Interactions between barrel cortex and primary somatosensory thalamus in the mouse

By

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Abstract

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In order to fully understand how thalamus and cortex work together to process sensory information, we need to examine not only the well-known feedforward connections between them but also the numerous feedback projections that originate in cortex and terminate in the thalamus. Specifically, we are interested in the thalamic contribution to the construction and processing of spatial representation in a sensory space, as well as the somewhat more basic question of the effect of cortical activity on thalamic activity that underlies any kind of representation. In this dissertation, I detail my foray into these questions using the whisker system in an awake mouse model.

In Chapter 1, I will provide a broad overview and introduction to the questions at hand, giving a framework in which to understand both the motivation and results of the following studies. Chapter 2 focuses on examining how representation of space emerges in cortex and how that is different from thalamus. This is presented via a reproduction of a co-authored manuscript. Chapter 3 focuses on presenting a set of experiments utilizing cortical modulation and electrophysiology to probe the function of corticothalamic feedback in the awake animal. To this point, I include a first-author manuscript in preparation. Finally, Chapter 4 provides closing thoughts.
Preface

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As my time at Berkeley draws to an end, it is harder and harder to find words to, ostensibly, sum up and reflect on what has been an unflinchingly surprising 5 years. Grad school has seen me turn from a budding young scientist, sure of the existence of answers if you just look hard enough, to a young-at-heart explorer eager to engage with the world in a fundamentally different way – with openness to uncertainty, a more honest curiosity, a level of positivity that I couldn’t have imagined when I started, and an undeniable wish to pepper this page with asides that I’m pretty sure only I would find funny. Best of all, I’ve settled into a deep comfort with being as much “me” as I know I can be, and expressing it through a fullness of spirit that I can hope causes joy in others. Without doubt, so much of this change is because of the people I’ve met in my time here and the experiences that has led to. Here I thank some of those folks, all the while realizing that these words are only a poor approximation of the gratitude I feel.

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Figure 1. Graduation post-it
Publications related to this work


Chapter 1: Introduction to thalamocortical and corticothalamic circuits

Incoming sensory information from the periphery must necessarily pass through the thalamus in nearly every sensory modality before arriving at the cortex and undergoing processing that underlies perception of the outside world. Accordingly, being such an essential precursor, the thalamus and specifically its relationship with a primary sensory cortex has been of great interest to researchers for as long as cortex itself has been studied. The thalamus has many subdivisions, of course, and of most relevance to us is the primary thalamus which mediates ascending sensory information as opposed to those subdivisions that are more heavily connected to secondary sensory cortices and higher-level areas, simply because we assume this “primary” thalamus has the most direct impact on how and what information arrives at the cortex for the early processing stages.

Given decades of research into this part of the sensory circuit, we still do not have a good enough grasp of its full potential, and in some cases described later in the introduction, even some basic properties have not been agreed upon. This is likely due to a disparity in what the circuit looks like and what it is – at first glance, it seems an attractively “simple” arrangement, composed of only a few moving parts, but when properly delved into, it transforms into an exquisitely complex one that can be difficult to disentangle and understand.

The thalamic side of the circuit is composed of two major components: the primary sensory thalamus, which, in my research, is the ventral posterior medial nucleus (abbreviated as VPM) of the mouse whisker system, and the reticular nucleus (abbreviated as nRT). The VPM in the mouse is composed of almost exclusively excitatory cells, which receive ascending glutamatergic input from brainstem nuclei, descending glutamatergic input from the primary somatosensory cortex (from now referred to as S1), and GABAergic input from its only source of inhibition, the nRT (Houser et al. 1980). Unlike some other thalamic nuclei, such as the LGN, the VPM does not have intrinsic inhibitory cells and must therefore rely on the nRT for inhibition. Neurons in the VPM are arranged into functional subunits that also happen to be visible anatomically with staining – they cluster into curved tapered cylinders, each of which corresponds to a given whisker on the snout of a mouse. Providing sensory stimulation to a given whisker results in robust activation of the corresponding area of VPM (Diamond et al. 1992). Glutamatergic projections leave the VPM and arrive mainly in cortical layer 4 in a somatotopic fashion (Land et al. 1995; Agmon et al. 1995) (although it is important to note that nearly all layers receive some amount of thalamic input), from which point the incoming sensory information is presumably distributed through the layers and, in that process, undergoes a variety of transformations.

In fact, this somatotopic arrangement is preserved throughout the entire whisker system, making it an ideal choice for probing spatial representation – an array of peripheral sensors (whiskers) is easily accessible to us as researchers and is part of an active sensing system that, in an awake animal,
constantly sweeps out space; thus, the animal must therefore construct an internal representation of this space. Over the years, many studies have addressed such a representation, and specifically, how the whiskers work in tandem to represent a focal sensory input, albeit in anesthetized paradigms that focus on cortical effects (Armstrong-James et al. 1992; Brecht & Sakmann 2002; Ego-Stengel et al. 2005; Kwegyir-Afful 2005; Ghazanfar & Nicolelis 1999; Mirabella et al. 2001; Petersen et al. 2001). The study contained in Chapter 2 presents an exploration into this very question in an awake, behaving animal preparation that centers on cortical processing, but includes data from the VPM that sheds light on the inter-layer transformations that take place and the de novo emergent properties of layer 4.

On the corticothalamic side, projections originate from a subset of cells in layer 6, appropriately categorized as corticothalamic (CT) cells as opposed to other pyramidal cells in that layer that only project intracortically (corticocortical cells, CC). These connections descend, following a somatotopic arrangement, into the VPM (Lam & Sherman 2010) and some send collaterals into the nRT where they contact inhibitory cells (Liu & Jones 1999; Golshani et al. 2001) which, then, themselves project to the VPM (Pinault & Deschênes 1998; Pinault et al. 1995; Lam 2005). The direct L6–VPM projection is known to be glutamatergic (Deschênes & Hu 1990; De Biasi & Rustioni 1990; Bromberg et al. 1981; McCormick & von Krosigk 1992; Fonnum et al. 1981; Reichova & Sherman 2004; Sherman 2012), while the reticular-VPM synapses are GABAergic (Cox et al. 1997; Kim et al. 1997; Spreatico et al. 1991; Guillery & Harting 2003; Ohara 1988; Houser et al. 1980). Thus, it is reasonable to assume that cortical influence on the VPM, or any given primary thalamus, is a complex combination of the excitatory and inhibitory inputs.

Of paramount interest is the fact that corticothalamic connections outnumber thalamocortical ones by 10:1 (Deschenes et al. 1998), contributing anywhere from 30 to 50% of the thalamic synaptic input (Liu et al. 1995; Erışir et al. 1997; Van Horn et al. 2000). Given the rather large size of the feedback, it has been of great research attention since cortex can easily influence and, to an extent, control, its own input via these projections. Many studies have addressed this over the years, and the literature is full of intriguing yet contradictory results depending on which modality is being examined in which manner. Early pre-optogenetic approaches included cortical cooling, pharmacological manipulations, and cortical ablation to manipulate cortex while observing the impact on thalamus, with all of the experiments being done on anesthetized animals or in vitro (Waleszczyk et al. 2005; Ghosh et al. 1994; Przybyszewski et al. 2000; Andolina et al. 2013; Li & Ebner 2007; Rivadulla et al. 2003; De Labra et al. 2007; Funke et al. 1996; Hasse & Briggs 2017; Temereanca & Simons 2004; Wolfart et al. 2005). The results are mixed – although the general indication is that cortex seems to sharpen thalamic receptive fields and influences how effectively information is transmitted through the thalamus, some basic principles, like the overall sign of cortical effect, has not been determined.

One might think that, with the advent of optogenetics and advances in transgenic techniques that allow specific targeting of cell subpopulations, this circuit may have become easier to tease apart, yet the clashing results continue (Jurgens et al. 2012; Pauzin & Krieger 2018; Mease et al. 2014; Guo et al. 2017; Olsen et al. 2012), and my own work presented in Chapter 3 is no exception. The study included therein focuses on corticothalamic function in an awake, behaving animal paradigm during active
sensation, using cell-type specific optogenetic modulation, and will provide yet another stepping stone on the way to a fuller understanding of the circuit. Despite the current lack of a firm resolution to the question of corticothalamic function, such research clearly must continue, as new, emerging methods, technologies, and understanding will inform and refine future approaches to the dissection of corticothalamic communication.
Chapter 2: Surround integration organizes a spatial map during active sensation

Foreword
This study was conducted mainly by my wonderful colleagues, Dr. Scott Pluta, and Evan Lyall, and took place during the early portion of my doctorate research. I also had the pleasure of directly working with Gregory Telian at this time. I performed all thalamic experiments and contributed VPM data to the paper to provide context for determining emergent properties of cortical transformations that happen between cortical layers in barrel cortex.


Summary
During active sensation, sensors scan space in order to generate a representation of the outside world. However, since spatial coding in sensory systems is typically addressed by measuring receptive fields in a fixed, sensor-based coordinate frame, the cortical representation of scanned space is poorly understood. To address this question, we probed spatial coding in the rodent whisker system using a combination of two photon imaging and electrophysiology during active touch. We found that surround whiskers powerfully transform the cortical representation of scanned space. On the single neuron level, surround input profoundly alters response amplitude and modulates spatial preference in the cortex. On the population level, surround input organizes the spatial preference of neurons into a continuous map of the space swept out by the whiskers. These data demonstrate how spatial summation over a moving sensor array is critical to generating population codes of sensory space.
Introduction

Cortical neurons represent sensory space through topographic projections of the peripheral sense organs, creating maps of the physical world in the brain. Sensory coding through maps is thought to make both the structure and function of neural circuits more efficient (Knudsen et al. 1987). In passive systems, maps can be probed by systematically stimulating different parts of the sensor array and measuring the receptive fields of individual neurons. In many sensory systems, such as the retina, integration over the sensor array is critical for receptive field formation (Hartline et al. 1956; Kuffler 1953). During active sensation, however, the sensors themselves move – scanning space to provide greater coverage of the outside world (Kleinfeld et al. 2006). How neurons in the cortex encode scanned space, and whether integration across the sensor array is involved, is not known. Furthermore, sensor scanning has the potential to create its own spatial map in the cortex, not of the sensor array itself, but of the space swept out by the sensors. Such a map of scanned space could provide a basis for fine object localization and identification needed for behaviors such as prey capture, predator avoidance, and navigation.

The rodent whisker system is an advantageous system to address this question (Brecht 2007; Feldmeyer et al. 2013; Petersen 2007). On one hand, the topographic and discretized representation of the rodent’s whiskers along the sensory hierarchy facilitates detailed analysis for how sensory neurons perform multi-whisker integration (Woolsey & Van der Loos 1970). On the other, the stereotyped pattern of whisking during spatial exploration facilitates investigation into the sensorimotor processes underlying active sensation (Diamond et al. 2008; Hartmann 2011). Decades of physiological analysis have quantified how spatial summation across the whisker array influences the cortical representation of touch (Armstrong-James et al. 1992; Boloori 2006; Brecht et al. 2003; Brecht & Sakmann 2002; Joshua C Brumberg et al. 1996; Brumberg et al. 1999; Chen-Bee et al. 2012; Ego-Stengel et al. 2005; Estebanez et al. 2012; Krupa et al. 1999; Goldreich et al. 1999; Higley & Contreras 2003; Hirata & Castro-Alamancos 2008; Kwegyir-Afful 2005; Mirabella et al. 2001; Ramirez et al. 2014; Shimegi et al. 2000; Zhu & Connors 1999; Moore & Nelson 1992; Moore et al. 1999; Petersen et al. 2001). Yet nearly all these investigations have utilized passive whisker stimulation, which can only probe receptive fields in discretized whisker space, and not in the continuous space scanned by the whiskers. An artificial whisking paradigm in anesthetized animals has allowed investigators to probe spatial coding during active touch, albeit in a reduced brain state (Brown & Waite 1974; Castro-Alamancos & Bezudhnaya 2015; Szwed et al. 2003; Wallach et al. 2016; Yu et al. 2015). These studies have revealed how spatial summation and the vibrissotopic map evolve across the sensory hierarchy or change dynamically with experience (Feldman & Brecht 2005; Fox 2002; Oberlaender et al. 2012).

Surprisingly, despite the well-ordered anatomical topography of the barrels in L4 (Woolsey & Van der Loos 1970), two-photon imaging in layer 2/3 (L2/3) has revealed that on the cellular scale, the whisker map breaks down, exhibiting a salt and pepper tuning for whisker preference (Clancy et al. 2015) with some spatial correlation on the more global level (Sato et al. 2007). Similar receptive field studies in other rodent cortical areas, such as the auditory and visual cortices, have also found local breakdowns in maps of sensory space (Bandyopadhyay et al. 2010; Rothschild et al. 2010; Smith &
Häusser 2010), despite some evidence of an underlying organization (Ringach et al. 2016) Nonetheless, these works analyzed maps of a fixed sensor array and not of scanned space. It remains uncertain whether an orderly map of scanned space exists in the barrel cortex or elsewhere.

