Experience-Dependent Refinement of Maps and Population Codes in Primary Somatosensory Cortex.

By

Amy M. LeMessurier

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Committee in charge:

Professor Daniel E. Feldman, Chair
Professor Yang Dan
Professor Hillel Adesnik
Professor Marla Feller

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Abstract

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Sensory experience can powerfully alter the spatial and temporal organization of population codes in the brain throughout an animal's lifetime. In this dissertation I focus on how changes in sensory experience, by learning or environmental enrichment, alter sensory map topography in rodent primary sensory cortex.

In Chapter 1 I review recent advances in our understanding of how perceptual learning and related sensory manipulations shape the structure of primary sensory cortex maps across multiple sensory modalities. Classic studies of map plasticity in primary sensory cortex have shown that experience shapes sensory tuning in individual neurons and at the average population level. But effective neural population codes depend on more than just sensory tuning in individual cells averaged over time. With population calcium imaging, activity can be measured simultaneously in large ensembles of neurons during behavior. This and related techniques make it possible to study changes in the single-cell level organization of sensory coding, as well as high-dimensional codes that depend on the activity of large ensembles of cells, and which may change with behavioral context. Here I review recent population calcium imaging and recording studies that have characterized population codes in sensory cortex, and tracked how they change with sensory manipulations and training on perceptual learning tasks. These studies confirm average sensory tuning changes observed in earlier studies, but also reveal other features of plasticity, including sensory gain modulation, restructuring of firing correlations, and differential routing of information to output pathways. Unexpectedly strong day-to-day variation exists in single-neuron sensory tuning, which stabilizes during learning. These are novel dimensions of plasticity in sensory cortex, which refine population codes during learning, but whose mechanisms are unknown.

Most of what is known about how sensory experience shapes maps is based on studies that have used robust sensory manipulations, such as deprivation, chronic over-stimulation, or explicit pairing of a sensory stimulus with a reward or punishment, as in the studies discussed in Chapter 1. However, much less is known about how maps are
refined by natural behavior-driven sensory experience. To understand how natural tactile experience influences map development, we studied the effects of tactile enrichment on the organization of the whisker map in mouse primary somatosensory cortex (S1), which is highly plastic throughout life and which contains an anatomically well-defined map of the whiskers. Mice were raised with enrichment (EN) or normal housing (CT) beginning at weaning (P21). We focused our study on Layer 2/3 (L2/3), which is particularly plastic and which has a “salt-and-pepper” organization of whisker tuning, and Layer 4 (L4), which contains the anatomical map of the whiskers. The genetically-encoded calcium indicator GCaMP6s was expressed virally in L2/3 or L4 excitatory cells using cell-type and layer-specific Cre mice. At P62 ± 13 days, we measured neuronal responses to 9 whiskers using 2-photon imaging through a chronic cranial window. After imaging was complete, cells were localized relative to anatomical barrel column boundaries corresponding to the whiskers in the stimulus set. Within a single anatomical column in both L2/3 and L4 excitatory cells, cells were heterogeneously tuned to different whiskers. Enrichment increased somatotopic precision (the fraction of cells within a certain radius that were anatomically tuned) in both layers near the centers of anatomical columns. Enrichment also sharpened whisker tuning curves in both layers, and essentially increased signal-to-noise of whisker coding in L2/3 by decreasing spontaneous activity while maintaining response magnitudes for columnar whiskers. In L2/3, point representations (the density and spatial spread of neurons responding to a whisker) were sharpened by increased whisker responses among cells located close to column centers.

To study the impact of enrichment on the spatial and temporal structure of population coding, we compared pairwise noise and signal correlations in whisker-evoked activity within each column. In L2/3, signal correlations – which reflect overall tuning similarity – were similar in EN and CT for pairs within columns, though signal correlations were higher in EN for pairs located further apart. However, for pairs of neurons located across column boundaries but at similar distances, signal correlations were dramatically decreased in EN, while comparable to in-column levels in CT. In both groups, noise correlations – which reflect shared trial-by-trial variability that is independent of the stimulus – decreased sharply with distance between neurons. For pairs of cells across column boundaries in CT mice, signal and noise correlations exhibited the same relationships with inter-cell distance as within column pairs; however, in EN mice, cross-column signal and noise correlations were substantially reduced compared to in-column pairs. Essentially, enrichment induced reorganization of functional correlations along anatomical column boundaries. This suggests that enrichment may selectively alter connectivity and/or shared synaptic input according to column boundaries.

These findings demonstrate a strong impact of juvenile sensory experience on functional columnar topography and organization in S1, and indicate that enrichment sharpens whisker representations at the population level in L2/3.
# Table of Contents

## Introduction

| Text | 1 |
| References | 3 |

## Chapter 1

1.1 Summary | 5
1.2 Introduction | 5
1.3 Plasticity of sensory tuning | 6
1.4 Principles of population coding in sensory cortex | 6
1.5 Learning by changes in response gain and reliability | 7
1.6 Learning by changes in firing correlations between neurons | 8
1.7 Learning by reduction in daily tuning variation | 9
1.8 Learning by changing routing of information down sensorimotor pathways | 10
1.9 Sites and mechanisms for changing population codes | 11
1.10 Acknowledgments | 14
1.11 References | 15

## Chapter 2

2.1 Introduction | 18
2.2 Methods | 19
2.3 Results | 24
2.4 Discussion | 32
2.5 References | 38
List of Tables and Figures

Ch.1, Figure 1. *Four ways to adjust a population code in sensory cortex.* (p. 13)

Ch.2, Figure 1. *In vivo 2-photon calcium imaging of pyramidal cell responses to whisker deflections in L2/3 and L4 barrel cortex.* (p. 42)

Ch.2, Figure 2. *Enrichment increases the proportions of somatotopically-tuned ROIs located near barrel column centers.* (p. 44)

Ch.2, Figure 3. *Enrichment sharpens whisker tuning curves.* (p. 46)

Ch.2, Figure 4. *Enrichment sharpens point representations of single whiskers in L2/3, but not L4, by increasing pyramidal cell responses to the columnar whisker.* (p. 48)

Ch.2, Figure 5. *Enrichment alters the spatial structure of signal and noise correlations along column boundaries in L2/3.* (p. 50)

Ch.2, Figure 6. *Enrichment increases signal and noise correlations within columns in L4.* (p. 52)

Ch.2, Figure 7. *Enrichment improves population coding of columnar whisker deflections.* (p. 54)

Ch.2, Figure S1. *Measurements of somatotopy based on absolute best whisker.* (p. 56)

Ch.2, Figure S2. *Non-normalized whisker tuning curves and evoked response distributions.* (p. 58)

Ch.2, Figure S3. *Non-normalized point representations of single whisker in L2/3 and L4* (p. 60)

Ch.2, Table 1. *Mouse Information.* (p. 61)
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Introduction

In the mammalian brain, peripheral sensory input across multiple modalities is represented topographically in the central nervous system (CNS). Maps of sensory input – the visual field, tactile input to glabrous and hairy skin, sound frequencies – can be found at multiple levels of the brain as information about sensory stimuli is extracted from patterns of activity in peripheral sensory receptor neurons and integrated to form complex representations of an animal’s environment. In this thesis I will focus on sensory coding and map topography in primary sensory cortex. Topographic organization – in which neurons that fire in response to stimuli that are closely spaced on the skin, in the visual field, etc. or are similar in other features, e.g. orientation or sound frequency, are located near each other – exists at multiple sites of the pathways that carry sensory information from peripheral sensory receptors to the brain. Primary sensory cortical areas receive sensory input directly from the thalamus, and project laterally to higher-order sensory and associational areas of cortex, in which more complex features of sensory input are encoded and integrated. Compared to subcortical representations, primary sensory cortex generally contains more finely-grained maps of sensory input. Sensory maps are highly reproducible across animals, but they require experience to develop, and develop abnormally in animals that are deprived of sensory experience early in development [1], [2].

