not the location of stimuli to perform the task. Indeed, when we altered the timing between distal stimuli, the animal's performance dropped, though it was able to quickly adapt. It would be of great interest to examine somatosensory responses when these rules are changed.

Because of the rules of the one-back distal task, the tactile pattern was stopped during incorrect trials and stimuli were always presented to the distal and proximal pads of D3 and D4. A future experiment would present competing stimuli to other pads or to vary the animal's spatial attention, cueing it to respond to the properties of tactile stimuli on one (or more) digit(s) while ignoring tactile stimuli presented to other digits. Are responses in 3b modified by tactile spatial attention? If not, is spatial attention manifested

in other, perhaps higher, somatosensory regions? Utilizing a two-forced discrimination task, as opposed to a continuous temporal sequence where the animal can respond at any time, would allow future experimenters to examine the effect of tactile spatial attention on neural responses and describe this relationship to behavior. That is, tactile stimuli and animal responses would be consistent throughout, even when the animal responds incorrectly, and one could then ask if neural responses predict behavior on a trial-by-trial basis. It would be of great interest to examine how neural responses and the degree of experience-dependent plasticity change depending on the task rules and difficulty of a tactile task. This has been performed in primary auditory cortex; plasticity is observed in tasks of mid-range difficulty (Engineer et al., 2012). Does experience dependent plasticity in somatosensory cortices increase when the task is more difficult? We found greater attention modulation on longer trials (Figure 6.10), when the animal was more likely performed incorrectly (Figure 6.2), though it is unclear if those trials were truly more perceptually difficult or the animal was simply more likely to saccade early to the response cue in anticipation of upcoming target stimuli.

We consider the studies discussed in this thesis offer new and important insights into experience-dependent plasticity in the somatosensory system. These studies expand upon others' work by describing which tactile stimulus properties specify neural plasticity and changes in the tactile perception and how somatosensory experience dependent plasticity may be utilized to perform a tactile task. We hope future studies will further our work to determine the precise mechanisms by which attention, reward, and stimulus properties alter somatosensory cortical responses and improve or interfere with tactile perception.

REFERENCES.

Abraira VE, Ginty DD (2013) The sensory neurons of touch. Neuron 79:618–639.

Aghdaee SM, Battelli L, Assad J a (2014) Relative timing: from behaviour to neurons. Philos Trans R Soc Lond B Biol Sci 369:20120472.

Ahissar E, Vaadia E, Ahissar M, Bergman H, Arieli A, Abeles M (1992) Dependence of cortical plasticity on correlated activity of single neurons and on behavioral context. Science 257:1412–1415.

Allard T, Clark S a, Jenkins WM, Merzenich MM (1991) Reorganization of somatosensory area 3b representations in adult owl monkeys after digital syndactyly. J Neurophysiol 66:1048–1058.

Alloway KD, Rosenthal P, Burton H (1989) Quantitative measurements of receptive field changes during antagonism of GABAergic transmission in primary somatosensory cortex of cats. Exp Brain Res 78:514–532.

Amarasingham A, Harrison MT, Hatsopoulos NG, Geman S (2012) Conditional modeling and the jitter method of spike resampling. J Neurophysiol 107:517–531.

Ball K, Sekuler R (1982) A specific and enduring improvement in visual motion discrimination. Science 218:697–698.

Bao S, Chan VT, Merzenich MM (2001) Cortical remodelling induced by activity of ventral tegmental dopamine neurons. Nature 412:79–83.

Bara-Jimenez W, Catalan MJ, Hallett M, Gerloff C (1998) Abnormal somatosensory homunculus in dystonia of the hand. Ann Neurol 44:828–831.

Bara-Jimenez W, Shelton P, Hallett M (2000a) Spatial discrimination is abnormal in focal hand dystonia. Neurology 55:1869–1873.

Bara-Jimenez W, Shelton P, Sanger TD, Hallett M (2000b) Sensory discrimination capabilities in patients with focal hand dystonia. Ann Neurol 47:377–380.

Barbas H, Mesulam MM (1985) Cortical afferent input to the principalis region of the rhesus monkey. Neuroscience 15:619–637.

Bensmaia SJ, Denchev P V, Dammann JF, Craig JC, Hsiao SS (2008) The representation of stimulus orientation in the early stages of somatosensory processing. J Neurosci 28:776–786.

Berens P (2009) CircStat: A MATLAB toolbox for circular statistics. J Stat Softw 31:1–21.

Blake DT, Byl NN, Cheung S, Bedenbaugh P, Nagarajan S, Lamb M, Merzenich M (2002) Sensory representation abnormalities that parallel focal hand dystonia in a primate model. Somatosens Mot Res 19:347–357.

Blake DT, Heiser MA, Caywood M, Merzenich MM (2006) Experience-dependent adult cortical plasticity requires cognitive association between sensation and reward. Neuron 52:371–381.

Blake DT, Strata F, Kempter R, Merzenich MM (2005) Experience-dependent plasticity in S1 caused by noncoincident inputs. J Neurophysiol 94:2239–2250.

Brody CD (1998) Slow covariations in neuronal resting potentials can lead to artefactually fast cross-correlations in their spike trains. J Neurophysiol 80:3345–3351.

Buonomano DV, Merzenich MM (1998) Cortical plasticity: from synapses to maps. Annu Rev Neurosci 21:149–186.

Burton H, Sinclair RJ (2000a) Tactile-spatial and cross-modal attention effects in the primary somatosensory cortical areas 3b and 1-2 of rhesus monkeys. Somatosens Mot Res 17:213–228.