During active touch, barrel cortex neurons are often well tuned to the horizontal location of an object (Pluta et al. 2015; Yu et al. 2015). Multiple mechanisms potentially contribute to their tuning. These include selectivity for the phase (Curtis & Kleinfeld 2009), deflection angle (Knutsen et al. 2008), inter-contact interval (Crochet et al. 2011), or contact forces (Bagdasarian et al. 2013; Yang & Hartmann 2016) at the moment of touch. These schemes can all operate at the single whisker level, and do not require multi-whisker integration, which is likely to occur in most natural contexts. Several studies have found that rodents perform better on whisker-guided behaviors when using multiple whiskers, suggesting that multi-whisker integration is critical for perceptual acuity (Knutsen et al. 2006; Krupa et al. 2001; Daniel H. O’Connor et al. 2010). Although spatial summation is not required for spatial tuning per se, multi-whisker integration could powerfully transform the cortical representation of space. This might be particularly true during active sensing, where neighboring sensors probe overlapping regions of space. This raises the possibility that multi-whisker integration during active sensing might transform a discretized vibrissotopic map into a continuous map of scanned space that could be highly advantageous for object localization and discrimination.

Whether such a map exists in the barrel cortex, and, more specifically, how multi-whisker integration could shape its organization, is unknown. Most prior studies of the barrel cortex during active sensation have either been done in unrestrained animals, when controlling the stimulus is challenging, or in head-fixed mice where only a single whisker is left intact. One study in unrestrained animals quantified tactile responses before and after removing select whiskers surrounding the principal whisker (PW) column and found opposing effects in the cortex and the thalamus (Kelly et al. 1999). Yet in these freely behaving conditions, precise measurements of neuronal receptive fields could not be obtained.

We used two photon imaging and multi-electrode array physiology to address spatial summation and map organization in the somatosensory thalamocortical system. First, we tracked how spatial summation evolves across four stages of the sensory hierarchy, from the thalamus through three cortical layers. We found that neurons in the cortex, but not in the thalamus, exhibited an asymmetric, rostro-caudal gradient of summation over surround whiskers. Surround modulation not only had dramatic impacts on firing rates, but also generated a heterogeneous and substantial shift in the spatial preference of most neurons. On the population level, our data reveal a highly ordered and continuous map of scanned space in L2/3 of the barrel cortex. This map was nearly absent when only a single whisker was intact, indicating that summation over surrounding whiskers is critical to map organization. These data demonstrate that multi-whisker integration in the cortex organizes the spatial preference of neurons to create a continuous map of scanned space. Maps of scanned space may contribute to high fidelity encoding of the location and shape of objects during natural exploration.
Results
Quantifying spatial coding and summation during active sensation

To address how barrel cortex neurons encode scanned space and summate over whiskers in naturally whisking mice, we employed a head-fixed preparation in which mice ran on a free-spinning circular treadmill while we presented a vertical bar to the whiskers at fixed locations for 1.5 seconds (Fig. 1A).

Mice were habituated to run for extended periods, a condition in which they move their whiskers in a highly rhythmic fashion (Pluta et al. 2015; Sofroniew et al. 2014) (Fig 1G). Under these conditions we could measure and quantify spatial representations with high precision. Neural activity was recorded with two-photon calcium imaging in the upper cortical layers or multi-electrode arrays in the lower
cortical layers and the ventro-posterior medial nucleus of the thalamus (VPM). Neural data was analyzed in the final 500 ms of stimulus presentation, during which neural activity and whisking kinematics had returned to a stable state after abrupt positioning of the stimulus bar (Fig. S1). Experimental trials were selected based on the velocity and consistency of treadmill running to minimize variation in whisking behavior (Fig. S1E, and see Methods). This strict sampling of running behavior ensured consistent, repetitive touches with the stimulus throughout the object presentation period (Fig. S1F). Prior to each experiment, we first identified the location of the C2 whisker’s representation in each mouse using intrinsic optical imaging. In both imaging and electrophysiology experiments, we found neurons across all layers of the barrel cortex whose tactile-evoked responses were tuned to the horizontal location of the vertical pole (Fig 1B-D). By labeling a single ‘principal whisker’ (PW) in a subset of mice with reflective paint we could track this whisker reliably in the presence of all other whiskers (Fig. 1E-H, Fig. S1). Using high-speed whisker tracking we found that across the full ‘whisking field’ the PW made rhythmic contact with the stimulus bar throughout the stimulus period at central but not lateral locations, where only adjacent whiskers (AWs) contacted the bar, defining aprincipal whisker contact zone (PWCZ) and an adjacent whisker contact zone (AWCZ, Fig. 1E,F).

To explore spatial summation during active sensation, we sought to quantify the contribution of the PW and the AWs to each neuron’s spatial representation. We reasoned that we could measure this by comparing a neuron’s spatial tuning function before and after acutely trimming off all the surround whiskers. The difference in these two measurements would reveal the parallel contributions of the AWs and PW to each neuron’s spatial receptive field. Towards this aim, we collected spatial tuning curves both before and after trimming all but the principal whisker in a single experimental session (< 1 hour), so that after trimming, only the PW could contact the stimulus bar. Importantly, whisker trimming on such an acute time-scale is much shorter than required for the induction of sensory-deprivation induced plasticity (Bender 2006; Glazewski & Fox 1996; Wen et al. 2013). The dataset consisted of 1016 neurons in L4 (340±120 ROIs/mouse; 3 mice), 2572 neurons in L2/3 (640±120 ROIs/mouse; 4 mice), 172 regular spiking (RS) units in L5 (10±2 units/mouse; 16 mice), and 90 units in VPM (11±2/mouse; 8 mice). Since acute whisker trimming might alter an animal’s pattern of whisking during active sensation, in a subset of mice we tracked the PW both before and after surround whisker trimming and found that trimming did not significantly alter the kinematics of the animals’ whisking patterns, except for a minute difference in amplitude (Fig. S1, mean ± s.e.m: 0.90 ± 0.20 degrees, far smaller than the 10–15 degrees between presented stimuli). This indicates that any changes we observed in neuronal response functions were due to changes in neural computation and not to changes in whisking behavior.

Spatial summation in L4

First we addressed spatial coding and summation in excitatory neurons in L4 of the barrel cortex. To record from a large population of L4 excitatory neurons across the spatial map in S1 we expressed GCaMP6s (Chen et al. 2013) in excitatory neurons in L4 using a Cre-dependent AAV and a
L4-specific Cre line (Madisen et al. 2010; Pluta et al. 2015) (Fig. 2A). Prior to whisker trimming we observed

contact-evoked responses across the entire imaging field. Following removal of the surround whiskers, sensory evoked responses were essentially abolished outside of the PW ‘column’ (68 ± 9% decrease in number of significantly driven units, n = 3 mice, for column identification see Methods and Fig. S2), demonstrating that the PW preferentially drives touch responses within its anatomically aligned column, consistent with prior observations under both passive and active conditions (Goldreich et al. 1999; Hires et al. 2015). Strikingly, in the rostral position of the PWCZ the majority (56 ± 8%, n = 3 mice) of L4 neurons within the PW column exhibited significant enhancements in their contact-evoked activity following surround whisker trimming (4.0 ± 1.3 fold increase in population mean, n = 231, Wilcoxon sign rank, p < 0.001, Fig. 2B-E). We computed a ‘trimming index’ as a metric for how surround whiskers influenced the evoked firing rate of each given neuron, defined as the difference over the sum of evoked activity between pre and post-trimming conditions. In the rostral PWCZ position, nearly all neurons
had a positive trimming index, indicating pronounced disinhibition following surround whisker trimming (trimming index = 0.33 ± 0.03, n = 231, p < 0.001, Wilcoxon sign rank, Fig. 2F). In contrast, in the caudal PWCZ position, most neurons showed a reduction in tactile evoked response (0.8 ± 0.1 fold decrease in population mean, trimming index = -0.30 ± 0.03, n = 231, p < 0.001, Wilcoxon sign rank, Fig. 2F). These data indicate that surround input from more caudal whiskers provides facilitation, whereas input from the more rostral whiskers primarily provides suppression. To address how surround whisker input influences spatial coding, we computed an index of spatial preference (the center of mass of the spatial tuning curve in the PWCZ). We found that for nearly all L4 neurons that exhibited spatial tuning (1-way ANOVA), spatial preference shifted forwards (1.77 ± 0.09 mm mean shift, n = 139, p < 0.001, t-test, Fig. 2G).

**Spatial summation in cortical projection layers**

Next we addressed spatial coding and summation in L2/3 and L5, the two major output layers of the barrel cortex. In L2/3 we used two-photon imaging (110-195 microns deep) to sample a large number of L2/3 neurons across the spatial map in S1.

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**Figure 3: Spatial summation in L2/3 neurons.** A) Example image of GCaMP6s-expressing L2/3 neurons. The anatomic aligned C2 column is at center. The red outline indicates the position of the example neuron in B, C. B) Top: schematic of the pre- and post-surround whisker trimming conditions. Bottom: Example tuning curve (mean ± s.e.m.) of a single L2/3 neuron before (black) and after (grey) trimming all but the C2 whisker. C) Example ‘raster’ plot of calcium responses of the cell from B). Top: before trimming. Bottom: after trimming. Responses from all 8 stimulus positions are presented in both cases. D) Example image of the mean change in dF/F for each neuron in the field of view in L2/3 between post and pretrimming conditions for stimulus position 5. Red indicates an increase in mean evoked responses, blue indicates a decrease. A Gaussian blur was applied. E) Plot of the fraction of cells in the C2 barrel that show significant increases (red) or decreases (blue) across each of the four stimulus positions within the PWCZ (n = 631 cells in 4 mice). F) Plot of the average trimming index for the same cells across the same stimulus conditions. G) Histogram of the change in spatial preference for all imaged neurons in the C2 column that exhibited significant spatial tuning both before and after surround whisker trimming (n = 413 cells across 4 mice, p < 0.001, Wilcoxon sign rank).
In L5 we employed laminar multi-channel electrodes that spanned the complete depth of L5. The laminar position of the electrode in each experiment was confirmed with a combination of depth readings off a precise micromanipulator, current source density analysis of the touch-induced local field potential (LFP), and post-hoc histology of the electrode track (Fig. S2). Prior to any trimming, we observed that L2/3 and L5 neurons in the PW column very often exhibited substantial evoked activity in the AWCZ, the region where the PW makes no contact (Fig S2), consistent with prior imaging studies showing that a single whisker could evoke broad activity across multiple barrel columns in L2/3 (Clancy et al. 2015; Peron et al. 2015). This is in contrast to neurons in L4 and in VPM which responded more specifically (but not exclusively) to stimuli within the PWCZ (see Fig. S2). This suggests that surround whisker input in L2/3 and L5 might be particularly important for spatial representations in these cortical projection layers.

Figure 4: Spatial summation in touch responsive regular spiking units of L5. A) Top: schematic of the pre- and post-surround whisker trimming conditions. Bottom: Example tuning curve (mean ± s.e.m.) of a single L5 RS unit before (black) and after (grey) trimming all but the principal whisker. B) Example raster of the unit from A) before trimming to the principal whisker. C) As in B) but for after trimming. Responses from all 8 stimulus positions are presented in both cases. D) Left: Plot of the fraction of L5 RS units in the spared whisker column that show significant increases (red) or decreases (blue) across each of the four stimulus positions within the PWCZ. Right: Plot of the average trimming index for the same cells across the same stimulus conditions (n = 48 units in 8 mice). E) Histogram of the change in spatial preference for all recorded L5 RS units in the spared column with significant spatial tuning both before and after surround whisker trimming (n = 39 units across 8 mice, p = 0.001, Wilcoxon). F) Example image from a recorded animal showing the Dil track (red) of the multi-electrode array extending into L5.
To address this hypothesis, we recorded tactile evoked responses in both layers prior and 
subsequent to trimming all but a single whisker, as above. L2/3 exhibited suppression in the anterior 
PWCZ, but nearly exclusive facilitation in the caudal PWCZ (rostral position trimming index = 0.14 ± 
0.02, n = 631, p < 0.001, Wilcoxon sign rank; caudal position trimming index = -0.37 ± 0.02, n = 631, p < 
0.001, Wilcoxon sign rank, Fig. 3B-F). As a consequence, surround input altered the spatial preference of 
L2/3 neurons, but did so somewhat more heterogeneously than L4, with most neurons shifting rostrally, 
but some shifting caudally in their preference (1.42 ± 0.07 mm mean shift, n = 413, p < 0.001, Wilcoxon 
sign rank, Fig. 3G). In L5, similar to L2/3, the predominant impact of surround input was to facilitate 
responses at the caudal PWCZ position (37 ± 7% mean decrease in spike rate, mean trimming index = - 
0.29 ± 0.06, n = 48, p < 0.001, Wilcoxon sign rank, Fig. 4A-D), which likewise had the net effect of 
altering spatial preference in most neurons (0.6 ± 0.2 mm mean shift forward, n = 39, p = 0.001, 
Wilcoxon sign rank, Fig. 4E).