The research I present here primarily focuses on rodent Vibrissal Sensory Cortex (vS1), or Barrel cortex, which is a specialized area of primary somatosensory cortex (S1) that represents the whiskers. Topography in vS1 is visible in anatomically discrete barrel-columns. Neurons located within each anatomically-defined barrel in layer 4 (L4), the primary thalamo-recipient layer, primarily represent input to a corresponding whisker on the face. This columnar structure extends in L2/3, in which cells on average respond preferentially to their anatomically-defined “columnar whisker”, but also respond to surrounding whiskers and exhibit broader receptive fields. When measured at population level, in which sensory responses are averaged across many neighboring neurons, this somatotopy appears quite uniform and is reproducible across animals (reviewed in [3]). However, the topography of whisker tuning is much more heterogeneous at the single-neuron level. Studies examining whisker tuning in individual L2/3 neurons have shown that many cells within a column are tuned for whiskers surrounding the CW, and average tuning across neurons varies with a gradient over cortical area [4],[5],[6]. This “salt and pepper” organization has also been observed in other primary sensory areas with respect to sensory feature tuning: in mouse primary visual cortex (V1), the spatial organization of orientation tuning appears topographic when averaged over many neurons, but is much more granular at the single cell level [7], [8]. Similarly, tonotopy in mouse primary auditory cortex (A1) is only observable when frequency tuning is averaged over many neurons [9].
In order for an animal to navigate and respond to a complex and changing environment, the brain must maintain stable representations, but it must also be able to flexibly accommodate any changes to an animal’s environment or sensory organs that occur throughout its lifetime. A large body of research has found that primary sensory cortex is especially plastic when sensory input is changed, even in adult animals. S1 is particularly convenient for studying sensory plasticity, because input to the whiskers can be easily deprived and restored by whisker plucking/trimming. After input to a single whisker or row of whiskers is deprived for several days, neurons within and near the column corresponding to the deprived whisker(s) exhibit reduced responses to the deprived whisker(s), and over time, increase their responses to neighboring spared whiskers. This effect is reversed after input to the deprived whisker is restored. Conversely, when a whisker is chronically overstimulated, the area and density of neurons in the cortex representing that whisker shrinks (reviewed in [1]). Map plasticity is also observed in V1 [2], [10] and A1 [11] when sensory input is deprived or chronically overexposed.

Classic studies have mostly examined map plasticity resulting from drastic changes to sensory input, such as total deprivation of input to a sensory organ (as described above) and have seen robust population-level changes in maps. However, the sensory landscape that animals are exposed to in naturalistic settings change over time in much more subtle ways. For example, as a mouse explores its environment, it may learn that certain sensory stimuli – odors, sounds, patterns of whisker deflections – indicate the presence of a particular food source, or a dangerous predator. The salience of these sensory stimuli may lead to changes in sensory representation in cortex. More recently, many studies of sensory plasticity have explored the effects of associative learning on encoding of sensory features in primary sensory cortex, and have found more complex and context-dependent changes in single neuron tuning and population-level codes. In Chapter 1, I review the findings of these studies and their implications for our understanding of how learning shapes sensory coding in primary sensory cortex. In particular I focus on studies that use population 2-photon calcium imaging, a technique in which activity in large populations of individual neurons can be simultaneously measured. This has revealed fine spatial structure and ensemble-level sensory coding that cannot be measured by techniques that require averaging across populations of neurons and/or which do not provide information about the spatial organization of the neurons measured.

It is clear from the studies I review in Chapter 1 that primary sensory cortex undergoes significant plasticity when animals learn associative sensorimotor tasks. However, these tasks require repeated delivery of non-naturalistic stimuli that animals might not otherwise experience. In S1, it is known that chronic overstimulation of individual whiskers without an aversive or appetitive stimulus pairing leads to downregulation of responses to that whisker in its corresponding column [1]. This is an effect that is unlikely to occur with natural sensory behavior. While these findings can tell us about the capacity of sensory cortex to undergo major plasticity, it is difficult to predict what
changes are likely to occur in sensory circuits as a result of natural sensory experience. Additionally, compared to naturalistic settings, the sensory experience of most laboratory mice is relatively deprived: animals are generally housed in rectangular cages with bedding, food and water, and few to no other objects. Thus, it is possible that the map organization observed in experimental animals may reflect an inexperienced cortex in which sensory circuits have not been fully refined in an activity-dependent manner.

Some studies have examined the effects of natural sensory experience on encoding by exposing animals to naturalistic or enriched environments. Compared to a standard laboratory cage, a mouse exploring such an environment likely exposes its whiskers to a broader distribution of whisker deflections, and may learn associations between complex stimuli that cannot be accurately predicted or replicated by existing sensorimotor learning tasks. Studies in which adult mice or rats are exposed to enriched environments as juveniles or adults have found broad, column-level plasticity in barrel cortex, as well as the forepaw representation in S1 [12], [13], [14]. All of these studies were largely limited to population-average measurements of somatotopy. We wondered whether the salt-and-pepper tuning that has been revealed in S1 by population calcium imaging would be refined by enrichment. In Chapter 2, I use population 2-photon calcium imaging to interrogate the effects of sensory enrichment on the fine-scale structure of somatotopy in S1. Our findings indicate that enrichment has robust effects on the sub-columnar organization of the whisker map, as well as population coding for input to the whiskers.

References


Plasticity of population coding in primary sensory cortex

Amy M. LeMessurier and Daniel E. Feldman

This chapter, in full, is a replication of the material as it appears in LeMessurier, A.M., Feldman, D.E. 2018. Plasticity of population coding in primary sensory cortex. Current Opinion in Neurobiology, 53; 50-56

1.1 Summary

That experience shapes sensory tuning in primary sensory cortex is well understood. But effective neural population codes depend on more than just sensory tuning. Recent population imaging and recording studies have characterized population codes in sensory cortex, and tracked how they change with sensory manipulations and training on perceptual learning tasks. These studies confirm sensory tuning changes, but also reveal other features of plasticity, including sensory gain modulation, restructuring of firing correlations, and differential routing of information to output pathways. Unexpectedly strong day-to-day variation exists in single-neuron sensory tuning, which stabilizes during learning. These are novel dimensions of plasticity in sensory cortex, which refine population codes during learning, but whose mechanisms are unknown.