Burton H, Sinclair RJ (2000b) Attending to and remembering tactile stimuli: a review of brain imaging data and single-neuron responses. J Clin Neurophysiol 17:575–591.

Byl NN, Merzenich MM, Cheung S, Bedenbaugh P, Nagarajan SS, Jenkins WM (1997) A primate model for studying focal dystonia and repetitive strain injury: effects on the primary somatosensory cortex. Phys Ther 77:269–284.

Byl NN, Merzenich MM, Jenkins WM (1996) A primate genesis model of focal dystonia and repetitive strain injury: I. Learning-induced dedifferentiation of the representation of the hand in the primary somatosensory cortex in adult monkeys. Neurology 47:508–520.

Candia V, Wienbruch C, Elbert T, Rockstroh B, Ray W (2003) Effective behavioral treatment of focal hand dystonia in musicians alters somatosensory cortical organization. Proc Natl Acad Sci U S A 100:7942–7946.

Carter AW, Chen SC, Lovell NH, Vickery RM, Morley JW (2014) Convergence across Tactile Afferent Types in Primary and Secondary Somatosensory Cortices. PLoS One 9:e107617.

Chapman CE, Meftah E-M (2005) Independent controls of attentional influences in primary and secondary somatosensory cortex. J Neurophysiol 94:4094–4107.

Chatagny P, Badoud S, Kaeser M, Gindrat AD, Savidan J, Fregosi M, Moret V, Roulin

C, Schmidlin E, Rouiller EM (2013) Distinction between hand dominance and hand preference in primates: A behavioral investigation of manual dexterity in nonhuman primates (macaques) and human subjects. Brain Behav 3:575–595.

Chen LM, Friedman RM, Roe AW (2003) Optical imaging of a tactile illusion in area 3b of the primary somatosensory cortex. Science 302:881–885.

Chubykin AA, Roach EB, Bear MF, Shuler MGH (2013) A Cholinergic Mechanism for Reward Timing within Primary Visual Cortex. Neuron 77:723–735.

Cipolloni PB, Pandya DN (1999) Cortical connections of the frontoparietal opercular areas in the rhesus monkey. J Comp Neurol 403:431–458.

Clancy KB, Schnepel P, Rao a. T, Feldman DE (2015) Structure of a Single Whisker Representation in Layer 2 of Mouse Somatosensory Cortex. J Neurosci 35:3946–3958.

Clark SA, Allard T, Jenkins WM, Merzenich MM (1988) Receptive fields in the body-surface map in adult cortex defined by temporally correlated inputs. Nature 332:444–445.

Cohen MR, Kohn A (2011) Measuring and interpreting neuronal correlations. Nat Neurosci 14:811–819.

Cohen MR, Maunsell JHR (2009) Attention improves performance primarily by reducing interneuronal correlations. Nat Neurosci 12:1594–1600.

Cohen MR, Maunsell JHR (2011) Using neuronal populations to study the mechanisms underlying spatial and feature attention. Neuron 70:1192–1204.

Connor CE, Hsiao SS, Phillips JR, Johnson KO (1990) Tactile roughness: neural codes that account for psychophysical magnitude estimates. J Neurosci 10:3823–3836.

Connor CE, Johnson KO (1992) Neural coding of tactile texture: comparison of spatial and temporal mechanisms for roughness perception. J Neurosci 12:3414–3426.

Connor CE, Preddie DC, Gallant JL, Van Essen DC (1997) Spatial attention effects in macaque area V4. J Neurosci 17:3201–3214.

Conte A, Rocchi L, Ferrazzano G, Leodori G, Bologna M, Li Voti P, Nardella A, Berardelli A (2014) Primary somatosensory cortical plasticity and tactile temporal discrimination in focal hand dystonia. Clin Neurophysiol 125:537–543.

Costanzo RM, Gardner EP (1980) A quantitative analysis of responses of direction-sensitive neurons in somatosensory cortex of awake monkeys. J Neurophysiol 43:1319–1341.

Cousineau D (2005) Confidence intervals in within-subject designs: A simpler solution to Loftus and Masson's method. Tutor Quant Methods Psychol:2–5.

Craig JC, Johnson KO (2000) The Two-Point Threshold: Not a Measure of Tactile Spatial Resolution. Curr Dir Psychol Sci 9:29–32.

Craig JC, Xu BH (1990) Temporal order and tactile patterns. Percept Psychophys 47:22–34.

Crist RE, Kapadia MK, Westheimer G, Gilbert CD (1997) Perceptual learning of spatial localization: specificity for orientation, position, and context. J Neurophysiol 78:2889–2894.

Crist RE, Li W, Gilbert CD (2001) Learning to see: experience and attention in primary visual cortex. Nat Neurosci 4:519–525.

Dahmen JC, King AJ (2007) Learning to hear: plasticity of auditory cortical processing. Curr Opin Neurobiol 17:456–464.

Diamond ME, Huang W, Ebner FF (1994) Laminar comparison of somatosensory cortical plasticity. Science 265:1885–1888.

DiCarlo JJ, Johnson KO (2000) Spatial and temporal structure of receptive fields in primate somatosensory area 3b: effects of stimulus scanning direction and orientation. J Neurosci 20:495–510.

DiCarlo JJ, Johnson KO, Hsiao SS (1998) Structure of receptive fields in area 3b of primary somatosensory cortex in the alert monkey. J Neurosci 18:2626–2645.

Ego-Stengel V, Shulz DE, Haidarliu S, Sosnik R, Ahissar E (2001) Acetylcholine-dependent induction and expression of functional plasticity in the barrel cortex of the adult rat. J Neurophysiol 86:422–437.