As a control for these changes, we performed a separate set of experiments where we sham 
trimmed the whiskers (total experimental time equal to trimming experiments), and observed no 
significant effects on the population, demonstrating that the neural responses were stable over the 
recording session (Fig. S3). In addition, to assess the stability of spatial preference in each neuron in the 
trimming datasets, we analyzed the first and second halves of the control and trimmed whisker trials 
separately. We found that the spatial preference of neurons within each condition were stationary over 
time (Fig. S3), further indicating that slow changes in neuronal response properties independent of 
surround whisker trimming cannot explain our results. To determine how spatial preference evolves 
over the time course of object presentation, we analyzed each neuron’s activity during eleven different 
time windows during object presentation. We found that the trimming-induced forward shift in spatial 
preference plateaued for analysis periods starting more than 600 ms after object presentation (Fig. S4). 
This result agrees with our behavioral analysis of whisking set-point, which stabilized approximately 600 
ms after object presentation (Fig. S1C), also emphasizing the importance of analyzing the neural data in 
a time window of high behavioral consistency. It should also be noted that the temporal resolution of 
GCaMP6s as a reporter of neural activity is substantially lower than that of electrophysiology. 
Nevertheless, GCaMP6s activity during our analysis period displayed temporal dynamics not too 
dissimilar from electrophysiology (Fig. S4 E&F).

Spatial summation in the somatosensory thalamus

The data described above demonstrate that surround whisker input powerfully influences how 
cortical neurons represent scanned space. Which of these surround effects emerge in the cortex, and 
which are inherited upstream via the thalamus? Whisker pathways converge even at the brainstem level, 
and can contribute to multi-whisker receptive fields in the thalamus (Timofeeva et al. 2004). To answer 
this question, we recorded from thalamic neurons in the ventro-posterior medial nucleus (VPM, 
dorsomedial portion) and compared the impact of surround whisker input on VPM neurons to our 
observations in cortical neurons. We found that thalamic neurons showed robust spatial tuning like
their cortical counterparts (fraction of neurons tuned, VPM: 83%, L4: 86%, L2/3: 89%, L5: 67%, 1-way ANOVA), demonstrating that tuning, per se, is likely to be generated sub-cortically, perhaps as early as the primary mechanoreceptors, according to previous reports (Szwed et al. 2003; Yu et al. 2015) (Fig. 5A-C). Nevertheless, trimming the surround whiskers demonstrated that surround input modified thalamic responses, but weakly compared to L4 (Fig. 5D-E). A minority of VPM neurons exhibited a significant change in their evoked activity across the center of their spatial receptive field (within the ‘PWCZ’, Fig. 5D). As a population, VPM neurons displayed a reduction in their evoked firing rate at the rostral PWCZ position (trimming index = -0.13 ± 0.06, p = 0.047, n = 54, paired t-test, Fig. 5E). This distinctly contrasts to the robust enhancement we observed in L4 neurons at the rostral PWCZ position. Furthermore, unlike for cortical neurons, surround input did not change the spatial preference of VPM neurons (0.16 ± 0.14mm mean shift, n = 51, p = 0.23, t-test, Fig. 5F). These results imply that surround modulation of the spatial preference of cortical neurons emerges primarily in the cortex.

Figure 5: Weak surround modulation in thalamic neurons in VPM. A) Top: schematic of the pre- and post-surround whisker trimming conditions. Bottom: Example tuning curve (mean ± s.e.m.) of a single VPM unit before (black) and after (grey) trimming all but the C2 whisker. B) Example raster of the unit from A) before trimming to the C2 whisker. C) As in B) but for after trimming. Responses from all 8 stimulus positions are presented in both cases. D) Plot of the fraction of units in the C2 barreloid that show significant increases (blue) or decreases (red) across each of the four stimulus positions within the PWCZ (n = 54 units across 8 mice). E) Plot of the average trimming index for the same cells across the same stimulus conditions. F) Histogram of the change in spatial preference for all recorded units in the C2 barreloid with significant spatial tuning both before and after surround whisker trimming (n = 51 units across 8 mice, p = 0.23, paired t-test). G) Example image from a recorded animal showing the DII track (red) of the multi-electrode array extending into VPM.
As a whole, the data above demonstrate that surround input uniquely transforms the cortical representation of space. Conversely, we sought to determine the importance of principal whisker (PW) input to spatial tuning in an output layer of the cortex, L5. Towards this end, in a separate set of mice, we measured spatial tuning functions before and after trimming off only the PW, leaving all the surround whiskers intact (Fig. S5). Following removal of the PW, we observed a pronounced reduction in the evoked firing rates of neurons that were facilitated by touch, consistent with the expected function of the principal whisker (−28 ± 5% change, trimming index: −0.20 ± 0.03, Fig. S5a, n = 36, p < 0.001, paired t-test). Even though almost all (95%) L5 units retained significant touch-evoked firing after removal of their PW, they exhibited no change in spatial preference (Fig. S5C, n = 20, p = 0.53, Wilcoxon sign rank), in notable contrast to the effect of removing surround whiskers. However, the spatial selectivity of the population was significantly reduced, typified by flatter tuning curves (n = 50, p = 0.003, Wilcoxon sign rank, Fig. S5D). These data indicate that the PW is the primary, but not sole, contributor to the amplitude of a given neuron’s tactile response, while surround whiskers potently influence its spatial preference.

**Surround input organizes a map of scanned space in the barrel cortex**

The data above indicate that surround whisker input powerfully influences how individual neurons in the barrel cortex encode scanned space. How might spatial coding be organized on the more global level? On one hand, the spatial preference of nearby neurons might show little correlation, similar to the salt and pepper distribution of orientation tuning in rodent visual cortex (Ohki et al. 2005). Alternatively, the spatial preference of neurons might gradually shift across the rostro-caudal axis of cortex, constituting a continuous map of scanned space. To address this question in L2/3, we plotted spatial preference for each neuron across the entire field of view (1.06 ± 0.30 mm²), encompassing the region above several adjacent barrels (Fig. S6). Strikingly, we observed a topographic representation in the positional preference of neurons across the rostro-caudal axis of stimulus space, arranged approximately across the row axis of the barrel cortex (Fig. 6A, see Methods and Fig. S6 for a description of how the map axis was determined). The spatial resolution of the aggregated maps was 6.7 μm of physical space per micron of cortical tissue, as quantified by the slope of the linear regression of spatial preferences across all mice (Fig. 6D).

Since we did not observe clear discretization in any of the individual maps (see Fig. S7), it is possible that summation over surround whiskers help generate this continuous map. To test this idea, we asked how the spatial map changed following removal of all but one whisker. While many neurons across the entire field of view retained significantly evoked responses and spatial tuning, the spatial map all but disappeared (Fig. 6B, Fig. S7). We quantified this change in several ways. First we compared the correlation of neurons’ spatial preference across the axis of best fit before and after trimming (see Methods). Before trimming, the spatial preference of the imaged neurons exhibited a clear correlation along the rostro-caudal axis (Pearson’s R = 0.70, p < 0.001), implying the presence of a map; however, after trimming, this correlation disappeared (Pearson’s R = 0.00, p = 0.9, Fig. 6D-E). This relationship
held true both across the entire field of view and within a restricted zone that retained strong activity following trimming (Pearson’s R pre-trim = 0.48, p < 0.001, vs. Pearson’s R post-trim = 0.08, p = 0.063), most likely corresponding to the region directly above the spared L4 barrel (486 ± 70 microns along axis of best fit, n = 4 mice). Second, we computed correlations between the spatial preferences of all pairs of neurons within a given map as a function of cortical distance along the axis of best fit. For a map to exist, nearby neurons should display similar spatial preferences, while distant neurons should diverge. Consistent with this notion, before trimming, an analysis of pairwise correlations show that nearby
Figure 6: Surround whiskers organize a spatial map in L2/3 of the barrel cortex. A) Example spatial preference map in a mouse with all the whiskers intact, a field of view in L2/3 imaged with two photon microscopy. The color indicates the spatial preference of the stimulus bar’s position. Only neurons that exhibited significant activity and spatial tuning are shown. B) Same field of view as in A) but collected immediately after removing all but the C2 whisker. Again, only neurons that exhibited significant activity and spatial tuning are shown. C) Plot of the magnitude and direction of change in spatial preference for all imaged neurons within A) and B) that exhibited significant spatial tuning both before and after surround whisker trimming. Yellow: rostral shift, purple: caudal shift. The length of each arrow corresponds to the magnitude of change in spatial preference, and its direction indicates the sign of the change. The arrows are all aligned to the axis of best fit for preferred position calculated prior to trimming. D) Plot of the spatial preference of all significantly tuned L2/3 cells versus their position along the axis of best fit (1789 neurons, 4 mice). The red line is a linear regression to the data. E) As in D) but for after trimming to the C2 whisker (796 neurons, 4 mice). F) Binned plot of the pairwise correlation of spatial tuning curves for all pairs of significantly tuned L2/3 neurons within each mouse as a function of distance in cortical space. G) Cumulative distribution plots of spatial preference of significantly driven and tuned neurons before trimming as a function of cortical position along the axis of best fit. H) As in G) but for after trimming to the C2 whisker. I) Cumulative distribution plot of the change in spatial preference for all the recorded neurons.

neurons have much greater similarity in spatial preference than distant neurons (Fig. 6F). However, after trimming to a single whisker, the relationship between pair-wise cortical distance and spatial preference similarity dramatically decreased (Fig. 6F). As a third means to quantify this map, we constructed cumulative distribution functions of spatial preference along the axis of best fit before and after trimming. With surround input intact, there was a gradual and systematic tiling of spatial preference...
along the entire axis of cortical space (p < 0.001, ANOVA, n = 1486, Fig. 6G). Following trimming to the C2 whisker, these spatial preference distributions coalesced (Fig. 6H), due to an increasingly greater forward shift in caudal neurons (Fig. 6I), demonstrating that multi-whisker integration is critical for an organized map of the scanned region. The apparent disorganization of the map was not simply due to noisier responses in the cortex after trimming, since our analysis is restricted to neurons significantly tuned for space and significantly driven by the stimuli. Nor is it due to analyzing different total numbers of responsive and tuned neurons between the two conditions, since the results held true even when we restricted our analysis to the population of neurons that were significantly tuned both before and after trimming (Fig. S8). Lastly, we addressed whether behavioral variation, such as minute trial-to-trial differences in whisker set-point, could have affected the smoothness (Pearson’s R) of the sensory map in L2/3. However, in our L2/3 dataset, the faster the mouse ran on the treadmill (the narrower the range of whisker set-points, Fig. S1), the smoother the map became (Fig. S8). Therefore, behavioral variation is in fact detrimental to map smoothness.

Finally, we probed this spatial map electrophysiologically using multi-shank laminar electrodes (Fig. 7A, B). We inserted three 8-electrode shanks across the C-row axis of the barrel cortex (identified with intrinsic optical imaging and electrophysiologically verified, Fig. S2) and measured spatial tuning functions of cortical units across 3 barrel columns both before and after trimming to the C2 whisker (Fig. 7C, D). Across the electrode shanks, the rostro-caudal distributions of spatial preference could be quantified by plotting cumulative distribution functions. Before trimming, neurons in different cortical columns had significantly different spatial preferences that corresponded to their relative location in the cortex (p < 0.001, ANOVA, n = 70, Fig. 7C, E). After trimming, the spatial preference of the neurons that retained significant tuning coalesced onto a narrow region of space (p = 0.32, ANOVA, n = 45, Fig. 7D, F). Furthermore, the magnitude of the change in spatial preference varied with cortical location; neurons in the caudal cortical column shifted further forward than neurons in the rostral column (p = 0.03, ANOVA, n = 31). These results are not simply due to inferior measurements of spatial preference caused by a uniform reduction in response strength, because the spatial selectivity of neurons outside of the spared column did not systematically decrease after trimming (p = 0.73, n = 24, Wilcoxon sign rank). Although these electrophysiological recordings cannot reveal the same degree of continuity we observed with two photon imaging, they nevertheless further support the notion that surround whisker input distributes the spatial preference of neurons to generate a map of scanned space in the barrel cortex. Lastly, we asked if the map was centered on the head, rather than on the set point of the whisking envelope. If so, the spatial preference of neurons should stay the same, despite a shift in whisker set-point. However, we observed that spatial preference follows the set-point of whisking, implying that the map is not head-centered (Fig. S9).
Figure 7: Surround whiskers distribute spatial representations in L5. A) Schematic of multi-shank laminar recordings in L5. B) Example histological images of the electrode of three adjacent shanks in S1. C) Example spatial tuning curves from three units on three adjacent electrode shanks. D) As in C) but following trimming off the surround whiskers. E) Cumulative distribution plots of spatial preference of significantly tuned units on each electrode shank before trimming. F) As in E) but following trimming off the surround whiskers.
Discussion

This study examines how neurons across four sequential stages of the thalamocortical system integrate across a sensor array during active sensation to encode the space scanned by the sensors, in this case, the rodent’s whiskers. While many previous studies have addressed spatial summation in anesthetized, paralyzed, or fixating animals, how summation influences sensory coding when the sensors are actively and volitionally moving has remained largely unexplored. Several previous studies have compared neural responses between active and passive conditions and reported significant differences, including reduced response amplitudes and more restricted spatial or temporal spread of activity (Fanselow & Nicolelis 1999; Ferezou et al. 2007; Hentschke et al. 2006; Lee et al. 2008). Yet spatial summation, per se, has not been rigorously characterized in awake, volitionally whisking mice. In this study, we found that surround whisker input potently transformed barrel cortex neurons’ spatial tuning, strongly impacting firing rates, and shifting their spatial preference. In L2/3, these shifts acted to organize a sensory map of scanned space. Such a map – referenced not to the sensors, but instead to the space probed by the moving sensor array, has not been previously demonstrated in any sensory system to our knowledge. Although the whisker system bears many unique qualities that distinguish it from other sensory systems, this spatial map of scanned space in the barrel cortex raises the possibility that similar maps might exist in other cortical areas in rodents, and in other mammalian species. Primates move their hands across surfaces to localize and identify objects (Chapman & Ageranioti-Bélanger 1991), similar to how rodents use their whiskers, and a continuous map of scanned space in the primate somatosensory cortex might also exist.