1.2 Introduction

Sensory experience drives robust plasticity of sensory tuning and maps in sensory cortex. This well-studied process drives map development to match sensory statistics, and contributes to sensory perceptual learning [1], [2], [3]. But is there more to sensory cortex plasticity than changes in sensory tuning? Sensory areas use population codes that are based on coordinated spiking across many neurons. Large-scale population imaging and recording enable comprehensive analysis of population coding. Chronic longitudinal imaging allows plasticity to be directly observed, with cellular resolution, as it unfolds [4], [5], [6]. These methods provide new insight into neural coding and how it changes during plasticity. Recent studies confirm changes in sensory tuning, but also reveal plasticity in other aspects of population coding, including response gain and variability, firing correlations, and top-down modulation by task context. Here we review some key findings, which suggest novel sites and mechanisms for sensory cortex plasticity.
1.3 Plasticity of sensory tuning

In classical map plasticity, neurons adjust their sensory tuning to represent common or behaviorally relevant (i.e., reinforced) sensory features. This is confirmed by population imaging. In mouse V1, filtering out all but one visual orientation in juveniles increases the proportion of neurons tuned to that orientation [7]. In adults, monocular deprivation causes 60% of active neurons to shift ocular dominance toward the open eye, though a minority shift paradoxically to favor the closed eye [8]. In S1, a large majority of neurons similarly shift whisker tuning away from deprived whiskers and toward a spared whisker [9]. Hebbian plasticity mechanisms are thought to underlie many of these changes. The ability of Hebbian plasticity to imprint information in sensory cortex has been shown decisively in V1, where optogenetic co-activation of L2/3 pyramidal (PYR) cells induces Hebbian-like ensembles that are spontaneously active, exhibit pattern completion, and are spatially mixed with visual-related ensembles [10].

Cortical plasticity is also induced by training on sensory tasks. Sensory training often alters sensory tuning in neurons representing relevant sensory features [11], [12], [13], [1], [14], [15]. These include shifts or expansions in tuning toward trained features [16], [1], [14] or sharpened selectivity that improves population-level discrimination [11], [12] (Figure 1A). Training can also shape more complex integrative tuning features, such as tuning for visual contours [17], [13]. In some cases, tuning changes are small or absent in primary cortex, but observable in higher sensory or sensorimotor areas (e.g., [12], [18]), or occur in primary cortex only transiently [16]. Thus, tuning changes in primary cortex are one mechanism, but not the only mechanism, for perceptual learning.

1.4 Principles of population coding in sensory cortex

Sensation occurs on single trials, despite noisy spike data. Population codes are robust on single trials because they utilize statistical patterns of activity across large numbers of neurons. Both population spike recording and population imaging have been used to characterize population coding in sensory cortex. Here we focus on population imaging, which typically samples more neurons, often with cell type specificity, and has revealed several key features of population coding in sensory cortex.

First, single neurons in rodent cortex have low response probability and high trial-to-trial variability, but populations of ~100 neurons provide robust sensory information on single trials [19], [9], [20]. Second, primary sensory cortex represents not only sensory information, but also non-sensory task variables including movement, anticipation, and behavioral choice [21], [22], [23], [24], [15], which may reflect top-down input. Third, spatially mixed PYR cell subpopulations project to different downstream cortical targets and carry different sensory and non-sensory information [25], [23]. Fourth, longitudinal imaging reveals substantial day-to-day variability in sensory tuning by single neurons, even under nominally constant sensory conditions. In S1, whisker somatotopic tuning is
remarkably poorly correlated across days in single neurons [9], though other sensory tuning is more reliable [26], [20]. In V1, Rose et al. (2016) discovered pronounced day-to-day variability in both ocular dominance and orientation tuning. This is not measurement noise, because it exceeds the variability across trials on the same day. Tuning variability is similar over 4-day and 12-day intervals, and thus is not random drift, which would accumulate over days. Instead, it represents constrained variation near each neuron’s mean tuning [8]. Why these basal dynamics exist is unclear. It could represent plasticity driven by ongoing experience, or random ‘noise’ related to maintaining synapses and circuits. Population coding may filter out this variability to achieve perceptual constancy, e.g. by ignoring the most labile neurons or by using highly redundant population codes [27], [28]. Alternatively, it may represent active exploration of sensory representations for perceptual learning, similar to variability in motor systems for motor exploration [5], [29], [30].

Recent studies have identified experience-dependent changes in these and other features of population coding in primary sensory cortex.

1.5 Learning by changes in response gain and reliability

Sensory training can improve population coding by modulating sensory response gain and reliability (Figure 1B). Poort et al. [15] trained mice to discriminate vertical from angled gratings in a virtual corridor to guide the decision whether to lick for a reward. Calcium imaging during behavior showed that with training, V1 neurons became more selective for grating orientation, largely driven by increased single-trial reliability and amplitude (gain) to the preferred orientation. This improved population coding of orientation, and correlated strongly with the animal’s behavioral discrimination ability. This learned improvement in population coding was reduced in interleaved trial blocks when orientation was task-irrelevant, or when mice were anesthetized. This suggests that top-down input representing the task-dependent salience of specific stimuli gates these learned changes in response reliability and sensory tuning.

Chen et al. [31] trained mice to discriminate two textures, and imaged activity in S1 neurons over days of training. Learning gradually increased texture discrimination by S1 populations. For S1 neurons that project to secondary somatosensory cortex (S2p neurons), the learned improvement in stimulus discrimination was not due to shifts in stimulus selectivity by individual neurons, but reflected loss of touch sensitivity by some S2p neurons and gain of touch sensitivity by others across days of learning. This shifted population tuning for rough vs. smooth textures. Thus, learning gated which S2p neurons were active during the task, which may represent another form of gain regulation. When mice were not rewarded for discriminating, the proportion of cells that discriminated textures returned to pre-learning levels, suggesting that top-down task-dependent signals gated the improved population code [31], similar to V1 [15].
1.6 Learning by changes in firing correlations between neurons

Population coding is strongly impacted by noise correlations, which are co-variations in firing rate between neurons that are not due to shared sensory tuning. Correlated noise in similarly-tuned neurons impairs population coding because it mimics sensory-evoked signals. But when noise correlations are inversely related to tuning similarity, they can increase stimulus information at the population level [32], [33]. Several studies in high-level sensory areas demonstrate that learning can improve stimulus coding by altering noise correlations.

In the first study to examine this issue [34], macaques were trained to either discriminate heading direction based on optic flow, or to simply fixate on these same visual stimuli. In the medial superior temporal area (a multi-sensory area involved in heading perception), training did not alter neural tuning for optic flow, but did substantially reduce noise correlations.