Engineer ND, Engineer CT, Reed a C, Pandya PK, Jakkamsetti V, Moucha R, Kilgard MP (2012) Inverted-U function relating cortical plasticity and task difficulty. Neuroscience 205:81–90.

Fahle M (1997) Specificity of learning curvature, orientation, and vernier discriminations. Vision Res 37:1885–1895.

Fahle M (2004) Perceptual learning: a case for early selection. J Vis 4:879–890.

Fahle M (2005) Perceptual learning: Specificity versus generalization. Curr Opin Neurobiol 15:154–160.

Fahle M, Edelman S, Poggio T (1995) Fast perceptual learning in hyperacuity. Vision Res 35:3003–3013.

Feldman DE (2009) Synaptic mechanisms for plasticity in neocortex. Annu Rev Neurosci 32:33–55.

Feldman DE, Brecht M (2005) Map plasticity in somatosensory cortex. Science 310:810–815.

Fiorentini A, Berardi N (1980) Perceptual learning specific for orientation and spatial frequency. Nature 287:43–44.

Fitzgerald PJ, Lane JW, Thakur PH, Hsiao SS (2004) Receptive field properties of the macaque second somatosensory cortex: evidence for multiple functional representations. J Neurosci 24:11193–11204.

Fitzgerald PJ, Lane JW, Thakur PH, Hsiao SS (2006a) Receptive field properties of the macaque second somatosensory cortex: representation of orientation on different finger pads. J Neurosci 26:6473–6484.

Fitzgerald PJ, Lane JW, Thakur PH, Hsiao SS (2006b) Receptive field (RF) properties of the macaque second somatosensory cortex: RF size, shape, and somatotopic organization. J Neurosci 26:6485–6495.

Flor H, Elbert T, Knecht S, Wienbruch C, Pantev C, Birbaumer N, Larbig W, Taub E (1995) Phantom-limb pain as a perceptual correlate of cortical reorganization following arm amputation. Nature 375:482–484.

Florence SL, Taub HB, Kaas JH (1998) Large-scale sprouting of cortical connections after peripheral injury in adult macaque monkeys. Science 282:1117–1121.

Foffani G, Chapin JK, Moxon K a (2008) Computational role of large receptive fields in the primary somatosensory cortex. J Neurophysiol 100:268–280.

Friedman RM, Chen LM, Roe AW (2008) Responses of areas 3b and 1 in anesthetized squirrel monkeys to single- and dual-site stimulation of the digits. J Neurophysiol 100:3185–3196.

Gardner EP, Costanzo RM (1980) Neuronal mechanisms underlying direction sensitivity of somatosensory cortical neurons in awake monkeys. J Neurophysiol 43:1342–1354.

Garraghty PE, LaChica EA, Kaas JH (1991) Injury-induced reorganization of somatosensory cortex is accompanied by reductions in GABA staining. Somatosens Mot Res 8:347–354.

Garraghty PE, Sur M (1990) Morphology of single intracellularly stained axons terminating in area 3b of macaque monkeys. J Comp Neurol 294:583–593.

Gentet LJ, Kremer Y, Taniguchi H, Huang ZJ, Staiger JF, Petersen CCH (2012) Unique functional properties of somatostatin-expressing GABAergic neurons in mouse barrel cortex. Nat Neurosci 15:607–612.

Ghose GM, Maunsell JHR (2002) Attentional modulation in visual cortex depends on task timing. Nature 419:616–620.

Ghose GM, Yang T, Maunsell JHR (2002) Physiological correlates of perceptual learning in monkey V1 and V2. J Neurophysiol 87:1867–1888.

Gibson GO, Makinson CD, Sathian K (2009) Tactile co-activation improves detection of afferent spatial modulation. Exp Brain Res 194:409–417.

Gilbert CD, Li W, Piech V (2009) Perceptual learning and adult cortical plasticity. J Physiol 587:2743–2751.

Godde B, Stauffenberg B, Spengler F, Dinse HR (2000) Tactile coactivation-induced changes in spatial discrimination performance. J Neurosci 20:1597–1604.

Goldstone RL (1998) Perceptual learning. Annu Rev Psychol 49:585–612.

Gomez-Ramirez M, Trzcinski N, Hsiao SS (2010) Cross-cortical neural interactions between the vLPFC & somatosensory cortex during a feature attention task. In: Society for Neuroscience, pp 304.24/LLL40.

Gomez-Ramirez M, Trzcinski NK, Mihalas S, Niebur E, Hsiao SS (2014) Temporal Correlation Mechanisms and Their Role in Feature Selection: A Single-Unit Study in Primate Somatosensory Cortex. PLoS Biol 12:e1002004.

Grant AC, Fernandez R, Shilian P, Yanni E, Hill MA (2006) Tactile spatial acuity differs between fingers: a study comparing two testing paradigms. Percept Psychophys 68:1359–1362.

Graziano A, Jones EG (2009) Early withdrawal of axons from higher centers in response to peripheral somatosensory denervation. J Neurosci 29:3738–3748.

Grüsser SM, Winter C, Mühlnickel W, Denke C, Karl A, Villringer K, Flor H (2001) The relationship of perceptual phenomena and cortical reorganization in upper extremity amputees. Neuroscience 102:263–272.

Hodzic A, Veit R, Karim AA, Erb M, Godde B (2004) Improvement and decline in tactile discrimination behavior after cortical plasticity induced by passive tactile coactivation. J Neurosci 24:442–446.

Hsiao SS, Lane J, Fitzgerald P (2002) Representation of orientation in the somatosensory system. Behav Brain Res 135:93–103.

Hsiao SS, O'Shaughnessy D, Johnson KO (1993) Effects of Selective Attention on Spatial Form Processing in Monkey Primary and Secondary Somatosensory Cortex. J Neurophysiol 70:444–447.