The map we observed was not an ego-centric map – i.e., a head-centered map – but rather a map centered on the set-point of the scanned region (Fig. S9). Nonetheless, a map of scanned space, as was observed here, may contribute to the generation of an egocentric (head-centered) map of space downstream that is independent of the scanned region (or ‘field of view’). Based on prior evidence in non-human primates, the posterior parietal cortex is a brain area that may be involved in this transformation (Andersen et al. 1985), but likely builds on cues present even at the mechanoreceptors themselves (Yang & Hartmann 2016).

How might a map of scanned space be generated? First, it is important to note that while the map depends on summation over multiple whiskers, spatial tuning for individual cortical neurons persists even with only a single whisker intact. This is largely consistent with prior reports that horizontal location can be computed by cortical neurons even with information from a single whisker (Curtis & Kleinfeld 2009; O’Connor et al. 2010), or even by neurons at very early stages of the somatosensory system (Szwed et al. 2003; Yu et al. 2015), a fact consistent with the strong tuning we observed in thalamic neurons. Thus horizontal tuning per se does not appear to depend on cortical computation. Instead, we propose that summation over the underlying whisker map, specifically in the cortex, is what helps create the map of scanned space. This computation might be analogous to local smoothing, and could be implemented by the broad dendritic trees and horizontal projections of L2/3 pyramidal neurons that cross cortical column boundaries, as well as the divergence of ascending L4 axons (Bender et al. 2003). Nevertheless, many other possibilities exist, including computations
involving efferent or re-afferent signals of whisker motion. While future experiments can address the underlying mechanisms that generate the map of scanned space in L2/3, we propose that the role of surround input in the cortex is not to generate spatial coding de novo, but rather to act on the global level to organize spatial preference across the horizontal axis of the cortex in such a way so as to generate a continuous map of space. Whether other maps that exist in the barrel cortex, such as for contact angle or for correlation selectivity, contribute to the generation of this spatial map, remains to be seen (Andermann & Moore 2006; Estebanez et al. 2016; Kremer et al. 2011; Peron et al. 2015).

In this study, owing to the highly stereotyped pattern of whisking that mice exhibit during head-fixed locomotion, we were also able to reliably quantify single neuron’s spatial tuning curves during active sensation. Although this preparation resembles in some respects anesthetized conditions where the whiskers are made to move artificially by electrical stimulation of the facial motor nerves (Brown & Waite 1974; Castro-Alamancos & Bezdudnaya 2015; Szwed et al. 2003), all of our data were collected in the awake, alert state. Since several studies have highlighted how brain state and the level of alertness can dramatically influence sensory processing and the firing of specific cortical subtypes (Adesnik et al. 2012; Castro-Alamancos 2004; Castro-Alamancos & Oldford 2002; Greenberg et al. 2008; Lee et al. 2013; Niell & Stryker 2010; Poulet & Petersen 2008; Reimer et al. 2014; Vinck et al. 2015), we consider it essential that we performed all of our experiments in the awake state while mice ran and whisked of their own volition.

The second key finding of this study with respect to spatial summation is the presence of an asymmetric rostro-caudal gradient of response modulation that emerges in the cortex. This modulation is most pronounced in L4, where contact with anterior whiskers powerfully suppresses responses to the PW, while contact with more posterior whiskers generate substantial facilitation. This effect is very likely to be related to the well-known impact of the temporal sequence of whisker-object contacts revealed in anesthetized recordings (Civilloco 2006; Drew & Feldman 2007; Higley & Contreras 2003; Shimegi et al. 2000). What is the utility of such across-whisker modulation? One possibility is that the combined action of anterior suppression and posterior facilitation strongly enhances spatiotemporal contrast in the population response in L4 during whisker contact. In other words, as an animal sweeps its whiskers forwards into an object, the largest neural responses will be in the barrel representing the first whisker to contact the stimulus, both because it gets no suppression from any anterior whisker and because it gets facilitation from the more posterior whisker that contacts the object second. However, at the same time, the L4 barrel representing the second whisker to touch will be suppressed by touch with the first whisker. The net effect of this scheme is to generate a high spatial gradient of evoked responses in L4 barrels that could sharpen the population representation of touch in the barrel cortex (JC Brumberg et al. 1996; Drew & Feldman 2007). This contrast-enhancing, asymmetric integration appears to be involved in generating the continuous map of scanned space we observed in L2/3, although it could be important on its own for other spatial computations. Additional factors unrelated to timing, such as asymmetry in forces on the PW across different object positions, likely shape the properties of surround integration.
Taken together, the results of this study reveal fundamental modes of cortical computation during active sensation, and shed light on key underlying neural mechanisms. Previous studies, primarily in anesthetized or sedated animals, have highlighted how summation across whiskers depends critically on the timing and spatial patterns of surround whisker stimulation (Brumberg et al. 1996; Shimegi et al. 2000). In at least two studies, coordinated waves of surround input, mimicking that which occurs naturally, can profoundly alter the response properties of cortical neurons (Drew & Feldman 2007; Jacob et al. 2008). In this study, since the animals whisked freely, the timing and pattern were not under experimental control, but our results are nevertheless consistent with prior experiments under anesthesia. A previous study, in anesthetized animals, demonstrated that the degree of correlated whisker movement across the array could profoundly influence single unit responses – with some units enhanced and other suppressed by global correlations (Estebanez et al. 2012). Furthermore, recent work showed that the enhanced neurons in L2/3 are clustered above the edges of the L4 barrels (Estebanez et al. 2016). In our study, since the mice naturally whisked in a coherent fashion at a vertical bar, the stimulus we used is likely to be more similar to the global correlation condition. In any condition, the precise spatiotemporal pattern of multi-whisker touch likely has a profound influence on sensory integration. Similar to previous studies that investigated active sensation with a single whisker (Ferezou et al. 2007; Hentschke et al. 2006; Lee et al. 2008), our results have the advantage that they are drawn from volitionally whisking mice, and thus within the ethologically relevant range of multi-whisker contact patterns. However, this naturalistic approach prevented us from identifying the precise moments of multi-whisker touch, thereby obscuring the effects of multi-whisker integration on the fine temporal structure of spiking. Future studies, using technological advances that permit the imaging and quantification of multi-whisker contacts during exploration of objects with complex surface geometry (Hobbs et al. 2016), in combination with the physiological approaches here, could address how a spatial map in S1 facilitates the encoding of higher order stimulus features. Furthermore, processing stages downstream of S1 could integrate topographic information of scanned space with sensorimotor signals conveying whisking set point to construct an egocentric map of space.
Author Contributions

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Methods
Contact for Reagent and Resource Sharing

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Hillel Adesnik (hadesnik@berkeley.edu).

Experimental Model and Subject Details

Wild-type adult ICR white (Charles River) mice between 6 and 10 weeks of age and of either gender were used for all experiments, except for those involving imaging cortical layer 4, for which the scnn1-tg3-Cre line (JAX), outcrossed to the ICR line for several generations, was used. All procedures were approved by the Animal Care and Use Committee of UC Berkeley. Both female and male animals were used and maintained on a 12:12 reversed light:dark cycle. For supplemental figure 6, we used a Thy1-GCaMP6s (4.3) mouse.

Methods Details

Preparation for in vivo electrophysiology

Anesthesia was induced with 5% isoflurane and then maintained at 1 – 3% during surgery. Respiratory rate and response to toe/tail pinching was monitored throughout surgery to ensure adequate anesthetic depth. 0.05 mg/kg of buprenorphine was administered for post-operative analgesia. After disinfecting the scalp with 70% alcohol and 5% iodine, the skin and fascia above the sensory cortices were removed with surgical instruments. Following application of Vetbond (3M) to the skull surface and wound margins, a custom stainless steel headplate was fixed to the skull with dental cement (Metabond). Two days after surgery, mice were habituated over increasing durations for 4 – 8 days to head-fixation on a free-spinning circular treadmill, until they freely ran at a fast and steady pace (>35 cm/s). Intrinsic optical imaging was performed to localize one or two barrel columns of interest (C1 – C3). In preparation for electrophysiology, mice were briefly (10 – 15 minutes) anesthetized with isoflurane, the skull over S1 was thinned with a dental drill (Foredom), and a small (<200 μm for a single shank) craniotomy was made with a 27 gauge needle. For multi-shank experiments a long, thin craniotomy was opened over S1 in a similar fashion. The small size of the craniotomy minimized motion of the brain during electrode penetration and animal movement. For recordings from the cortex, a 16 or 32-channel linear silicon probe (NeuroNexus) was guided into the brain using a micromanipulator (Sutter Instruments) and a stereomicroscope (Leica) to the desired barrel column (C1 – C3) by aligning the intrinsic optical signal (Fig. S2) with superficial blood vessels. For multi-shank experiments, a Neuronexus Buzsak32 probe was used. The principal whisker was verified electrophysiologically by deflecting individual whiskers and listening to multiunit activity (MUA). There was an audibly clear difference in MUA between principal and surround whisker contact. For recordings of the thalamus, a 16-channel linear silicon probe (NeuroNexus) was guided into the brain at 1600 μm posterior and 2000 μm lateral from bregma. The electrode was lowered until strong whisker responses were detected, usually around 2700-2800 μm, indicating the border of the ventro-posterior medial nucleus. The
electrode was lowered further until it reached a barreloid corresponding to C2 or B2, where that whisker caused the strongest response from deflection. In all cases, electrical contacts on the probe spanned the C1 – C3 or B1 – B3 barreloids, as verified by electrophysiology.

**Preparation for in vivo two photon imaging**

The surgery was as described above, but with the following modifications for transcranial imaging through a glass window. 2 mg/kg of dexamethasone were administered as an anti-inflammatory. A 3 mm diameter craniotomy over the left primary somatosensory cortex was drilled, and a Nanoinject II nanoliter injector was used to inject 18.4 nL of AAV-GCaMP6s at ten to twenty sites within the craniotomy at an overall rate of 0.5 nL/s. AAV9-synapsin-GCaMP6s (UPenn Vector Core) was injected into wildtype ICR mice (Charles River) for L2/3 datasets, and AAV9-flexed-CAG-GCaMP6s (UPenn Vector Core) was injected into scnn1-tg3-Cre mice (JAX) for L4 datasets. After viral injection a window plug consisting of two 3mm diameter coverslips glued to the bottom of a single 5mm diameter coverslip using Norland Optial Adhesive #71 was placed over the craniotomy and sealed permanently using Orthojet. Mice were head-fixed on a freely spinning running wheel under a Nikon 16x-magnification water immersion objective and imaged with a Neurolabware two-photon resonant scanning microscope within a light tight box. Image acquisition was at 15.45 Hz with fields of view (FoVs) ranging from 600 μm by 650 μm to 1.25 mm by 1.15 mm. To obtain large fields of view in all cases, in some experiments four adjacent FoVs were imaged sequentially. Wide-field reflectance imaging with a white LED was used to illuminate the vasculature and center the FoV on the region the intrinsic signal identified as corresponding to the C2 barrel. For L2/3 imaging, imaging depth was 100 – 300 μm, and for L4 imaging, depth was 400 – 500 μm deep.

**Tactile Stimulus presentation**

During continuous two-photon imaging or electrophysiological recording, a modified 0.7mm Hex key (McMaster-Carr) was presented vertically at 8 locations along an axis perpendicular to whisking motion and ~1 cm away from the mouse’s face. The pole was presented to the whiskers for 1.5 seconds during each trial using a stepper motor (Oriental Motor) to quickly move the pole in, hold the pole stationary for the entire stimulus period, and then move it back out. There was an interval of 3 - 4.5 seconds between trials for imaging to allow the evoked calcium response to return to baseline. At the beginning of each inter-trial interval the stepper motor and pole were translated to the next trial’s horizontal position using a motorized linear stage (Zaber). Stimuli were randomized in batches such that no stimulus was presented more than twice in a row. After >15 repetitions of the stimulus batches, data collection was paused and all but the principal whisker (always C2 for imaging experiments) were trimmed such that only the remaining whisker could contact the vertical pole stimulus at any position. Data collection immediately recommenced and at least 16 new batches of stimuli were presented. After conclusion of the experiment, the vertical pole was presented at each of the stimulus positions, and the PWCZ positions were identified by high speed camera acquisition or by visual inspection using
stereomicroscope. This was verified post-hoc by determining which stimulus positions evoked significant activity throughout the object presentation period after trimming the surround whiskers.