More recently, Jeanne and Gentner [35] trained songbirds to discriminate complex song motifs to earn a reward. After learning, single units were recorded in CLM, a higher-order auditory area that encodes song features. For task-relevant motifs, noise correlation was lowest for pairs of neurons tuned to similar song features, and highest for neurons tuned to dissimilar features. The opposite was true for task-irrelevant motifs and novel, untrained motifs. Thus, learning altered noise correlations and their relationship to tuning similarity in a way predicted to specifically improve encoding of relevant motifs. A classifier trained to discriminate motifs from the spiking of cell pairs performed better when noise correlations were intact vs. when they were eliminated, confirming that the learned changes in correlation structure improved sensory coding.

It is unclear whether noise correlations are altered by learning in primary sensory cortex, but they are modulated by attention during discrimination tasks. Ramalingam and Gilbert [36] recorded single unit spiking in V1 in macaques trained to discriminate bar position and perform contour detection. During the task, noise correlations for pairs of neurons were lower when animals attended to stimuli within the receptive fields, compared to when attention was shifted to a different location. This effect only occurred when animals were engaged in the task, and was largest for units with similar visual tuning, which is predicted to maximally increase visual information. Another recent study found different noise correlation structure in V1 when macaques performed different orientation discrimination tasks, arguing that top-down inputs conveying task context determine noise correlations [37].

Thus, sensory training can alter noise correlations in sensory areas to improve coding. This is likely guided by top-down feedback, similar to learned changes in sensory gain [37]. Changes in noise correlations have also been observed in V1 of anesthetized mice after monocular deprivation [8], but their functional relevance and circuit basis remain unknown.
1.7 Learning by reduction in daily tuning variation

In the motor system, variability in movement-related activity is robust, and drives variable motor output that explores the space of useful movements. During motor learning, circuit activity that commands successful movements is reinforced, thus selecting an optimal movement for completing the task. As learning occurs, neural variability decreases [29], [30], [38]. Does the day-to-day variability in single-neuron sensory tuning play a similar role in sensory learning?

Several studies show that day-to-day tuning variability in sensory cortex decreases over the course of learning (Figure 1C). In V1, Poort et al. [15] examined session-to-session variability in single-neuron selectivity between two oriented gratings, as mice learned the grating discrimination task. Early in training, stimulus selectivity was highly variable for individual neurons from session to session. With learning, day-to-day variability decreased, so that selectivity was more stable across sessions. This stability developed in parallel with improved grating selectivity, population coding and behavioral performance.

Similar changes occur during learning in S1. Chen et al. [31] tracked whether neurons responded to touch, non-touch cues, or were inactive as mice learned the texture discrimination task. Single neurons showed day-to-day variability in this response category, which decreased as animals became experts. Peron et al. [22] trained mice to detect a bar using a single whisker, and imaged ~75% of all L2/3 PYR cells during learning. Different cells encoded touch, whisker movement, and other task parameters. While the proportion of cells encoding touch remained constant over the course of learning, which specific cells were touch-responsive changed from session to session. This variability decreased as behavioral performance improved, so that touch-related and whisking-related activity became more predictable day to day.

Interestingly, the increase in tuning stability with learning was associated with improved population coding of the stimulus in the Poort study of visual discrimination [15], but not in the Peron study of whisker touch detection [22]. Instead, population coding of touch remained stable throughout learning, and consistently better than the mouse’s behavioral performance. This suggests that the touch information needed for task performance was always present in S1, and that learning was accomplished by improved processing of this information in downstream areas. This supports a general hypothesis that when learning requires fine discrimination between similar stimuli, or detection of a subtle feature from noise, reorganization of population coding in primary sensory cortex occurs to improve the low-level sensory representation of task-relevant features (perceptual learning). But when animals must simply learn to use a robust sensory cue to trigger a specific behavioral response, optimization of sensory coding is not required, and learning occurs by downstream changes that trigger appropriate behavioral responses (sensorimotor learning).
The specific role of tuning variability in sensory learning remains unclear. It may reflect the formation of transient Hebbian ensembles by recent sensory experience patterns, which exist for a brief time but are then reversed by subsequent experience. Alternatively, novel ensembles may be formed stochastically by random circuit variation or noisy patterns of spontaneous activity, and once formed, could provide a basis for selection and reinforcement by sensory activity. Such random variation could help cortex escape from existing ensemble structure to establish new representation patterns [39], [28]. Once learning is complete, stabilization of tuning is likely to allow more reliable readout of task-relevant information by downstream areas for better task performance.

While sensory tuning stabilizes in sensory cortex once learning is complete, single-neuron tuning for task features remains dynamic in higher-order, memory-related cortical and hippocampal regions [40], [41]. For example, in posterior parietal cortex of mice performing a navigation task, single-cell coding of task-relevant visual stimuli reorganizes over days and weeks, even when mice are expert at the task [40]. This suggests that while early sensory areas develop stable representations in consistent behavioral conditions, associative areas maintain more flexible codes that can readily incorporate changing information and behavioral associations.

### 1.8 Learning by changing routing of information down sensorimotor pathways

Learning of sensorimotor associations requires plasticity downstream of sensory cortex to transform sensory signals to appropriate behavioral responses. Recently, Le Merre et al. [42] tracked macroscopic changes in sensory-evoked population activity along cortical pathways while animals learned to lick in response to whisker deflection. Recording chronically in an array of sensory, associative, and motor areas, they found that sensory-evoked potentials were initially present in S1 and S2, but not medial prefrontal cortex (mPFC) or CA1 hippocampus. During learning, sensory responses stayed constant in S1 and S2, but developed strongly in mPFC, CA1, and M1 in proportion to behavioral learning. Neural activity in mPFC or CA1 was necessary for learned task performance. Thus, changes in sensory routing from sensory to associative and motor cortical areas can be directly observed during sensorimotor learning.

A first stage of this routing may occur in primary sensory cortex itself, by altering information coding in the PYR subclasses that project to different downstream target areas (Figure 1D). In Chen et al.’s study of texture discrimination learning [31], learning increased the proportion of M1-projecting (M1p) neurons in S1 that encoded touch, due to recruitment of previously inactive cells, and shifted texture tuning within S2p cells. Both M1p and S2p populations became better at discriminating texture stimuli, suggesting that task-relevant information is more effectively relayed to M1 and S2 after learning. Decision-related signals are also found preferentially in S2p cells after training on a whisker detection task [24]. Thus, learning can affect sensory representations in
primary sensory cortex in an output pathway-specific way, which may help transfer task-relevant information to downstream areas related to choice and motor control.