Hubel DH, Wiesel TN (1970) The period of susceptibility to the physiological effects of unilateral eye closure in kittens. J Physiol 206:419–436.

Hubel DH, Wiesel TN, LeVay S (1977) Plasticity of ocular dominance columns in monkey striate cortex. Philos Trans R Soc Lond B Biol Sci 278:377–409.

Hyvärinen J, Poranen A, Jokinen Y (1980) Influence of attentive behavior on neuronal responses to vibration in primary somatosensory cortex of the monkey. J Neurophysiol 43:870–882.

Iwamura Y, Tanaka M, Sakamoto M, Hikosaka O (1983) Functional subdivisions representing different finger regions in area 3 of the first somatosensory cortex of the conscious monkey. Exp Brain Res 51:315–326.

Jain N, Florence SL, Qi HX, Kaas JH (2000) Growth of new brainstem connections in adult monkeys with massive sensory loss. Proc Natl Acad Sci U S A 97:5546–5550.

Jenkins WM, Merzenich MM, Ochs MT, Allard T, Guíc-Robles E (1990) Functional reorganization of primary somatosensory cortex in adult owl monkeys after behaviorally controlled tactile stimulation. J Neurophysiol 63:82–104.

Johnson KO, Hsiao SS (1992) Neural mechanisms of tactual form and texture perception. Annu Rev Neurosci 15:227–250.

Johnson KO, Phillips JR (1981) Tactile spatial resolution. I. Two-point discrimination, gap detection, grating resolution, and letter recognition. J Neurophysiol 46:1177–1192.

Jones EG (2000) Cortical and subcortical contributions to activity-dependent plasticity in primate somatosensory cortex. Annu Rev Neurosci 23:1–37.

Jones EG, Burton H (1976) Areal differences in the laminar distribution of thalamic afferents in cortical fields of the insular, parietal and temporal regions of primates. J Comp Neurol 168:197–247.

Jones EG, Friedman DP (1982) Projection pattern of functional components of thalamic ventrobasal complex on monkey somatosensory cortex. J Neurophysiol 48:521–544.

Kalisch T, Tegenthoff M, Dinse HR (2007) Differential effects of synchronous and asynchronous multifinger coactivation on human tactile performance. BMC Neurosci 8:58.

Karni a, Sagi D (1991) Where practice makes perfect in texture discrimination: evidence for primary visual cortex plasticity. Proc Natl Acad Sci U S A 88:4966–4970.

Kilgard MP (1998) Cortical Map Reorganization Enabled by Nucleus Basalis Activity. Science (80-) 279:1714–1718.

Killebrew JH, Bensmaïa SJ, Dammann JF, Denchev P, Hsiao SS, Craig JC, Johnson KO (2007) A dense array stimulator to generate arbitrary spatio-temporal tactile stimuli. J Neurosci Methods 161:62–74.

Kim SS, Gomez-Ramirez M, Thakur PH, Hsiao SS (2015) Multimodal Interactions between Proprioceptive and Cutaneous Signals in Primary Somatosensory Cortex. Neuron 86:555–566.

Kostopoulos P, Albanese M-C, Petrides M (2007) Ventrolateral prefrontal cortex and tactile memory disambiguation in the human brain. Proc Natl Acad Sci U S A 104:10223–10228.

Lacruz F, Artieda J, Pastor M a, Obeso J a (1991) The anatomical basis of somaesthetic temporal discrimination in humans. J Neurol Neurosurg Psychiatry 54:1077–1081.

Li W, Piech V, Gilbert CD (2004) Perceptual learning and top-down influences in primary visual cortex. Nat Neurosci 7:651–657.

Lipton ML, Liszewski MC, O'Connell MN, Mills A, Smiley JF, Branch C a, Isler JR, Schroeder CE (2010) Interactions within the hand representation in primary somatosensory cortex of primates. J Neurosci 30:15895–15903.

Luck SJ, Chelazzi L, Hillyard S a, Desimone R (1997) Neural mechanisms of spatial selective attention in areas V1, V2, and V4 of macaque visual cortex. J Neurophysiol 77:24–42.

Ma W, Liu B, Li Y, Huang ZJ, Zhang LI, Tao HW (2010) Visual representations by cortical somatostatin inhibitory neurons--selective but with weak and delayed responses. J Neurosci 30:14371–14379.

Markram H, Toledo-Rodriguez M, Wang Y, Gupta A, Silberberg G, Wu C (2004) Interneurons of the neocortical inhibitory system. Nat Rev Neurosci 5:793–807.

Martin a. B, von der Heydt R (2015) Spike Synchrony Reveals Emergence of Proto-Objects in Visual Cortex. J Neurosci 35:6860–6870.

McLaughlin D, Shapley R, Shelley M, Wielaard DJ (2000) A neuronal network model of macaque primary visual cortex (V1): orientation selectivity and dynamics in the input layer 4Calpha. Proc Natl Acad Sci U S A 97:8087–8092.

Meftah E-M, Bourgeon S, Chapman CE (2009) Instructed delay discharge in primary and secondary somatosensory cortex within the context of a selective attention task. J Neurophysiol 101:2649–2667.

Meftah E-M, Shenasa J, Chapman CE (2002) Effects of a cross-modal manipulation of attention on somatosensory cortical neuronal responses to tactile stimuli in the monkey. J Neurophysiol 88:3133–3149.

Merzenich MM, Kaas JH, Wall JT, Sur M, Nelson RJ, Felleman DJ (1983) Progression of change following median nerve section in the cortical representation of the hand in areas 3b and 1 in adult owl and squirrel monkeys. Neuroscience 10:639–665.