Two photon imaging analysis

Raw two photon movies were first corrected for brain motion using Scanbox’s fourier transform-based sbxalign script, written in MATLAB, to correct for the 2D translation of individual frames. The mean of each motion-corrected video was used to translate and register the before and after trimming datasets to within a single pixel of each other. Regions of interest (ROIs) encompassing neurons were identified in a semi-automated manner using Scanbox’s sbxsegmentflood (MATLAB, Mathworks) which computes and thresholds the pixel-wise cross-correlation for all pixels within a 60 by 60 pixel window. If an ROI only appeared in one of the datasets via the semi-automated method, then the ROI was copied over to its relative location in the dataset in which it was not identified. The ROI’s signal ($R_i$) was taken as the mean value across all pixels within and unique to that ROI (Fig. S2). This signal is assumed to be a mixture of the cell’s actual fluorescence signal and a contaminating neuropil signal resulting from scattering producing off-target excitation, high illumination powers producing out of focus fluorescence, or unresolvable neurites passing through the microscope’s point spread function. The neuropil signal ($N_i$) for each ROI was computed by averaging over an annulus of pixels surrounding the ROI but excluded pixels assigned to other ROIs as well as a smaller annulus of pixels that acted as a buffer in case any motion artifact was not perfectly accounted for (Fig. S2). This buffer annulus existed for all ROIs and was excluded from any neuropil calculation. As a result the max diameter of the neuropil annulus varied per ROI in order to ensure a similar number of usable pixels to average over. Each neuron’s true fluorescence signal ($F_i$) was computed per ROI by the following equation:

$$F_i(t) = R_i(t) - k_i * N_i(t)$$

The amount of contamination ($k_i$) was assumed to be constant per ROI, but vary between ROIs as a result of local differences in expression and scattering. Each $k_i$ was defined by assuming that the neuron’s true fluorescence signal ($F_i$) can never be negative (i.e. $k_i * N_i(t) \leq R_i(t)$), and that there must be a maximal bound for contamination. The contamination coefficient per neuron was defined as follows:

$$k_i = \min\left(\frac{R_i(t)}{N_i(t)}\right); \text{ if } k_i > .65, k_i = .65$$

The true signal was then converted into a trial-wise change in fluorescence ($\frac{f(t) - f_0}{f_0}$ or $\Delta f/f$) to capture the stimulus-evoked changes in neural activity while compensating for any fluctuations in baseline fluorescence. The baseline fluorescence ($f_0$) for a trial was taken to be the mean fluorescence over the one second prior to stimulation.

High-Speed Whisker Tracking
In a subset of experiments the whiskers were tracked at high speed (~500 frames per second). Previous data, confirmed here (Fig. S1), indicate a tight correlation between run-speed of the mouse and whisker set-point, which plateaus above 35 cm/s (Sofroniew et al., 2014). A high-speed camera (Basler, acA2000-340kc) was placed below the running wheel; the principal whisker was imaged from below using a mirror angled at 45 degrees. The base of the PW was painted with a thin layer of Titanium White (Liquitex) paint and illuminated from below using a bright red LED, providing contrast from the other whiskers. High-speed videos were acquired at 500 fps with a 100 μs exposure and were synchronized with neural data acquisition via external triggers. Videos were processed in MATLAB using custom tracking software. An ROI was placed over the sector that the painted whisker swept out, cropping out other reflective surfaces (e.g. mouse's nose) that would otherwise interfere with tracking. All frames were luminance-thresholded to create a binary image, and the center of the painted region was calculated; the angle between the center of the painted region and a user defined position on the face was calculated for all frames. Angle traces were created from these measurements to calculate the whisker kinematic features in Figure S1: set-point (median angle of envelope), amplitude (half-width of envelope), speed (distance/time), and frequency (cycles/second). The image of the PWCZ and the AWCZ in Figure 1 was created from tracking a mouse with a single row of whiskers illuminated from the top. The whisker traces were manually traced for display purposes only. It was not possible to detect contacts between the painted whisker and the stimulus bar, since only the base of the whisker was painted to avoid adding substantial weight to this whisker or altering its curvature.

**Spike Sorting**

16-32 channels of electrodes were amplified (AM Systems), filtered (0.1-5 kHz) and digitized at 30 kHz (National Instruments) using custom acquisition software (MATLAB, Mathworks). Spike detection was performed using the UltraMegaSort2000 package in MATLAB (Hill et al., 2011) (Mathworks). After detection, spikes were automatically sorted into clusters of units. Units were then further sorted manually to meet inclusion criteria and prevent pseudo-replication. Quality metrics included analysis of spike amplitude, spike rate, auto- and cross-correlation, inter-spike interval, outlier removal, distance from threshold, and cortical depth of largest waveform. With the exception of a small subset of fast-spiking or bursting units, included units had no more 1% of their individual waveforms violating a refractory period of 2.5 ms. The surround whisker trimming data was collected from 8 mice for the L5 RS population and 8 mice for the thalamus population. The principal whisker trimming data was collected from 8 separate mice.

**Spike Waveform classification**

Fast-spiking units were separated from regular spiking units using a k-means cluster analysis of two waveform components. One component was the normalized difference between the two positive-going peaks. The other component was the trough-to-peak latency of the large negative-going deflection. Fast-spiking units were categorized by a larger 2nd positive-going peak (positive difference), and a short (less than 0.33 ms) trough-to-peak latency, following previously established approaches. Units on the
border between the classification as FS or RS was excluded from analysis. FS neurons were excluded from the paper.

**Trial inclusion criteria and layer boundaries**

In sorted units, firing rates were computed by counting spikes in the final 500 ms of stimulus presentation. This window was chosen because within 1000 ms of the bar entering the whisker field, neuronal firing rates and behavior reached steady-state. Trials containing stimulation periods where the animal’s mean run speed during the stimulus period dropped below 1.3 standard deviations of its population mean were excluded, to ensure consistency in whisking behavior across trials. In addition, trials where the standard deviation of an animal’s run speed was more than 0.8 standard deviations from the population mean were excluded. Trials where the animal was not moving, thresholded by the animal’s run speed being below 3 cm/s, were completely excluded from analysis. The depth of each unit was assigned based on the calculated depth of the electrode on the linear array that exhibited its largest waveform. Layer boundaries were confirmed post-hoc using current source density analysis (CSD, Fig. S2) and labeling of the electrode track with a dye. CSDs were calculated from the trial-averaged local field potential (0.5 – 300 Hz) measured at each electrode contact, as previously published. We estimated the layer 4/5 boundary as the base of the current sink corresponding to layer 4.

**Analytical Metrics**

A *Trimming Index* for each condition was computed as the difference between the mean evoked firing rates during post-trimming (T) and control (C) conditions, divided by the sum of their mean evoked firing rates:

\[
TRIMMING INDEX = \frac{T - C}{T + C}
\]

The *Spatial Preference* of a neuron was determined by calculating the center of mass (CM) on the absolute value of its spatial tuning curves. FR, the mean evoked firing rate (or delta F) at position, P, at stimulus locations 1 through n:

\[
SPATIAL PREFERENCE (CM) = \frac{FR_1 * P_1 + FR_2 * P_2 + \ldots + FR_n * P_n}{FR_1 + FR_2 + \ldots + FR_n}
\]

Statistically significant changes in spatial preference at the level of single units was computed using a standard permutation test. For each unit, a null distribution of change in spatial preference was created by randomly sampling values among both conditions 5000 times. Significance (p < 0.05) was observed if the experimental effect was beyond the 97.5 percentile or below the 2.5 percentile of the null (two-tailed) distribution.
The relationship between the center and surround of the horizontal receptive fields of neurons was calculated as the difference between the max evoked firing rate (or delta F) in the PWCZ and the max evoked firing rate in the AWCZ divided by their sum.

\[
Cent:Surr \text{ Index} = \frac{\max(\abs(PWCZ)) - \max(\abs(AWCZ))}{\max(\abs(PWCZ)) + \max(\abs(AWCZ))}
\]

The spatial selectivity of neurons was calculated from the normed (Euclidean) vector of the peak normalized spatial tuning curves. This value was then divided by square root of \( n \) dimensions – 1 to restrict its range from 0 to 1. Larger values signify higher spatial selectivity (lower broadness). Raw spike rates were used.

\[
Spatial \text{ Selectivity} = 1 - \left( \frac{||x||}{\max(x) - 1} \right)
\]

**Map analysis**

Within each dataset, the centroids of all significantly driven and tuned ROIs were whitened and projected onto 1800 axes spanning from 0 to pi. The centroids of the ROIs were whitened to minimize spurious correlations derived from the structure of the ROIs sampled. A linear regression was computed between the projected location of the ROIs and their preferred positions (calculated over the entire tuning curve) for each axis. The axis of best fit was determined to be the axis whose linear regression had the largest \( R^2 \) value. This axis of best fit was then transformed into cortical space via the inverse of the whitening transform. The center of the axis was located to the center of the spared whisker column (as identified above) allowing for data across mice to be aggregated.

A Pearson’s correlation was computed for the significantly driven and tuned neurons between their projected locations on the axis of best fit and their preferred positions, both before and after trimming. A linear regression was performed to compute the slope of that correlation. The mean pairwise correlation in tuning over the PWCZ (Pearson’s R) was computed as a function of their pairwise difference of their projections along the axis of best fit and binned within 20 \( \mu \text{m} \) bins. Cumulative distribution functions were created by binning the location of the neurons along the projection into 18 equally sized bins. Only the central 8 bins, which had more than 145 neurons each (the expected value if the distribution of ROIs along the axis were uniform), are shown.

**Quantification and Statistical Analysis**
Statistically significant differences between conditions were determined using standard parametric or nonparametric tests in MATLAB, including a 1-way ANOVA, student’s t-test, rank sum, and a Wilcoxon sign-rank test. Tests for normality were performed with a Lilliefors test. Units were defined as tuned for space if their evoked spike rate changed as a function of object position, determined by a 1-way ANOVA. Analysis of spatial preference changes was restricted to neurons that were significantly tuned for the stimulus both before and after trimming. The number of neurons that significantly changed their response per position was defined as neurons whose pre- and post-trimming response distributions were significantly different via a rank sum test. All “n” values are referring to the number of cells present in an analysis except when explicitly stated that the n is referring to the number of mice used.

**Electrophysiology**

Unless stated otherwise, analyses were performed from evoked spike rates. The spontaneous firing rate of a neuron in the 500 ms window preceding stimulation was subtracted from its firing rate of the last 500 ms of active touch, on a trial by trial basis. Neurons in L5 and the thalamus were classified as touch-facilitated or touch-suppressed. Touch facilitated neurons had a positive mean evoked spike rate in the principal whisker contact zone (PWCZ), while touch-suppressed neurons had a negative mean evoked rate in the PWCZ.

**Two-photon calcium imaging**

Analyses were performed on trial-wise dF/F. Analysis was limited to ROIs that met several criteria: they must be significantly driven by at least one stimulus, be larger than 50 μm², and for Figs. 2 and 3 have been within the principle whisker column. A significant response for a position had to meet two criteria: have a mean df/f greater than .2, and pass a t-test between the evoked responses at that position and the measured df/f values during control trials. The Benjamini & Hochberg false discovery rate correction was used to correct for the multiple comparisons taken across the multiple stimuli. Outlier responses per stimulus position were identified by the median rule, where values further than 2.3 times the inter-quartile range from the median are determined to be outliers, and were removed prior to any analysis. Neurons were identified to be within the spared principle whisker column or to be in a surrounding column by using a custom MATLAB (Mathworks) algorithm to segment the pixels that exhibited a significant response post-trimming (t-test between control trials and the mean of PWCZ stimulus trials) which is putatively localized to the spared column (Fig. S2). The neural response for a single trial was calculated as the average df/f during the last 500 ms of stimulation.
Supplementary Figures

A

B

C

D

E

F
Figure S1. Related to Figure 1.

Acute whisker trimming minimally impacts basic whisking kinematics.

(A) Plots from an example mouse of mean run speed, whisker set-point, amplitude, speed, and frequency for the C2 whisker against stimulus position before (black) and after (red) trimming all of the surrounding whiskers (mean ± 95% C.I.). FW: free whisking.

(B) Population histograms of change in the same kinematic variables as in A) (n = 8 mice, 9 conditions per mouse). For whisking kinematics, only a very slight, but significant change in amplitude was observed (0.93 ± 0.21 degrees, mean ± s.e.m), much smaller than the inter-stimulus spacing (10 - 15 degrees).

(C) Top: Example whisking trace with set-point (green line) overlaid. Whisker set-point is the midpoint of the whisking envelope. Bottom: Average set-point of the tracked PW during free whisking and for three stimulus positions over the course of the trial. Shaded areas represent 95% confidence intervals. Cyan shaded area represents the analysis period.

(D) As in C) but for whisk amplitude. Whisk amplitude is defined as half the distance between the peak and trough of the whisking envelope.

(E) Mean (± s.e.m) whisker set-point (top) and amplitude (bottom) as a function of mouse run speed. Data are averaged across eight mice that were imaged once with their full whisker pad intact and then with only their C2 whisker intact.