1.9 Sites and mechanisms for changing population codes

These changes in population coding are likely to reflect a mix of classical and novel circuit plasticity mechanisms. Hebbian plasticity in local circuits will form and strengthen ensembles of coactive neurons, driven either by bottom-up sensory statistics, top-down inputs, or their interaction. This likely explains shifts and narrowing of sensory tuning for task-relevant sensory features. Pattern completion within Hebbian ensembles could explain increased response reliability. As demonstrated by Carrillo-Reid et al. [10], coactive ensembles are readily formed within existing structures of connectivity, and layered on pre-existing ensembles. Together, this local Hebbian plasticity will contribute strongly to improved population codes for discriminating relevant stimuli. Top-down input may contribute to reactivating learned ensembles, explaining why learned improvement of population coding is often strongest during task performance.

Other learning-related changes in sensory cortex population codes likely reflect plasticity that takes place in higher sensory, decision, or motor areas, and feeds back to sensory cortex via top-down projections. This may explain how perceptual learning increases choice and reward-related activity in primary sensory cortex [31], [24], and how task salience and attention modify the structure of noise correlations in sensory cortex [36], [37]. Learning could alter top-down activation of primary sensory cortex by changing stimulus representation in higher-order areas, thus changing spiking patterns in existing feedback connections, or by driving synaptic plasticity at feedback synapses in primary cortex.

Some population coding changes could also reflect learned changes in sensory strategy, mediated by plasticity in cognitive or motor areas controlling sensory behavior. For example, in both the Peron and Chen studies [22], [31], mice learned to optimize whisking behavior and to produce more consistent whisking during learning, presumably to extract relevant touch signals more efficiently. This change in active sensory exploration may have contributed to improved stimulus selectivity, reliability, and decreased day-to-day variability during learning. In Poort and Hofer [15], however, alterations in eye position, pupil size, or running speed could not explain improvements in sensory coding. Thus, plasticity in motor systems for active sensor control can affect sensory cortex population coding, and it is critical to monitor behavior precisely to assess this possibility.

Together, these findings indicate that experience-dependent changes in sensory cortex population codes reflect a mixture of plasticity in local cortical circuits, in ascending sensory pathways to cortex, in top-down projections carrying cognitive information, and even in distant motor pathways. In this sense, each local sensory area is a window onto large-scale brain plasticity. Monitoring plasticity in the different inputs to sensory areas
(e.g., by calcium imaging in specific populations of axon terminals), will be a critical step to unravel these different contributions to sensory cortex plasticity.
Figure 1. Four ways to adjust a population code in sensory cortex. A, Systematic changes in sensory tuning by single neurons. Each curve is tuning of a different neuron, along a sensory feature axis. Neurons shift or sharpen their tuning to better represent common or reinforced stimulus features. B, Changes in sensory gain and response reliability. Each circle represents activity of a neuron on a given trial (filled: spiking, open: not spiking). Subpopulations of neurons are tuned for stimulus 1 or 2. Before training, response gain and reliability are relatively low, leading to poor discrimination on the population level. During learning, gain and reliability to the reinforced stimulus increase, increasing reliability of the population code. C, Reduction in day-to-day tuning variability. Under normal sensory conditions, sensory tuning of individual neurons changes from day to day. During learning, this variability decreases, so that population decoding becomes more stable and accurate. D, Routing of information on output pathways from primary sensory cortex. Intermixed
subpopulations of neurons project to different output pathways. With learning, one subpopulation increases stimulus selectivity or responsiveness, thus routing information preferentially down one pathway.

2.10 Acknowledgments

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Tactile enrichment drives emergence of functional columns and improves population coding in L2/3 of mouse S1

Amy M. LeMessurier, Daniel E. Feldman

2.1 Introduction

Sensory experience drives plasticity that is critical for refinement of cortical sensory maps in postnatal development. Such plasticity has primarily been studied using sensory deprivation or by pairing specific stimuli with positive or negative reinforcement [1]. These approaches reveal substantial plasticity, but do not demonstrate how natural sensory experience transforms maps during development. An alternative is to study environmental enrichment, which increases the amount, complexity and salience of sensory stimuli. Enrichment promotes cortical plasticity and can refine tuning and map topography (S1: [2], [3]; A1: [4], [5], [6]).

A rich sensory environment may be particularly relevant in development of whisker somatosensory cortex (S1). In rodents, whiskers are the primary tactile sensors and are represented in S1 by a discrete, topographic array of cortical columns, each centered on a cell cluster (barrel) in layer (L) 4. Extracellular single-unit recordings suggested a precise functional topographic map in S1, in which nearly all neurons in each column are tuned for the anatomically corresponding columnar whisker (CW). In contrast, population calcium imaging studies that characterized the map in L2/3 at cellular resolution found a high proportion of neurons that responded better to a surround whisker (SW) than the CW, indicating a highly heterogeneous, salt-and-pepper somatotopic organization [7],[8]. In this organization, each whisker activates a broad region in S1 that is centered on its column, without rigid functional borders between columns [7], [8], and whisker-evoked activity spreads over substantial regions [9], [10]. Thus, functional activation of L2/3 of S1 may not obey columnar boundaries, in conflict with the columnar hypothesis.

Salt-and-pepper organization has been observed in rat and mouse V1 for orientation tuning and retinotopy [11],[12], and in rat A1 for frequency tuning [13], and thus has been suggested to be a defining feature of rodent sensory cortex [11]. However, these measurements were made in rodents reared in standard laboratory housing, which may be considered a deprived sensory environment. L2/3 is a particularly robust site of experience-dependent plasticity [14]. Thus, we wondered whether weak columnar organization and salt-and-pepper tuning structure in L2/3 of rodent sensory cortex may be a product of inadequate activity-dependent development.
We tested this question in whisker S1, by raising mice in either standard laboratory housing with only nesting materials and a single littermate, or in an enriched environment with novel tactile toys and an additional littermate (whisker contact is an important aspect of social interaction in rodents [15]), from P21 for 25-50 days. We then characterized sensory responsiveness, receptive field structure, and map organization using 2-photon calcium imaging in L2/3 or L4 excitatory cells, with layer-specific Cre lines and conditional expression of GCaMP6s. In control (CT) mice, we confirmed the existence of weakly-responsive, salt-and-pepper maps in L2/3, with a weak spatial gradient of tuning similarity and noise correlations that did not respect columnar boundaries. L4 had moderately more topographic precision. Enrichment increased tuning homogeneity for the CW, strengthened and sharpened the point representation of single whiskers, and strikingly created distinct functional columns measured by tuning similarity and noise correlations. These effects were mostly found in L2/3, not L4. Thus, enrichment dramatically sharpens functional columnar representations in L2/3 of S1, creating functional topography that better matches anatomical columnar structure.

2.2 Methods

Animals

All procedures were approved by the University of California Berkeley Animal Care and Use Committee and follow NIH guidelines. We used 17 Drd3-Cre mice (JAX), including 11 males and 6 females, and 11 Scnn1-Cre mice (JAX), including 4 males and 7 females (Table 1).