Merzenich MM, Nelson RJ, Stryker MP, Cynader MS, Schoppmann A, Zook JM (1984) Somatosensory cortical map changes following digit amputation in adult monkeys. J Comp Neurol 224:591–605.

Mitchell JF, Sundberg K a, Reynolds JH (2009) Spatial attention decorrelates intrinsic activity fluctuations in macaque area V4. Neuron 63:879–888.

Motter BC, Health S (1994) Neural Correlates of Feature Selective Extrastriate Area V4 Memory and Pop-Out in. Physiology 14.

Mountcastle VB, Powell TP (1959) Neural mechanisms subserving cutaneous sensibility, with special reference to the role of afferent inhibition in sensory perception and discrimination. Bull Johns Hopkins Hosp 105:201–232.

Mountcastle VB, Steinmetz MA, Romo R (1990) Frequency discrimination in the sense of flutter: psychophysical measurements correlated with postcentral events in behaving monkeys. J Neurosci 10:3032–3044.

Négyessy L, Pálfi E, Ashaber M, Palmer C, Jákli B, Friedman RM, Chen LM, Roe AW (2013) Intrinsic horizontal connections process global tactile features in the primary somatosensory cortex: Neuroanatomical evidence. J Comp Neurol 521:2798–2817.

Niebur E, Koch C (1994) A model for the neuronal implementation of selective visual attention based on temporal correlation among neurons. J Comput Neurosci 1:141–158.

Owen AM, Hampshire A, Grahn J a, Stenton R, Dajani S, Burns AS, Howard RJ, Ballard CG (2010) Putting brain training to the test. Nature 465:775–778.

Pala A, Petersen CCH (2015) In Vivo Measurement of Cell-Type-Specific Synaptic Connectivity and Synaptic Transmission in Layer 2/3 Mouse Barrel Cortex. Neuron 85:68–75.

Pei Y-C, Denchev P V, Hsiao SS, Craig JC, Bensmaia SJ (2009) Convergence of

submodality-specific input onto neurons in primary somatosensory cortex. J Neurophysiol 102:1843–1853.

Pei YC, Hsiao SS, Craig JC, Bensmaia SJ (2011) Neural Mechanisms of Tactile Motion Integration in Somatosensory Cortex. Neuron 69:536–547.

Phillips JR, Johnson KO (1981) Tactile spatial resolution. II. Neural representation of Bars, edges, and gratings in monkey primary afferents. J Neurophysiol 46:1192–1203.

Phillips JR, Johnson KO, Hsiao SS (1988) Spatial pattern representation and transformation in monkey somatosensory cortex. Proc Natl Acad Sci U S A 85:1317–1321.

Pons TP, Garraghty PE, Ommaya AK, Kaas JH, Taub E, Mishkin M (1991) Massive cortical reorganization after sensory deafferentation in adult macaques. Science 252:1857–1860.

Preuss TM, Goldman-Rakic PS (1989) Connections of the Ventral Granular Frontal Cortex. Exp Brain Res 316.

Pruszynski JA, Johansson RS (2014) Edge-orientation processing in first-order tactile neurons. Nat Neurosci.

Randolph M, Semmes J (1974) Behavioral consequences of selective subtotal ablations in the postcentral gyrus of Macaca mulatta. Brain Res 70:55–70.

Rausell E, Bickford L, Manger PR, Woods TM, Jones EG (1998) Extensive divergence and convergence in the thalamocortical projection to monkey somatosensory cortex. J Neurosci 18:4216–4232.

Recanzone GH, Jenkins WM, Hradek GT, Merzenich MM (1991) A behavioral frequency discrimination paradigm for use in adult primates. Behav Res Methods, Instruments, &amp Comput 23:357–369.

Recanzone GH, Merzenich MM, Jenkins WM, Grajski KA, Dinse AND, A H (1992a) Topographic reorganization of the hand representation in cortical area 3b of owl monkeys trained in a frequency-discrimination task. J Neurophysiol 67:1031–1056.

Recanzone GH, Merzenich MM, Schreiner CE (1992b) Changes in the Distributed Temporal Response Properties of SI Cortical Neurons Reflect Improvements in Performance on a Temporally Based Tactile Discrimination Task. Neurophysiology 67:1071–1091.

Recanzone GH, Schreiner CE, Merzenich MM (1993) Plasticity in the frequency representation of primary auditory cortex following discrimination training in adult

owl monkeys. J Neurosci 13:87–103.

Reed A, Riley J, Carraway R, Carrasco A, Perez C, Jakkamsetti V, Kilgard MP (2011) Cortical map plasticity improves learning but is not necessary for improved performance. Neuron 70:121–131.

Reed JL, Qi H-X, Zhou Z, Bernard MR, Burish MJ, Bonds a B, Kaas JH (2010) Response properties of neurons in primary somatosensory cortex of owl monkeys reflect widespread spatiotemporal integration. J Neurophysiol 103:2139–2157.

Reis J, Schambra HM, Cohen LG, Buch ER, Fritsch B, Zarahn E, Celnik P a, Krakauer JW (2009) Noninvasive cortical stimulation enhances motor skill acquisition over multiple days through an effect on consolidation. Proc Natl Acad Sci U S A 106:1590–1595.

Ringach DL, Bredfeldt CE, Shapley RM, Hawken MJ (2002a) Suppression of neural responses to nonoptimal stimuli correlates with tuning selectivity in macaque V1. J Neurophysiol 87:1018–1027.

Ringach DL, Shapley RM, Hawken MJ (2002b) Orientation selectivity in macaque V1: diversity and laminar dependence. J Neurosci 22:5639–5651.