(F) Top: raster plot of touches between the PW and the stimulus bar. Bottom: Average number of contacts per second the C2 whisker makes with the bar during a trial. Cyan shaded area indicates the analysis period.
Figure S2. Related to Figures 2, 3, 4, & 5.

Localization and unit identification methods and surround responses
(A) Left: Example field of view (FoV) from L4 color coded by pixels that are significantly driven for PWCZ stimuli after trimming (computed via a t-test with control trials). An automated segmentation algorithm was applied to the images to produce the red dashed lines. ROIs whose centroids fall within or on the line are taken to be neurons located within the spared column. Right: same but for a FoV from L2/3.

(B) Mean FoV from one L2/3 mouse with 7 example ROIs and their corresponding neuropil ROIs overlaid. All pixels that are located within two or more ROIs are ignored from all ROIs. All pixels within any ROI are ignored from neuropil ROIs.

(C) Normalized fluorescence traces for the 7 ROIs from (B) captured during a series of stimulus presentations (gray bars).

(D) Fluorescence traces for the 7 neuropil ROIs from B) captured during the same time period as C) and normalized to the same value.

(E) Neuropil-corrected versions of the traces in C).

(F) Schematic of two example units, green: fast-spiking (FS), black, regular-spiking (RS), and criteria used for classification.

(G) Plot of amplitude asymmetry vs waveform duration for all L5 units in the study. Units are colored (green, FS, black, RS) according to cluster analysis.

(H) Example intrinsic optical signal overlaid on a picture of the cortical vasculature. Intrinsic signals were collected in anesthetized mice for the C1 and C3 whisker to identify the C row and location of the C2 whisker column.

(I) Example current source density from a laminar 32-channel multi-electrode array in the C2 column. The early sink (blue) in the middle layers correspond to L4. The dash line indicates the approximate L4/L5 boundary. Only units with their largest waveform on an electrode below this boundary were included in this study.

(J) Percent of units/cells in the VPM, L4, L2/3 and L5 that were significantly driven in the adjacent whisker contact zone (AWCZ) indicating they could be driven when only adjacent whiskers contact the stimulus.

(K) Center:Surround modulation index across VPM, L4, L2/3, and L5 showing a general trend towards a greater surround contribution across the conventional feed-forward circuit (mean ± s.e.m.)
Figure S3. Relate to Figures 2, 3, 4, & 5.

Sham trimming and within-condition control tests show that spatial representations are stable over the recording duration.

(A) Example spatial tuning functions (mean ± s.e.m) and raster plots of a unit before (black) and after (grey) the sham trim. Experimental time in each half is identical to the experiments in which the whiskers were actually trimmed.

(B) Sham indices (mean ± s.e.m) for data that were collected using electrophysiology (n = 60 units in 3 mice).

(C) & (D) Same as A, B) except data was collected using two-photon GCaMP6s imaging (n = 151 ROIs in 1 mouse).

(E) Percent of individual units that displayed a significant shift in spatial preference using a permutation test with 5000 resamples (see methods). A small minority of units in the sham (green) and within-condition (black) data displayed a significant shift in spatial preference. In contrast, after trimming (blue bars), a large fraction of units displayed a significant shift in spatial preference.
(F) Boxplot illustrating the effect of sham trimming, within-condition consistency, and real trimming on the spatial preference of the neuronal populations. After sham trimming, no significant shift in the spatial preference of the population was observed (L4 GCaMP6s, n = 151, p = 0.3, paired t-test; E-phys, n = 28, p = 0.12, paired t-test).
Figure S4. Related to Figures 2, 3, 4, & 5.

Spatial preference stabilizes ~600 ms after stimulus onset.

(A) Diagram of the methodology used to determine the evolution of spatial preference over the time course of stimulus presentation. Eleven overlapping 500 ms windows were analyzed, starting 0 - 1000ms after object presentation.

(B) Example L5RS unit showing the slight progressive shift forward in spatial preference for the specified analysis start points.

(C) The evolution of spatial preference of the population using GCaMP6s imaging (mean ± 95% C.I.). Relative spatial preference is related to the analysis window used in the main Results section (starting 1000 ms after presentation).

(D) Same as C), except from data collected using electrophysiology in the cortex and the thalamus.
(E) Temporal dynamics of L4 population activity (n = 840 ROIs) for the stimulus position immediately caudal to the PWCZ. During the analysis period for the single whisker condition, activity returned to near baseline levels (mean ± 95% C.I.).

(F) Same as E, except for cortical electrophysiology population data (n = 190 units). During the analysis period for the single whisker condition, activity returned to a level indistinguishable from baseline.

(G) Maps of spatial preference in L2/3 that were calculated during the initial (0 - 500ms) and main Results (1000 - 1500) analysis periods.

(H) Pearson’s correlation coefficient between the spatial preference and cortical location of the neurons along the axis of best fit across the analyzed time windows shown in A) for the 4 L2/3 mice in supplementary figure 10.
Figure S5. Related to Figure 4.

Principal whisker contribution to spatial representations in L5 RS units facilitated by touch.

(A) An example spatial tuning curve (mean ± s.e.m.) of a L5 RS unit before (black) and after (grey) trimming the principal whisker.

(B) Histogram of the trimming index in touch-facilitated units (n = 36 units in 8 mice, p < 0.001, Wilcoxon).

(C) Histogram of the spatial preference change (n = 20 units, p = 0.53, Wilcoxon).

(D) Histogram of the spatial selectivity change (n = 50 units, p = 0.003, Wilcoxon).
Figure S6. Related to Figure 6.

Determining the map’s axis and example map overlaid on underlying barrels.

(A) The centroids of all significantly driven and tuned ROIs in an example field of view. The center of the spared column (as identified in Fig. S2) is shown by the red asterisk.

(B) Same as in A) but the centroids have been whitened. Three example axes that were projected upon have been overlaid.

(C) The linear regression’s $r^2$ value between the neurons’ preferred position and its projected location onto all 1800 axes tested. The $r^2$ value of the example axes in B) are shown by corresponding asterisks of the same color. The red axis is the axis of best fit as the projection of the whitened ROI centroids produces the linear regression with the highest $r^2$ value.

(D) The spatial preference vs. the projected location of the given ROIs for the example axes in B), separated by their corresponding color. The linear regressions of the data are overlaid.

(E) Example thresholded intrinsic optical signal resulting from deflecting the C2 whisker registered to an image of the cranial window for a 4.3 Thy1-GCaMP6s mouse (JAX). The yellow x marks the center of the two-dimensional Gaussian fit to the intrinsic signal.

(F) Cytochrome oxidase staining of the barrels in tangential sections from same mouse as in E).

(G) The centroids of the Gaussian fits to the intrinsic optical signals for the stimulated whiskers are shown as yellow x’s. The centroids of the barrels as determined from the histology were registered to the intrinsic signals via an affine transform and are overlaid in red.

(H) Image of the vasculature directly above the two-photon imaging field of view overlaid on the image of the cranial window.

(I) The outline of the underlying L4 barrels are overlaid on the L2/3 map of the same mouse.
Figure S7. Related to Figure 6.

Four L2/3 maps of scanned space.

(A) Fields of view (FoVs) from four L2/3 mice showing significantly driven and tuned ROIs colored by the center of mass of their pre-trimming tuning curve. The axis of best fit (see Methods & Fig. S9) between location on that axis and spatial preference is overlaid in red and centered on the spared column.

(B) Same FoVs as A) but for data acquired post-trimming.

(C) Scatter plots of spatial preference to location along the axis of best fit for the four FoVs and ROIs in A). A linear regression was performed and its slope (m) and coefficient of determination (r²) are presented. A Pearson’s correlation was also performed, and its correlation coefficient (R) and significance value (p) are also presented.

(D) Same analysis as C) but for the post-trimming data presented in B).
A. Analysis of ROIs significantly driven in both conditions

Spatial Preference (mm)

Full

PW only

B. Analysis of Shuffled ROIs

Spatial preference (mm)

Full

PW only

m=0.38μm/μm
r²=0.87
p=0.001

m=0.38μm/μm
r²=0.87
p=0.001

C. Analysis of Shuffled ROIs

Spatial preference (mm)

Full

PW only

m=0.34μm/μm
r²=0.88
p=0.001

m=0.34μm/μm
r²=0.88
p=0.001

D. Treadmill run speed (cm/s)

Set point standard deviation (days)

0 20 40 60 80 100 120

E. Correlation (Pearson's R)

Slow

Fast

Correlation

0.2 0.4 0.6 0.8 1

F. Spatial Preference (mm)

Slow

Fast

100 μm

100 μm
Figure S8. Related to Figure 6.

L2/3 map produced via multi-whisker integration is not a result of sampling, chance, or behavioral variability.

(A) Example maps from mouse shown in Fig. 6, but now limited to only the ROIs that are significantly driven and tuned both before and after trimming.
(B) Scatter plot of preferred position to location along the axis of best fit before trimming for all ROIs significantly driven and tuned both before and after trimming across all 4 mice. Same analysis as D) but for data acquired after trimming.
(C) Same scatter plots as those presented in Fig. 6 but presented after randomly shuffling spatial preference across ROIs within each FoV. F) Same shuffle as in E) but for data after trimming.
(D) Plot of the standard deviation of whisker set-point as a function of run speed (mean ± s.e.m.). Note the standard deviation of the whisker set point decreases as the run speed increases. Trials from two different bins of run speed (blue: slow, and red: fast) were used in the subsequent analyses.
(E) The Pearson’s correlation value calculated for the slow and fast run speed bins for two mice. Note the map correlation is higher at higher run speeds where the whisker set-point is less variable.
(F) Example maps generated for a single mouse when the trials are restricted to slower run speeds (left) and faster run speeds (right).
Figure S9. Related to Figures 6 & 7.

The map of spatial preference in L2/3 depends on whisker set-point.

(A) Distribution of whisker set-points for an example mouse across all (n = 360) trials in an experiment. The trials were arbitrarily divided into two groups with a dividing line of 110 degrees.

(B) Mean (± s.e.m.) whisker set-point as a function of object position for the low and high whisker set-point group.

(C) Example spatial tuning curves from a unit in S1 created from trials less than 110 degrees (green) or greater than 110 degrees (black) (mean ± s.e.m.).

(D) The difference in each neuron’s spatial preference between the two groups (protruded spatial preference minus retracted spatial preference). A negative value indicates that a neuron’s spatial preference was more rostral for trials containing more protruded (> 110 deg.) whisker set-points.

(E) Example two photon map in L2/3 of spatial preference for slow running trials.

(F) As in E), but for faster running trials.

(G) As in D but for the two photon imaging data (fast spatial preference minus slow spatial preference).
Chapter 3: Sensory cortex controls the strength and tuning of its thalamic input

Foreword
This work comes from the latter part of my graduate research – after completing the prior project and dabbling in other thalamus-adjacent questions for a while, I wanted to focus mostly on the very interesting question of corticothalamic feedback. This is presented in the form of a manuscript in preparation, in which I detail cortical modulation experiments and their effect on thalamic activity aiming to resolve that very question.

Summary
Sensory information for touch, vision, and hearing must pass through the thalamus on its way to the cortex. Although corticothalamic ‘top-down’ projections outnumber ‘bottom-up’ projections from the sensory periphery, the function of cortical feedback on thalamic sensory coding remains poorly understood, particularly in awake, actively sensing animals. We combined multi-site, multi-electrode array physiology with optogenetic suppression of layer 6 corticothalamic (CT) neurons in mice actively palpating objects with their whiskers to address this question. Suppressing L6 CT neurons reduced thalamic activity in both the ventroposterior medial nucleus (VPM) and in the reticular nucleus (nRT), and sharpened spatial representations in many VPM neurons. Optogenetic activation of L6 CT neurons also reduced thalamic activity, suggesting that artificially elevating CT feedback may lead to spurious conclusions. Our results imply that cortex enhances thalamic activity during active sensation and broadens how thalamic neurons encode tactile space. These results suggest that the cortex can control both the amplitude and the spatial tuning of its own input.
Introduction

The function of the massive cortical innervation of the thalamus remains uncertain. The monosynaptic connection between L6 and the primary sensory thalamus is glutamatergic (Montero & Wenthold 1989; Fonnum et al. 1981; Reichova & Sherman 2004; Sherman 2012; Deschénes & Hu 1990; De Biasi & Rustioni 1990) and the disynaptic route through the reticular nucleus (nRT) is GABAergic (Spreafico et al. 1991; Guillery & Harting 2003; Ohara 1988; Houser et al. 1980; Pinault & Deschénes 1998; Cox et al. 1997; Kim et al. 1997). The overall effect of cortex on the thalamus is presumably a finely tuned interaction of these two opposing components of the descending corticothalamic projection, and could therefore give rise to a diversity of effects depending on the stimulus and brain state. Many studies have probed this connection through targeted manipulations, perturbing it chemically, electrically, or more recently, optogenetically, yet little consensus has emerged.