Enrichment protocol

Starting at weaning (P20-P21), littermates were separated into control and enriched cages. Enriched animals were housed with 2-3 littermates in enriched cages, while control animals were housed with a single littermate under standard laboratory conditions. Enriched cages were standard mouse cages (bedding, food, water, etc.) with the addition of toys/manipulanda which were swapped out every 2-4 days. Each set of toys included an enclosure for nesting, one other large toy, and several small toys (wood sticks, blocks of various shapes, etc.; Fig. 1A). All types of toys varied in materials, which included wood, ridged plastic, PVC, rubber, and rough cement. All toys were cleaned and sterilized between uses, either with chlorine bleach or in an autoclave. Neither enriched nor control animals received running wheels.

Surgery

We injected virus and implanted cranial windows and headplates 19 ± 5 days (Drd3-Cre mice) [16] or 34 ±11 days (Scnn1-Tg3-Cre mice) [17] before performing imaging experiments. During surgery, mice were anesthetized with isoflurane and kept at a body
temperature of 37°C. We affixed a stainless steel head-holder containing a 5 mm aperture to the skull, centered over S1. To target viral expression specifically to the D2 column and surrounding columns, we mapped the location of the D1, D2, and D3 columns using transcranial intrinsic signal optical imaging (ISOI). After making a 3 mm diameter craniotomy centered over D2, we delivered virus (AAV1-Syn-Flex-GCaMP6S-WPRE for expression in Drd3-cre mice; AAV9-CAG-Flex-GCaMP6s-WPRE for expression in Scnn1-Cre, (via Janelia GENIE/UPenn Viral Vector Core [18]) to three locations surrounding D2 at two depths each (~450 µm and ~350 µm ventral to the pial surface for L4 experiments; ~300 µm and 200 µm for L2/3), using a nanoinjector. The craniotomy was then covered with a 3 mm diameter #1 thickness (0.15 ± 0.02 mm) glass coverslip cemented to the skull with dental cement. Before surgery mice were given dexamethasone (2 mg/kg), meloxicam (10 mg/kg) and enrofloxican (5 mg/kg) administered intraperitoneally to reduce inflammation and prevent infection. Following surgery mice were given buprenorphine (0.1 mg/kg) intraperitoneally for analgesia.

**Imaging**

Because GCaMP expression developed over time, we checked expression levels each day starting about 2 weeks post-surgery and performed imaging experiments once the number of visibly-expressing cells in our target area changed minimally from day to day, but before many cells exhibited nuclear expression, which can lead to aberrant fluorescent responses [19]. 2-photon imaging experiments were conducted on a Sutter Moveable-Objective Microscope with one resonant scanner and one galvo scanner for scanning in X and Y respectively. We used a 16x, 0.8 NA water-dipping objective (Nikon). Excitation was delivered with a Coherent Chameleon Ti-Sapphire pulsed laser tuned to 920 nm. Fluorescence emission was filtered with a Chroma HQ 575/50 filter and detected with a Hamamatsu photomultiplier tube (H10770PA-40). Single Z-plane images were collected at a frame rate of 30 Hz for 512x512 pixels using ScanImage [20].

During imaging, mice were anesthetized using a low concentration (<1%) isoflurane in oxygen combined with intraperitoneally-injected chlorprothixene (0.08 mg). We imaged activity through cranial windows in single planes in ~400 µm² at depths between ~100-250 µm ventral from the dura. Mice were kept at a body temperature of 37°C using a closed-loop heating pad (Harvard Apparatus). During imaging we used an array of 9 piezo-electric actuators to individually deflect 9 whiskers on right whisker pad, including D2 and the surrounding whiskers. Whiskers were kept intact, and were threaded through and glued to tubes attached to the piezos, positioned at approximately 5 mm from the face to avoid disturbing facial hair. Non-stimulated whiskers were isolated from the piezo array using a plastic shield enveloping the piezos. Each stimulus train consisted of 5 300 µm-amplitude rostro-caudal deflections spaced 100 ms apart. Each individual deflection had rise and fall times of 2 ms (from resting position to 300 µm caudal), and a duration of 10 ms. We used trains of deflections, rather than single deflections, to increase the probability of multi-spike responses which would be more easily detectable with GCaMP (Fig. 1B). Whiskers were stimulated in pseudo-random
order 5 seconds apart to minimize short-term plasticity/adaptation. We also interleaved “blank” trials in which no whisker was deflected to measure baseline activity. Each whisker was deflected 60 ± 10 times. For each experiment we collected approximately 40 80-second long movies to acquire 50-70 total repetitions of each stimulus.

Histology and imaging field localization

Following imaging experiments, we performed histology to recover the location of each imaging field relative to each of the 9 barrels corresponding to the stimulated whiskers. After image data acquisition, we collected Z-stacks projecting dorsally from the imaging field location to the dural surface in order to document the location and appearance of blood vessels above each imaging field. We also collected wide-field fluorescence images of the surface blood vessels above the imaging field under 1p excitation. We then created small lesions of approximately 50 µm² near the surface of the brain by scanning a small area for ~1 minute continuously at high power and 800 nm. Following lesion creation, animals were sacrificed and brains were fixed in 4% paraformaldehyde. To obtain tangential cortex sections, the cortex was removed and flattened before being sectioned at 50 µm thickness on a freezing microtome. We performed cytochrome oxidase staining to visualize the barrels, and matched 2photon images to histological sections using patterns of blood vessels in superficial sections as well as the lesion. We then projected the boundaries of the barrels onto the imaging field using Adobe Photoshop and used a custom MATLAB program to measure the X-Y position of each ROI in the imaging field relative to the centroids of the 9 barrels corresponding to the stimulated whiskers.

Analysis

All data analysis was conducted in MATLAB using custom-written routines unless otherwise noted.

Image processing and ROI definition

Imaging data was corrected for slow XY motion in Matlab using dftregistration ([21], matlab file exchange). We did not observe substantial motion in the Z axis. Registered fluorescence stacks were smoothed in the time axis with a moving-median filter across 4 frames. Neuronal regions-of-interest (ROIs) were identified manually, by selecting ellipsoid ROIs over somata that appeared in all movies. Activity was quantified for each ROI as the mean fluorescence on each frame within this ROI. Neuropil ROIs were created individually for each soma as a 5 pixel-wide ring beginning 2 pixels away from the somatic ROI. Any pixels within this ring that were correlated with any soma ROI with a correlation coefficient > 0.2 were removed, and the mean fluorescence of the remaining pixels was measured for each frame. For L4 ROIs, the neuropil mask raw fluorescence was scaled by 0.3 and subtracted from the somatic raw fluorescence. Neuropil subtraction was not performed for L2/3 ROIs. Fluorescence time series for all individual 80 sec movies and identified ROIs were inspected manually to remove movies in which brightness decreased by more than ~10% from the average across
movies (for example, due to loss of water meniscus under the objective lens). Fluorescence time series were then converted to \( \Delta F/F \), computed for each ROI within each 80 second movie as \((F_t-F_0)/F_0\), in which \( F_0 \) is the 20\(^{th}\) percentile of fluorescence across the entire movie and \( F_t \) is the fluorescence on each frame.