Romo R, Brody CD, Hernández A, Lemus L (1999) Neuronal correlates of parametric working memory in the prefrontal cortex. Nature 399:470–473.

Romo R, Hernández A, Zainos A, Lemus L, Brody CD (2002) Neuronal correlates of decision-making in secondary somatosensory cortex. Nat Neurosci 5:1217–1225.

Roy A, Steinmetz PN, Hsiao SS, Johnson KO, Niebur E (2007) Synchrony: A Neural Correlate of Somatosensory Attention. J Neurophysiol 98:1645–1661.

Rutkowski RG, Weinberger NM (2005) Encoding of learned importance of sound by magnitude of representational area in primary auditory cortex. Proc Natl Acad Sci U S A 102:13664–13669.

Saal HP, Bensmaia SJ (2014) Touch is a team effort□: interplay of submodalities in cutaneous sensibility. Trends Neurosci 37:689–697.

Salinas E, Hernandez a, Zainos a, Romo R (2000) Periodicity and firing rate as candidate neural codes for the frequency of vibrotactile stimuli. J Neurosci 20:5503–5515.

Sanger TD, Tarsy D, Pascual-Leone A (2001) Abnormalities of spatial and temporal sensory discrimination in writer's cramp. Mov Disord 16:94–99.

Sasaki Y, Nanez JE, Watanabe T (2010) Advances in visual perceptual learning and

plasticity. Nat Rev Neurosci 11:53–60.

Sathian K, Zangaladze A (1996) Tactile spatial acuity at the human fingertip and lip: bilateral symmetry and interdigit variability. Neurology 46:1464–1466.

Schoups A, Vogels R, Qian N, Orban G (2001) Practising orientation identification improves orientation coding in V1 neurons. Nature 412:549–553.

Scontrini a, Conte A, Defazio G, Fiorio M, Fabbrini G, Suppa A, Tinazzi M, Berardelli A (2009) Somatosensory temporal discrimination in patients with primary focal dystonia. J Neurol Neurosurg Psychiatry 80:1315–1319.

Seitz A, Watanabe T (2005) A unified model for perceptual learning. Trends Cogn Sci 9:329–334.

Seitz AR, Kim D, Watanabe T (2009) Rewards evoke learning of unconsciously processed visual stimuli in adult humans. Neuron 61:700–707.

Seitz AR, Watanabe T (2003) Psychophysics: Is subliminal learning really passive? Nature 422:36.

Seitz AR, Watanabe T (2009) The phenomenon of task-irrelevant perceptual learning. Vision Res 49:2604–2610.

Seol GH, Ziburkus J, Huang S, Song L, Kim IT, Takamiya K, Huganir RL, Lee H-K, Kirkwood A (2007) Neuromodulators control the polarity of spike-timing-dependent synaptic plasticity. Neuron 55:919–929.

Shapley R, Hawken M, Ringach DL (2003) Dynamics of orientation selectivity in the primary visual cortex and the importance of cortical inhibition. Neuron 38:689–699.

Shuler MG, Bear MF (2006) Reward timing in the primary visual cortex. Science 311:1606–1609.

Smith M a, Kohn A (2008) Spatial and temporal scales of neuronal correlation in primary visual cortex. J Neurosci 28:12591–12603.

Spengler F, Roberts TP, Poeppel D, Byl N, Wang X, Rowley H a, Merzenich MM (1997) Learning transfer and neuronal plasticity in humans trained in tactile discrimination. Neurosci Lett 232:151–154.

Sripati AP, Yoshioka T, Denchev P, Hsiao SS, Johnson KO (2006) Spatiotemporal receptive fields of peripheral afferents and cortical area 3b and 1 neurons in the primate somatosensory system. J Neurosci 26:2101–2114.

Steinmetz PN, Roy A, Fitzgerald PJ, Hsiao SS, Johnson KO (2000) Attention modulates synchronized neuronal ® ring in primate somatosensory cortex. :187–190.

Sur M (1980) Receptive fields of neurons in areas 3b and 1 of somatosensory cortex in monkeys. Brain Res 198:465–471.

Sur M, Garraghty PE, Bruce CJ (1985) Somatosensory cortex in macaque monkeys: laminar differences in receptive field size in areas 3b and 1. Brain Res 342:391–395.

Sur M, Merzenich MM, Kaas JH (1980) Magnification, receptive-field area, and "hypercolumn" size in areas 3b and 1 of somatosensory cortex in owl monkeys. J Neurophysiol 44:295–311.

Thakur PH, Fitzgerald PJ, Hsiao SS (2012) Quadratic receptive fields reveal multi-digit interactions in area 3b of the macaque monkey. J Neurophysiol.

Thakur PH, Fitzgerald PJ, Lane JW, Hsiao SS (2006) Receptive Field Properties of the Macaque Second Somatosensory Cortex: Nonlinear Mechanisms Underlying the Representation of Orientation Within a Finger Pad. J Neurosci 26:13567–13575.

Tinazzi M, Frasson E, Bertolasi L, Fiaschi a, Aglioti S (1999) Temporal discrimination of somesthetic stimuli is impaired in dystonic patients. Neuroreport 10:1547–1550.

Tong J, Mao O, Goldreich D (2013) Two-point orientation discrimination versus the traditional two-point test for tactile spatial acuity assessment. Front Hum Neurosci 7:1–11.

Van Boven RW, Johnson KO (1994) The limit of tactile spatial resolution in humans: grating orientation discrimination at the lip, tongue, and finger. Neurology 44:2361–2366.