Notably, no study has yet addressed the impact of corticothalamic activity through a combination of cell-type specific optogenetic inactivation, in awake animals, and during active sensation. Prior work, largely in anesthetized animals or using less specific manipulations, has found that cortical suppression leads to mixed effects on thalamic activity (Waleszczyk et al. 2005; Ghosh et al. 1994; Denman & Contreras 2015). Likewise, cortical stimulation can also drive suppression or facilitation (Guo et al. 2017; Olsen et al. 2012; Jurgens et al. 2012; Mease et al. 2014; Crandall et al. 2015). Many studies have explored the effects of cortical perturbations on receptive field properties and other characteristics of information transfer through the thalamus and have noted that cortex streamlines thalamic processing or regulates receptive field size (Andolina et al. 2013; Li & Ebner 2007; Rivadulla et al. 2003; De Labra et al. 2007; Wolfart et al. 2005; Funke et al. 1996; Hasse & Briggs 2017; Temereanca & Simons 2004).

Despite its specificity, optogenetic manipulation of L6 CT neurons via the highly selective Cre recombinase line Ntsr1-Cre has likewise not yielded a coherent picture of the impact of cortex on the thalamus. Even Ntsr1-Cre experiments across visual, somatosensory and auditory cortices have yielded conflicting conclusions as to the impact of L6 CT manipulation (Jurgens et al. 2012; Olsen et al. 2012; Crandall et al. 2015; Pauzin & Krieger 2018; Mease et al. 2014).

Since these prior studies only investigated the impact of L6 CT feedback on the thalamus in anesthetized animals, during passive sensory stimulation, or in brain slices, we sought to determine the net impact of this pathway specifically in awake, actively sensing mice. Since both cortical and thalamic activity can be dramatically altered by anesthetic states and vary between different awake states, it is imperative that the question of cortical impact on the thalamus be addressed in awake, actively sensing animals. In addition, we also addressed the impact of L6 CT activity on the thalamic reticular nucleus (nRT), which is arguably critical for understanding the magnitude and sign of the effect of cortical activity on the thalamus, as the nRT plays a large role in shaping thalamic sensory responses and receptive fields (Desilets-Roy et al. 2002; Hartings et al. 2003; Lee et al. 1994).

In our study, we combine the L6 CT-selective Cre driver line, optogenetic suppression, and in vivo multielectrode array electrophysiology in VPM and nRT to address the question of corticothalamic feedback in an awake, actively sensing animal. We found that L6 CT cells enhance activity in the VPM.
and reticular nucleus both during free whisking and during active touch. The effect of L6 CT feedback on the VPM and the nRT can be described as additive gain, whose net impact is to broaden VPM spatial representation during active sensation.
Results

In order to probe the effect of cortical connections on thalamic activity in awake mice, we performed extracellular recordings of cortical cells in L6 of barrel cortex, thalamic relay cells in the VPM, and cells in the reticular nucleus (nRT) while simultaneously measuring whisking activity during both free whisking activity and active touch of a stimulus bar. All data analyzed in this study comes from head-fixed mice locomoting on a free spinning running wheel, a condition in which mice rhythmically sweep their whiskers in a highly stereotyped fashion. This paradigm facilitates quantitative measurement of neural activity during free whisking and during active touch. This paradigm has been extensively described elsewhere (Pluta et al. 2015; Pluta et al. 2017) (and see Methods).

To probe how reducing L6 CT feedback impacts thalamic activity, we injected Ntsr1-Cre mice with an adeno-associated virus (AAV) driving the optogenetic silencer eNpHR3.0 fused to YFP, hereafter referred to as ‘halorhodopsin’ (Fig. 1A). To confirm that halorhodopsin could be used to optically suppress L6 activity, we quantified the change in firing rates of regular spiking ‘RS’ units isolated from electrodes placed into L6 (see Methods). L6 is composed of several major cell types, including multiple cell classes that would be classified as RS by our criteria (see Methods), but only one of which projects to VPM and is labeled in the Ntsr1-Cre line (Olsen et al. 2012; Mease et al. 2014; Pauzin & Krieger 2018; Guo et al. 2017). Since our extracellular recording cannot identify the subtype of each recorded unit, we compared the net impact of illumination of the cortex between mice injected with an AAV driving halorhodopsin-YFP, or YFP alone. During free whisking, the average reduction in the firing rates of RS units that showed suppression during cortical illumination was 48.91 ± 28.44 % (Fig. 1B, D). In YFP-expressing mice, using otherwise identical experimental conditions and analysis, we observed only a 12.17 ± 12.83% reduction (p << 0.001 for comparison with opsin data), confirming the efficacy of our manipulation (Fig. 1C, D). During sensory evoked activity, the mean reduction of suppressed RS units was similar (Fig. 1D, E, p = 0.14), and the reduction of the control YFP population was still negligible in comparison (15.94 ± 14.18 % reduction, p = 0.85, Fig. 1D, F). The incompleteness of suppression of L6 RS units in halorhodopsin-expressing animals is likely to be a combination of incomplete infection of L6 CT neurons by the AAV virus, insufficient hyperpolarization of infected L6 CT neurons, and L6 non-CT neurons that are not labeled in the Ntsr1-Cre – notably the substantial fraction of L6 principal cells that project intra-cortically.
Next, we asked how reducing L6 activity influenced thalamic activity. We found that it modestly but significantly decreased VPM activity, both during free whisking and during active touch (touch: 8.31 ± 31.99% reduction, n = 77, signrank p = 0.007; free whisking: 15.41 ± 34.73% reduction, n = 77, signrank p = 0.02, Fig. 2B, D). In mice expressing YFP alone we observed no significant changes for either condition (touch: 1.51 ± 19.41% reduction n = 46, signrank p = 0.64; free whisking: 3.09 ± 18.34% increase, n = 46, signrank p = 0.33, Fig. 2C, D), confirming that the effects of L6 CT suppression on the VPM were specific and not due to the illumination light alone.

The reduction in VPM activity during suppression of L6 CT neurons could be due to reduced excitation from the direct L6-VPM connection, increased inhibition from the nRT onto VPM, or both. As nRT neurons are the only source of inhibition to the VPM we recorded from the nRT under the same conditions. Like in the VPM, optogenetically suppressing L6 CT neurons decreased activity of most nRT
units (22/30 units) both during free whisking (16.72 ± 29.14 %, n = 30, signrank p = 0.02) and active touch 11.12 ± 22.32 %, signrank p = 0.02, n = 30, Fig. 2D-F). Illumination of the cortex in mice expressing YFP alone showed no effect of light in either condition (free whisking: 0.21 ± 20.84 % decrease, n = 37, signrank p = 0.53; touch: 3.64 ± 20.12 %, n = 37, signrank p = 0.95, Fig. 2D-F). Based on these results, it is likely that the reduction in activity in the VPM results from a loss of the direct excitatory input, and not an increase in GABAergic inhibition, since nRT neurons likewise showed reduced firing rates.

**Figure 2**. A Top and bottom: slices from an Ntsrt-Cre mouse injected with AAV9-eNpHR-EYFP, top showing the end of a recording track in the VPM (dashed white line), and bottom showing a track in nRT. B Comparison of sensory-driven firing rates in VPM with and without L6 modulation. Inset: C VPM cell activity during sensory stimulation, with and without light in a YFP-expressing control population of Ntsrt-Cre mice. D Comparison of average decrease with light in VPM and nRT between an opsin-expressing population (Halo) and a control population (YFP). E Reticular nucleus activity comparison with and without L6 suppression. Inset: raster plot of activity in an nRT cell during presence of sensory stimulus (black bar) with cortical suppression (red bar). F nRT cell activity during sensory stimulation, with and without light in the YFP control population.

Next, we sought to determine the impact of L6 CT suppression on spatial representations in the VPM. To estimate the spatial tuning of VPM units we presented the vertical stimulus pole in one of eight locations in the whisking field of the mice. We constructed spatial tuning curves of touch-evoked activity for each unit, and applied a vector norm-based metric that described spatial selectivity for the eight pole positions (see Methods). This selectivity metric takes into account the “negative” components of tuning curves that result from a unit being suppressed at certain positions, and ranges from 0 to 1, with 0 describing a unit that has no preference (i.e., a ‘flat’ tuning curve), and 1 describing a unit a significant
touched induced response to just one pole position. When optogenetically suppressing L6 CT neurons, VPM cells markedly increased their spatial selectivity for pole position (off: 0.32 ± 0.14, on: 0.39 ± 0.17, n = 33, signrank p < 0.001, Fig. 3B, C). nRT units did not show a significant change (off: 0.36 ± 0.14, on: 0.35 ± 0.16, n = 30, signrank p = 0.26, Fig. 3D).

The change in spatial selectivity of VPM unit during L6 suppression could potentially be due to a light-induced change in whisker movement. To address this potential confound, we quantified both set point and amplitude of whisking across during L6 CT suppression by analyzing high-speed videos of whisking (Fig. 4A). No significant differences were observed (Fig. 4D, E). We likewise observed no impact of L6 CT photo-suppression on the phase-locking of VPM units to the whisk cycle (Vinck et al. 2010), or the frequency with which VPM neurons firing in bursts (Fig. 4B, C).

Finally, to complement our suppression experiments which reduced intrinsically generated L6 CT activity during free whisking and active touch, we asked how artificially elevating L6 CT neurons activity would impact thalamic firing rates to better compare our results to the wider literature on corticothalamic activation. We injected Ntsr1-Cre mice with AAVs driving ChR2 and illuminated the cortex with blue light (Fig. 5A). As above, we first confirmed the efficacy of our manipulation by measuring the light-induced changes in L6 RS units. Blue light increased the activity of 9/16 recorded RS units, but suppressed the remaining 7 RS units (Fig. 5B). Although it is impossible to determine their exact identity with our extracellular recording approach, the units showing potent firing rate elevation are likely to be ChR2-expressing L6 CT neurons, while the suppressed units are more likely to be L6
intracortically projecting units or local regular spiking GABAergic units not labeled in the Ntsr1-Cre line (686.83 ± 1469.04 % increase, n = 16, signrank p = 0.76).

Having confirmed the efficacy of L6 CT neuron photo-stimulation, we addressed its impact on thalamic activity. Our experiments with L6 CT suppression above would predict that photo-stimulating L6 CT neurons should enhance activity in the VPM. However, we instead observed that L6 CT photostimulation potently suppressed VPM firing rates (free whisking: 37.19 ± 48.63 % reduction, n = 50, signrank p << 0.001; touch: 29.62 ± 47.65 % reduction, n = 50, p << 0.001, Fig. 5C-F). The suppression of VPM during L6 CT photo-stimulation could be due to artificially powerful recruitment of nRT GABAergic neurons, which would disynaptically inhibit VPM. However, we found that L6 CT photo-stimulation also suppressed activity of nearly all recorded nRT units (free whisking: 7.44 ± 76.09 % reduction, n = 36, signrank p < 0.001; touch: 26.54 ± 50.75 % reduction, n = 36, p = 0.001, Fig. 5E). We
address possible explanations for this discrepancy between loss-of-function and gain-of-function experiments of L6 CT activity below.

Figure 5. A Top: Ntsr1-Cre mouse injected with AAV9-ChR2-tdTOM in barrel cortex. Bottom: slice showing terminals in VPM and nRT as well as tracks in VPM and nRT with a dashed white line. B L6 RS firing rates in a sensory-driven condition compared to periods with light stimulation during sensory drive. Inset: raster from a putative ChR2-expressing cell showing sensory-driven activity (black bar) with light stimulation (blue bar). C Effect of L6 stimulation on VPM firing rates – comparison of firing rates with the light off and on. D Reticular nucleus activity with and without L6 stimulation. E Top: raster plot of activity from a VPM cell during sensory activation with suppression. The blue bar indicates the presence of the light and black bar indicates presence of sensory stimulus. Bottom: raster plot of activity from an nRT cell. F Average percent change across regions with L6 stimulation.
Discussion

This study shows, for the first time, that decreasing corticothalamic output from L6 in S1 has a measurable and significant effect on primary thalamic activity, specifically in awake, actively sensing animals. Based on our experiments in which we optogenetically suppress L6 CT neurons, we conclude that the net impact of L6 CT feedback on the VPM is to facilitate its activity and broaden its representation of the space the whiskers sweep out.

It is important to put our results into the broader context of a number of studies that have optogenetically manipulated cortical activity and monitored its impacts on thalamic dynamics. Pauzin et al., did not find significant effects of optogenetic suppression of L6 CT neurons on thalamic activity, and when they photo-stimulated L6 CT neurons via ChR2, they instead observed enhancements of VPM activity (Pauzin & Krieger 2018), also in disagreement with our findings. These differences might be explained by the use of anesthesia in this study, which blocks active touch and free whisking, and is well known to globally alter cortical dynamics. The anesthetic removal of re-afferent input to the VPM during self-motion of the whiskers (Urbain et al. 2015) might dramatically alter how L6 CT feedback influences thalamic unit activity, as this input may contribute a substantial amount of their ongoing synaptic conductance. Another study in anesthetized mice found that VPM shows biphasic effects to L6 CT photo-stimulation – a fast depolarization followed by transient inhibition (Mease et al. 2014). This study observed that L6 CT stimulation suppressed the VPM response to a single whisker deflection, but reduced VPM adaptation to repeated whisker deflection. Thus, an alternative explanation to reconcile these findings is that the impact of L6 CT feedback is dynamic – suppressive at the onset and facilitator during repeated whisker stimulation. This notion is broadly consistent with a brain slice study from the whisker system which showed that the sign of the impact of L6 CT photo-activation is frequency dependent: suppressive at low frequencies, but facilitating at higher frequencies. In our awake, active sensing paradigm, the mouse’s whiskers make repeated contact with the stimulus bar over the course of the 1000 ms that we optogenetically suppressed L6 CT neurons (Pluta et al. 2015; Pluta et al. 2017). It is possible that we might observe an opposite effect on VPM activity for the very first touch in a series of touches, although we did not parametrically alter our illumination conditions (the illumination light was always switched on 300 ms after initial presentation of the stimulus pole to obtain a within-trial baseline). Nevertheless, since rodents naturally make repeated contacts with tactile objects to determine their location, shape, and texture (Diamond et al. 2008; Prescott et al. 2011), the net impact of L6 CT feedback during a series of touches is arguably more ethologically relevant.