**Whisker response and receptive field characterization**

Whisker-evoked activity on each trial was measured as the mean \( \Delta F/F \) during a 1 second period following each stimulus onset, from which we subtracted the mean \( \Delta F/F \) in the 0.5 seconds prior to the stimulus (Fig. 1C). The average 9-whisker receptive field for each ROI was quantified as the median evoked \( \Delta F/F \) to each whisker over all stimulus repetitions (Fig. 1D). We considered an ROI responsive to a whisker if the distribution of evoked \( \Delta F/F \) was significantly greater than spontaneous activity, measured as the distribution of mean \( \Delta F/F \) on blank trials. Significance was computed using a permutation test of difference in means: for each whisker, a vector of mean \( \Delta F/F \) following each stimulus iteration was combined with mean \( \Delta F/F \) on each blank trial (using the same frames as for measuring evoked activity). The combined distribution of means was split into two groups, and the difference in means of the two groups was measured. This was repeated 10,000 times, and the actual difference between the stimulus and blank distributions was compared to the distribution of permuted differences in means. A difference was considered significant if it was greater than the 95\(^{th}\) percentile of the permuted distribution. P values for each of the 9 whiskers were corrected for multiple comparisons by False Discovery Rate [22]. An ROI was considered whisker responsive if it was significantly responsive to at least one whisker above baseline activity.

**RF/whisker response normalization**

To compare whisker responses across multiple ROIs from multiple fields, in which GCaMP expression – and thus absolute measures of fluorescence intensity and \( \Delta F/F \) – were variable, we normalized whisker receptive fields within each ROI using the following procedure: for each whisker, the median was computed across all stimulus trials, then z-scored to the median \( \Delta F/F \) on blank trials.

**ROI position clustering**

For measurements made by position relative to column centers (Figs. 2E,4B,4D,5G) we rotated each barrel position and ROI locations relative to each barrel into the same orientation in order to compare across imaging fields and barrels. Each column center was coded as the origin, and ROI positions were coded in relation to each origin. For binned Voronoi plots, ROIs were combined into one population across columns and imaging fields, then binned based on position using k-means clustering constrained so that each bin contained the same number of ROIs. Averages were then computed by position bin.

**Signal and noise correlation analysis**

To quantify receptive field similarity between pairs of neurons, we computed signal correlations between all pairs of simultaneously imaged, whisker-responsive neurons.
Signal correlations were computed by creating a vector for each neuron composed of its mean response to each whisker over all stimulus repetitions, Z-scoring within this vector, and computing the Pearson’s correlation of these vectors between pairs. We also computed noise correlations between all pairs of simultaneously-imaged neurons as a measure of trial-by-trial response co-variability. For each neuron, we constructed a vector for each whisker containing the mean ΔF/F during the evoked window following each stimulus iteration. Each vector was individually z-scored. We computed the Pearson’s correlation for each pair of neurons separately for each stimulus, then computed the mean correlation for all whiskers.

We quantified the distance between each neuron pair as the Euclidean distance based on pixel location, then converted this to µm. Neuron pairs were considered co-columnar if they were within the boundaries of a barrel outline projected onto the imaging field. Cross-column pairs were all pairs separated by at least one column boundary.

**Neural Decoder**

We constructed two neural decoders – one to detect a whisker deflection compared to spontaneous activity, and one to discriminate stimulus identity among 9 whiskers - from single-trial mean ΔF/F of individual ROIs and ensembles of ROIs. For the discrimination decoder, each ROI was represented by a one-versus-all (OVA) classifier that was trained by logistic regression to report the probability of each stimulus given the mean ΔF/F during the evoked window following a single whisker deflection, selected randomly from each stimulus iteration (structured as in [23]). Each classifier was composed of 9 logistic functions, one for each stimulus. For each logistic function coefficients were fit by logistic regression and K-fold cross validation to relate the mean ΔF/F on each trial to the probability of given whisker having been deflected. Each iteration of model fitting was performed on 80% of trials, and performance was assessed on the remaining 20% of trials. For single ROI decoders, predictions were made by selecting the highest probability whisker on a given trial. The fitting and testing process was repeated 500 times for each single ROI decoder, and performance was averaged across iterations.

For ensemble decoding, simultaneously-imaged ROIs were clustered into ensembles either randomly or based on position within the imaging field using k-means clustering. To sample a range of ensemble sizes and positions, clustering was performed for each field while varying the number of ensembles between 2 to as many ensembles as responsive ROIs. This was repeated separately for each whisker, and positions used for clustering were normalized relative to the reference whisker. This yielded ensemble sizes of 2:42, with 4242 ± 1400 total ensembles per CT field, and 9630 ± 1501 ensembles per EN field. For ensemble decoders composed of random sets of ROIs, we trained and tested ensemble decoders of 1, 2, 3, 5,10, 15, 20, 25, and 30 ROIs. For each ensemble size, we tested the lesser of 500 or NchooseK ensembles (where N is the total number of responsive ROIs in the field and K is the ensemble size) randomly-drawn from each imaging field. We used a maximum of 30 ROIs in order to sample as many imaging fields as possible, since the number of responsive ROIs varied across
fields and several fields contained fewer than 30 responsive ROIs. To predict single-trial stimuli from ensembles, the output of each ROI classifier was normalized so that each unit had the same weight in population decoding. The population stimulus prediction was calculated by summing the probabilities of each stimulus over all units in an ensemble and selecting the stimulus with the maximal summed probability.

The detection decoder was constructed identically to the discrimination decoder, except that logistic functions were trained to discriminate one whisker from spontaneous activity, rather than one whisker vs. all other whiskers.

2.3 Results

Whisker map topography among excitatory neurons in L2/3 and L4

Because prior imaging studies of whisker map topography in L2/3 did not distinguish between excitatory and inhibitory cell types, map topography among pyramidal cells is not clear [8], [7]. In addition, whether L4 has similarly heterogeneous tuning as in L2/3, or contains a more precise, locally homogeneous map, as suggested by anatomical clustering of whisker-related thalamic afferents into segregated barrel, is not known. To address these questions, we measured whisker receptive fields and map topography using 2-photon calcium imaging of GCaMP6s expressed either in L2/3 pyramidal (PYR) cells or L4 excitatory cells, using Drd3-Cre or Scnn1a-Tg3-Cre mice, respectively [16],[17]. GCaMP6s was expressed using a Cre-dependent viral vector injected into a region centered on the D2 whisker column, localized by intrinsic signal optical imaging (ISOI), and a chronic cranial window was implanted. 19 ± 5 days later (for L2/3 imaging in Drd3-Cre mice) or 34 ±11 days later (for L4 imaging in Scnn1-Tg3-Cre mice), mice were lightly anesthetized with isoflurane and chlorprothixene, and neural activity was imaged through the cranial window. For L2/3 imaging, we imaged ~400 µm^2 fields, which contained ~50-100 GCaMP6s-expressing neurons. For L4 imaging, we imaged smaller fields of ~300 µm^2 to increase the signal-to-noise for each ROI, since imaging deeper in the cortex results in more tissue scattering than at shallower depths. Thus, L4 fields tended to have fewer cells than L2/3 fields. 1-2 fields were imaged in each mouse, several days apart.