Vega-Bermudez F, Johnson KO (2002) Spatial acuity after digit amputation. Brain 125:1256–1264.

Wall PD (1977) The presence of ineffective synapses and the circumstances which unmask them. Philos Trans R Soc Lond B Biol Sci 278:361–372.

Wang X, Lu T, Snider RK, Liang L (2005) Sustained firing in auditory cortex evoked by preferred stimuli. Nature 435:341–346.

Wang X, Merzenich MM, Sameshima K, Jenkins WM (1995) Remodelling of hand representation in adult cortex determined by timing of tactile stimulation. Nature 378:71–75.

Wang Z, Chen L, Négyessy L, Friedman R, Mishra A, Gore J, Roe A (2013) The Relationship of Anatomical and Functional Connectivity to Resting-State Connectivity in Primate Somatosensory Cortex. Neuron 78:1116–1126.

Watanabe T, Náñez JE, Sasaki Y (2001) Perceptual learning without perception. Nature

413:844–848.

Wong M, Gnanakumaran V, Goldreich D (2011) Tactile spatial acuity enhancement in blindness: evidence for experience-dependent mechanisms. J Neurosci 31:7028–7037.

Wong M, Peters RM, Goldreich D (2013) A physical constraint on perceptual learning: tactile spatial acuity improves with training to a limit set by finger size. J Neurosci 33:9345–9352.

Woo S-H, Lumpkin E a., Patapoutian A (2015) Merkel cells and neurons keep in touch. Trends Cell Biol 25:74–81.

Xerri C, Coq JO, Merzenich MM, Jenkins WM (1996) Experience-induced plasticity of cutaneous maps in the primary somatosensory cortex of adult monkeys and rats. J Physiol Paris 90:277–287.

Xing D, Ringach DL, Hawken MJ, Shapley RM (2011) Untuned Suppression Makes a Major Contribution to the Enhancement of Orientation Selectivity in Macaque V1. J Neurosci 31:15972–15982.

Yang G, Pan F, Gan W-B (2009) Stably maintained dendritic spines are associated with lifelong memories. Nature 462:920–924.

Yang T, Maunsell JHR (2004) The effect of perceptual learning on neuronal responses in monkey visual area V4. J Neurosci 24:1617–1626.

Yau JM, Connor CE, Hsiao SS (2013) Representation of tactile curvature in macaque somatosensory area 2. J Neurophysiol 109:2999–3012.

Yau JM, Pasupathy A, Fitzgerald PJ, Hsiao SS, Connor CE (2009) Analogous intermediate shape coding in vision and touch. Proc Natl Acad Sci U S A 106:16457–16462.

Zarzecki P, Witte S, Smits E, Gordon DC, Kirchberger P, Rasmusson DD (1993) Synaptic mechanisms of cortical representational plasticity: somatosensory and corticocortical EPSPs in reorganized raccoon SI cortex. J Neurophysiol 69:1422–1432.

Zénon A, Krauzlis RJ (2012) Attention deficits without cortical neuronal deficits. Nature 489:434–437.

Zhang LI, Tan AYY, Schreiner CE, Merzenich MM (2003) Topography and synaptic shaping of direction selectivity in primary auditory cortex. Nature 424:201–205.

Zhou Y Di, Fuster JM (1997) Neuronal activity of somatosensory cortex in a cross-modal

(visuo-haptic) memory task. Exp Brain Res 116:551–555.

Zhou YD, Fuster JM (1996) Mnemonic neuronal activity in somatosensory cortex. Proc Natl Acad Sci U S A 93:10533–10537.

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EDUCATION.

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PhD, Neuroscience
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Cornell University, College of Agriculture and Life Sciences, Ithaca, NY
Bachelor of Science, Magna Cum Laude with Distinction in Research
Biological Sciences, concentration in Neurobiology and Behavior
2004-2008

University College London, London, UK
Affiliate Student, Science and Technology Studies
2007

RESEARCH EXPERIENCE.

Graduate Research
Drs. Steven Hsiao and Charles Edward Connor, Johns Hopkins University, Zanvyl Krieger Mind/Brain Institute/ Department of Neuroscience, Baltimore, MD
2009- Present
Thesis work on the role of attention and neural mechanisms underlying cortical plasticity in somatosensory cortex and tactile perceptual learning.

Dr. Steven Hsiao, Zanvyl Krieger Mind/Brain Institute
2009
Performed neuronal extracellular single-unit recordings using rhesus macaques performing various visual and tactile discrimination tasks. Examined the effect of attention on the temporal correlation of feature-specific neuronal responses in secondary somatosensory cortex.

Dr. Amy Bastian, Johns Hopkins University School of Medicine, Kennedy Krieger Institute/ Departments of Neurology and Neurosurgery, Neuroscience, and Physical Medicine and Rehabilitation, Baltimore, MD
2009
Investigated the effect of variable walking pattern and optic flow on motor learning and generalization to over-ground walking following locomotor adaptation on a split-belt treadmill.

Dr. Barbara Landau, Johns Hopkins University, Department of Cognitive Science, Baltimore, MD
2008
Investigated the development of visually-guided reaching. Developed custom software for performing computer visual tasks.

Undergraduate Research
Dr. Carl Hopkins, Cornell University, Department of Neurobiology and Behavior, Ithaca, NY
2005- 2008: Undergraduate thesis project: "Electric Signaling and Knollenorgan Receptor Organ Properties In the Genus *Campylomormyrus* (Mormyridae)"

RESEARCH SUPPORT.

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Ruth L. Kirschstein National Research Service Awards for Individual Predoctoral Fellows, Role: PI
"Mechanisms and Perceptual Consequences of Somatosensory Cortical Plasticity"

TEACHING EXPERIENCE.