Our L6 ChR2-stimulation data do agree with results from a similar manipulation performed in the visual system, where stimulation of V1 L6 CT neurons suppressed the activity of activity in the primary visual thalamic nucleus, the dorsal lateral geniculate nucleus, dLGN. This study also examined the impact of suppressing the cortex on thalamic activity, but only when silencing the cortex much less specifically by photo-activating cortical parvalbumin (PV) neurons, rather than by optogenetically suppressing L6 CT neurons. Under these conditions they observed enhancements of dLGN activity, which is in contrast to our results in which L6 CT photo-suppression reduces VPM firing rates. This difference could be explained by various factors: the total suppression of all cortical layers via PV neuron
stimulation; the difference between active sensation versus passive sensory stimulation; or a modal difference between vision and somatosensation. Notably, dLGN, unlike VPM in rodents, has intrinsic GABAergic neurons which might also contribute to this difference.

In the auditory system Guo et al. explored the effect of L6 stimulation on the auditory thalamus (medial geniculate body, MGB) in awake animals (as well as on other cortical layers). They activated L6 CT neurons at various delays after a tone pip, and reported an enhancement of the sensory activity in MGB and auditory nRT when the tone and light were presented simultaneously. However, at other intervals after the auditory stimulus, photo-stimulating L6 CT neurons had either a weaker facilitatory or no effect on MGB units, while the nRT was inhibited at certain delay (Guo et al. 2017). This is broadly consistent with the aforementioned studies in the whisker system suggesting that the timing between sensory stimulation and light activation may cause varied dynamics in the corticothalamic loop (Crandall et al. 2015; Mease et al. 2014). Guo et al. also optogenetically suppressed L6 CT neurons while recording thalamic activity (to our knowledge the only other study to do so in awake animals), but did not observe a significant change in either MGB or nRT activity, which might also explained by the temporal delay between tone and light stimulation.

In our study we observed that optogenetic suppression and optogenetic stimulation of L6 CT neurons reduced activity in the VPM and the nRT. This leads to a difficulty in interpreting some of the optogenetic results. However, it is possible that artificially activating a wide swath of L6 CT neurons with ChR2 leads to highly unnatural dynamics in the CT system that might lead to spurious conclusions. Consistent with prior theoretical considerations (Phillips & Hasenstaub 2016), we believe one should interpret ChR2-based experiments with substantial caution. Halorhodopsin-based suppression experiments, because they only eliminate natural, ongoing activity, are more likely to lead to more meaningful conclusions. It is possible that a thorough exploration of the large parameter space of possible ChR2 stimulation paradigms of L6 CT neurons could reveal that the impact on VPM is a complicated, and non-monotonic function of L6 CT activity. Indeed, prior work alluded to above already suggests this is the case, as the sign of the impact on VPM activity depends highly on the light pulse number and pulse frequency. Rather than attempt to explore this space, we instead rely on the optogenetic suppression experiments which demonstrate that CT feedback positively modulates activity in both VPM and its inhibitory nucleus, nRT, effectively broadening how VPM units encode the tactile space swept out by the whiskers during active sensation. It is likely that, during L6 suppression, VPM not only loses both excitatory drive from the monosynaptic pathway and inhibitory drive from the disynaptic pathway, but the balance of excitation and inhibition also changes to shape the remaining activity. Based on our results, we conclude that the cortex can regulate the strength and spatial tuning of its own input. Since L6 neurons receive long range projections from higher cortical areas, as well multiple types of neuromodulatory input, L6 CT neurons are poised to dynamically control the flow of sensory input to the cortex.
Methods

Animals

We used Ntsr1-Cre mice (GN220, MMRRC) of both sexes for all experiments, with the mice being between 1.5 and 3 months of age. Prior to use, the line was outcrossed to ICR mice for several generations. Mice were injected at P3-P4 with AAV9.EF1a.DIO.eNPHR3.0-EYFP.WPRE.hGH for suppression experiments, AAV9.CAGGS.Flex.ChR2-ttdTomato.WPRE.SV40 or AAV9.EF1a.DIO.hChR2(H134R)-eYFP.WPRE.hGH for stimulation, or with just a fluorophore, AAV2/9-Ef1a-DIO-YFP for control experiments. Viruses were acquired from the University of Pennsylvania Vector Core; viral aliquots were loaded into a Drummond Nanoject injector. Neonates were briefly cryo-anesthetized and placed in a head mold. Injection coordinates for S1 were 1.9 mm anterior, 2.9 mm lateral, and 0.4 mm dorsoventral. All procedures were approved by the Animal Care and Use Committee of UC Berkeley.

In vivo electrophysiology

Animals were first anesthetized with 5% isoflurane, then maintained at 2% while their temperature and tail pinch response were monitored for the duration of the surgery. The scalp was disinfected with betadine (5%) and alcohol (70%), then removed to expose the skull. Vetbond (3M) was applied to the wound margins, and a headplate was then attached using dental cement, followed by Ortho-Jet for stabilization. The animal was given post-surgical medication (buprenorphine and meloxicam) and allowed to recover for at least 3 days while being monitored daily. After recovery, the animal was trained to run on a rotary treadmill with head fixation until it ran freely at a steady pace, usually 3-7 days. For electrophysiology, the animal was anesthetized, the skull above the intended target was thinned, and a craniotomy was made using a 27 ½ gauge needle. For thalamic recordings, the craniotomy was centered at 1600 µm posterior and 1800 µm lateral from bregma for VPM and 1600 µm posterior and 2300 µm lateral for nRT, and a 16-channel silicon probe (NeuroNexus) was lowered until whisker responses were detected, usually starting at 2600-2800 µm, which indicated entry into the VPM. The electrode kept being lowered until some contacts were located in an area representing a large whisker that would be easily stimulated, usually B2, C2, or D2. For cortical recordings, the craniotomy was centered at 1500 µm posterior and 3000 µm lateral and a 16-channel probe was lowered at an angle so as to be perpendicular to the surface. While responses to whisker deflections were probed, it was driven down until robust responses stopped when white matter was reached. After recording, electrodes were taken out, dipped in a fluorescent dye solution (DiI), and reinserted into the brain in order to mark electrode tracks for histological confirmation.

Sensory stimulus presentation

While the animal ran during electrophysiological recording, a modified 0.7mm Hex key (McMaster-Carr) was presented vertically at eight locations spanning the whisker field from rostral to caudal. This pole was presented ~ 1cm away from the mouse’s face, and there was about 2.2 mm difference between
adjacent positions. In addition to the eight positions, a ninth position was presented outside of the whisker field for baseline comparisons. During each trial, the stimulus was present in the whisker field for 1.5 seconds, and we used a stepper motor (Orienta Motor) to ensure quick movement of the pole in and out of the field. There was an interval of 1.7 seconds between trials. At the beginning of each inter-trial interval the stepper motor and pole were translated to the next trial’s horizontal position using a motorized linear stage (Zaber). Stimuli were randomized such that no stimulus was presented more than twice in a row.

**Optogenetics**

To activate eNpHR3.0 during electrophysiology experiments, we used a red LED (Thorlabs) coupled to a 1-mm diameter multimode optical fiber, outputting 30–80 mW mm\(^{-2}\) of red light, while for ChR2 experiments, we used a blue LED. In all cases, the fiber was as close as possible to the craniotomy.

**Analysis of multi-electrode extracellular data**

Spikes were detected using the UltraMegaSort2000 package (Hill et al. 2011) in MATLAB (Mathworks), then automatically sorted into unit clusters, after which, manual sorting was required to ensure that all clusters met inclusion criteria and no clusters were double-counted between adjacent contacts. Inclusion criteria were based on quality metrics, including inter-spike interval, spike amplitude, spike rate, and auto- and cross-correlation. In cortical sorting, we set a refractory period of 2.5 ms, and included units had no more than 1% of their individual waveforms falling into this window. In the thalamus, included units were capped at 2%. In cortex, regular-spiking units were identified and separated from fast-spiking units using a k-means cluster analysis of spike waveform components, one of which was the normalized difference between the two positive-going peaks and the other being the trough-to-peak latency of the negative deflection (Reyes-Puerta et al. 2015). A positive difference for the first metric and a short (< 0.33 ms) trough-to-peak time for the second indicated that a cell was a fast-spiking unit and was then excluded from analysis.

Bursting characteristics were determined using the Poisson surprise method (Legéndy & Salcman 1985), and quantified as the proportion of total spikes that were in bursts. Phase-locking between the whisking oscillation and neuronal spike times was computed using pairwise phase consistency (Vinck et al. 2010)

**Selectivity metric**

The spatial selectivity of neurons was calculated from the normed vector of the peak normalized spatial tuning curves using raw spike rates as previously published (Pluta et al. 2017). This value was then divided by square root of \(n\) dimensions – 1 to restrict its range from 0 to 1. Larger values signify higher spatial selectivity (higher sharpness and lower broadness).
Spatial Selectivity = 1 - \left( \frac{\|x\|}{\max(x)} - 1 \right) \frac{1}{\sqrt{n} - 1}

High-speed whisker tracking

For every mouse used in analysis, whiskers were tracked using a high-speed camera (Basler, acA2000-340kc) during electrophysiological recording. The camera is placed under the whiskers and running wheel and the whiskers are illuminated from above using an infrared light source and imaged from below using a mirror angled at 45 degrees. The videos were synchronized with neural data using external triggers, and was acquired at 500 frames per second. Video data was processed in MATLAB using Janelia Farms software which extracted whisker angle, and then further processed using custom-written software that produced set point, amplitude, and whisk frequency information.
Chapter 4: Closing thoughts

The corticothalamic circuit, despite years of research attention, has evaded being pinned down in terms of function. Of course, our ability to precisely define and pigeonhole any brain circuit, must, of course, be taken as a baseline and no circuit has just one easily definable purpose; even still, this particular connection is complicated and prior work reflects that. What could explain such variety of results? One potential reason, of course, is the different approaches that have been used to probe the question – in early days, predominant methods were those that were difficult to control spatially and temporally, and could thus lead to diffuse downstream effects. The introduction of optogenetics brought with it more precision, but also greater freedom and variability in experimental design, which is a good byproduct of the available technology, of course, but is a downside for comparing across studies. The other, and more concerning, potential source of confusion is that corticothalamic feedback serves a different function in different sensory modalities, and studies that approach it from one may not be studying a conserved mechanism that would then generalize to others. Nature doesn’t tend to reinvent an already working wheel from scratch too often, though, and it would be surprising if all studied animals, in all sensory modalities, were all truly distinct. I think the answer lies in methodological differences – if an approach were to be parametrized with respect to variables such as behavioral state (ranging from anesthetized to fully awake and states in between), strength of optogenetic modulation, and timing between optogenetic interventions and other stimuli, for starters, the circuit would be easier to dissect.

More specifically, if the line of research described in Chapter 3 were to be continued, it would benefit from exactly such suggestions – titrating levels of optogenetic modulation would allow us to draw more nuanced conclusions regarding similarities and differences between thalamic effects of cortical activation and suppression, as well as settle the issue of potential hyperactivation of cortical cells leading to spurious downstream effects. Exploring the differences in timing between optogenetic modulation and presentation of a sensory stimulus may also shed light on what may be a complicated interplay of activity that is extremely dynamic and difficult to describe by restrictively focusing on a static time period.

Yet another limiting factor, currently, is that the question of feedback has often been approached from a simplistic point of view, though necessarily so since we are only at the beginning of understanding this circuit despite many past studies. Instead of focusing on the sign of the feedback and its effect on firing rates, maybe the question should be reframed in terms of more global processing, like what kind of higher-order functions these connections could be mediating in the overall scheme of sensory processing. Viewed through this lens, perhaps prior work isn’t so much contradictory as it is indicative of us asking the wrong questions. With time, patience, and a continually refined approach, however, this, much like other complex circuits in neuroscience, can eventually be disentangled for a better understanding of how we process the sensory world.
Bibliography


Diamond, M.E. et al., 1992. Somatic sensory responses in the rostral sector of the posterior group (POm) and in the ventral posterior medial nucleus (VPM) of the rat thalamus: Dependence on the barrel field cortex. *Journal of Comparative Neurology, 319*, pp.66–84.