Course Director/Instructor
January 2015
Neuroscience in Popular Culture (AS.080.202.12)
Johns Hopkins University, Homewood Campus, Baltimore, MD
Developed and taught an intersession course exploring neuroscience topics commonly discussed in mass media (e.g. film, TV, books). Created lectures covering topics including consciousness, artificial intelligence, and brain-machine interfaces, contrasting current neuroscience knowledge and cutting edge techniques to the media's depictions. Developed and led small group activities throughout to enhance active learning and supervised small group presentations.

Preparing Future Faculty Teaching Academy
2013- 2015
Member of the inaugural cohort of advanced doctoral students selected to learn pedagogy related to higher-level education, educational models and assessment skills while working with faculty mentors. Program run through the School of Nursing and Center for Educational Resources.

Teaching assistant- Undergraduate course
August- December 2010
Brain Injury, Recovery, and Function (AS.080.330.01.FA10), Course Director, Dr. Linda Gorman
Johns Hopkins University, Homewood Campus, Baltimore, MD
Met with students to answer material, writing, and presentation questions regarding neurological processes underlying traumatic brain injury. Graded weekly writing assignments, student presentations, and a final end-of-term essay.

Teaching assistant- Graduate course
January- May 2010
Neuroscience and Cognition II (ME:440.812), Course Director, Dr. Amy Bastian
Johns Hopkins School of Medicine, Baltimore, MD
Attended lectures and prepared lecture notes, met with students and ran review sessions utilizing personally constructed review material in which lecture topics were covered in greater detail and graded written exams.

Undergraduate tutor
August - December 2007
Learning Strategies Center
Cornell, Ithaca, NY
Held weekly tutoring sessions on lecture, homework, and exam topics in introductory biology and genetics. Sessions were specifically tailored to the student or group of students seeking assistance.

PUBLICATIONS.

Trzcinski N.K., Gomez-Ramirez M, Hsiao SS. (Submitted) Functional consequences of experience-dependent plasticity on tactile perception. European Journal of Neuroscience.

Gomez-Ramirez M, **Trzcinski N.K.**, Mihalas S, Niebur E, Hsiao SS (2014) Temporal Correlation Mechanisms and Their Role in Feature Selection: A Single-Unit Study in Primate Somatosensory Cortex. PLoS Biol 12(11): e1002004. doi:10.1371/journal.pbio.1002004

Trzcinski, N.K., Hopkins, C.D. (2008) Electric Signaling and Electroreception Properties in Electric Fishes of the Genus *Campylomormyrus* (Mormyridae). Cornell Synapse: Undergraduate Journal of Neuroscience.

PRESENTATIONS/ POSTERS.

Trzcinski, N.K. , Gomez-Ramirez M, Hsiao, S.S. (2014) Dynamic changes in receptive field properties in primate primary somatosensory (area 3b). Poster, 339.08/HH16, Society for Neuroscience ,Washington, DC, November.

Trzcinski, N.K. , Hsiao S.S. (2013). Cortical plasticity and tactile attention in primate primary somatosensory cortex (SI). Presentation for HHMI Janelia Farms Conference: Mammalian Circuits Underlying Touch Sensation, Ashburn, Va. September.

Trzcinski, N.K. , Hsiao S.S. (2013). Mechanisms and Perceptual Consequences of Experience-Dependent Somatosensory Plasticity. Poster for HHMI Janelia Farms Conference: Mammalian Circuits Underlying Touch Sensation, Ashburn, Va. September.

Trzcinski, N.K. , Hsiao, S.S. (2013) Cortical plasticity and tactile attention in primate primary somatosensory cortex. Poster, 70.02/NN2, Society for Neuroscience, San Diego, Ca. November.

Trzcinski N.K. , Hsiao S.S. (2011). The perceptual consequences of training-induced somatosensory plasticity. Poster, 74.15/LL17. Society for Neuroscience, Washington, DC. November.

Gomez-Ramirez, M., **Trzcinski, N.K**., Hsiao, S. (2010) Cross-Cortical Neural Interactions between the vLPFC & Somatosensory Cortex during a Feature Attention Task. Poster, 304.24/ LLL40. Society for Neuroscience, San Diego, Ca., November.

Torres-Oviedo, G., **Trzcinski, N.K.,** Bastian, A.J. (2009). Does the source of motor variability affect learning and generalization of a new locomotor pattern? Presentation, 702.6. Society for Neuroscience, Chicago, Il., November.

Gomez-Ramirez, M. **Trzcinski, N.K.,** Yoshioka, T., Mihalas, S., Niebur, E., Hsiao, S. (2009) The neural mechanisms underlying the spotlight of attention: A selective attention study assessing orientation and frequency mechanisms in the somatosensory modality. Poster, 562.10/DD27. Society for Neuroscience, Chicago, Il., November.

Gregory, E., **Trzcinski, N.K.**, Hoffman, J., & Landau, B. (2009). The representation of action in memory: A developmental study. Poster, Vision Sciences Society, Naples, Fl. May.

AWARDS.

University of Maryland Postdoctoral Fellowship in Comparative and Evolutionary Biology of Hearing (2015), Miriam M. Salpeter Undergraduate Research Award in

Neuroscience (2008), High Honors in Biological Science Research (2008), Myron M. Fuerst Scholarship (2008), Cornell CALS Dean's list (2004-2008), Undergraduate HHMI Scholar (2007), UCLU Student Employee of the Year (2007), Polish Women's Alliance of America Academic Scholarship Winner (2006)

PROFESSIONAL MEMBERSHIP. Society for Neuroscience, 2008-Present