In each imaging session, we randomly interleaved deflections of 9 whiskers in a 3 x 3 grid centered on D2, plus no-stimulation ‘blank’ trials, using a 5-pulse train (100-ms interval) of rostrocaudal deflections for each whisker, and 5 sec intervals between whiskers. This yielded 50-70 repetitions of each stimulus. Trains are necessary due to sparse spiking in L2/3 [24], [25], and increase the likelihood of eliciting detectable GCaMP signals. After imaging was complete, the brain was removed and processed histologically to reveal the barrels in L4, and imaged neurons were localized relative to barrel column boundaries by alignment using surface blood vessels (see Methods). Whisker-evoked responses were analyzed in manually drawn regions of interest (ROIs). An ROI was considered significantly whisker-responsive if the distribution of whisker-
evoked ΔF/F (measured in a 1-sec window after whisker deflection onset) was greater than observed on blank trials (permutation test, alpha = 0.05, False Discovery Rate correction for comparison across 9 whisks). In control (CT) mice, 47.6% of L2/3 ROIs and 60.3% of L4 ROIs were significantly responsive to at least one whisker. All analyses were limited to whisker-responsive ROIs, unless otherwise noted.

We observed heterogeneous whisker tuning within each column among L2/3 PYR cells in CT mice (Fig 2A, Fig S1A). For each cell, we defined its Best Whisker (BW) as the whisker that evoked the largest median response. We calculated the fraction of cells tuned to a given whisker within spatial bins relative to the center of that whisker’s anatomical column. In L2/3, the fraction of cells tuned to one whisker (e.g. cells for which this whisker was the BW) was 49.5% (50/101 cells) in the bin at the center of the anatomical column for that whisker (0-91 µm). This fraction fell off gradually with cortical distance to 26.3% (132/502) of cells located 182-273 µm from the column center, and 2.6% (14/545) of cells located 455-546 µm from the center. In L4, the fraction of CW-tuned cells was 61.9% (39/63 cells) at the column center, which was statistically equivalent to the precision in L2/3 (p=0.15, Fisher’s Exact Test). However, this fraction fell off more sharply with distance in L4 compared to L2/3 (Fig. S1b), with 12% of L4 cells located 182-273 µm from the column center being tuned to the reference whisker (21/175 cells, p=0.0001, Fisher’s exact test), and no cells located 455-546 µm from the column center (0/98 cells, p=0.14, Fisher’s exact test).

A second analysis was designed to account for statistical uncertainty in identifying the BW given the limited number of stimulus repetitions. We identified, for each cell, all whiskers that evoked a mean ΔF/F that was statistically indistinguishable from the BW (assessed by permutation test of difference in means, alpha<0.05, False Discovery Rate correction for multiple comparisons). These whiskers were termed equal best whiskers (EBWs). In L2/3 of CT mice, 62.4% (63/101) of cells at the center of a column had the CW as an EBW. This fell to 48.6% (244/502 cells) at 182-273 µm and 14.1% (77/545 cells) at 455-546 µm away from column center (Fig. 2B). In L4, within 91 µm of a column center, 73.0% (46/63, p=0.18, Fisher’s exact test vs. L2/3) of excitatory neurons were somatotopically tuned, dropping to 24.6% (43/175 cells, p=0, Fisher’s Exact Test) at 182-273 µm and 1.0% (1/98 cells, p=0, Fisher’s Exact Test) at 455-546 µm (Fig. 2B). Thus, the whisker map was locally heterogeneous among L2/3 PYR cells in CT mice, and was spatially sharper among L4 excitatory cells.

**Enrichment decreases tuning heterogeneity in the salt-and-pepper map**

We developed a home-cage enrichment paradigm to examine whether environmental enrichment affects somatotopic map structure and receptive field heterogeneity in L2/3 or L4. Enriched mice (EN) were separated from their CT littermates at weaning, and housed with at least 2 littermates (compared to CT mice, which were housed with 1 littermate). EN mice were exposed to a variety of toys which were swapped out every 2-3 days to encourage exploration (Fig. 1A). We conducted imaging experiments in EN
mice after at least 3 weeks of enrichment. CT mice received no toys or manipulanda beyond their normal wood chip bedding and nesting materials.

Enrichment altered tuning heterogeneity in L2/3. In EN mice, the percentage of L2/3 cells tuned equivalently to the CW was 80.0% (168/210 cells) in the spatial bin at column center (0-91 µm), which is higher than the 62.4% in CT mice (p=0.0013, Fisher’s Exact Test) (Fig. 2C). With distance from the column center, the fraction of CW-tuned cells fell similarly in both groups, to 14.5% (EN: 164/1129 cells) and 14.1% (CT) in the 455-546 µm bin (p=0.88, Fisher’s Exact Test). Thus, enrichment increased tuning precision for cells near column centers. We also analyzed whether tuning precision was increased for all cells located within column boundaries (irrespective of absolute distance to column center). In EN mice, 74.7 ± 5.1% of whisker-responsive cells were tuned equivalently to the CW, compared to 57.7 ± 9.1% in CT mice (Fig. 2D). This difference was significant in the bulk population (77.1% or 468/607 cells in EN; 65.1% or 224/344 cells in CT; p=0.00008, Fisher’s Exact Test), but when analyzed across individual columns was only a non-significant trend (p=0.098, 2-tailed t-test). Thus, enrichment improves tuning homogeneity within each column, but by a modest amount relative to the endogenous variability across columns.

Despite the general expectation that L4 is less plastic than L2/3 [26],[14], we observed similar effects of enrichment on tuning accuracy in L4. The percentage of L4 cells tuned equivalently to the CW in the central spatial bin (0-91 µm) was 92.2% (71/77 cells) in EN mice, which was higher than the 73% in CT mice (p=0.0028, Fisher’s Exact Test). But this fell off normally with spatial distance outside the column, to 4% (8/204 cells) at 455-546 µm from the column center (compared to CT, p=0.28, Fisher’s Exact Test, Fig. 2C). Analyzing all responsive cells within a column, there was again a non-significant trend for a higher fraction of cells to be equivalently tuned to the CW (91.6 ± 5% in EN; 80.7 ± 9% in CT; p=0.3138, 2-tailed t-test, Fig. 2D). As in L2/3, this difference was significant in the bulk population (89.3% or 133/149 cells in EN; 75.6% or 65/86 cells in CT; p=0.0086, Fisher’s exact test). Thus, enrichment also increased tuning precision in column centers in L4.

**Enrichment sharpens whisker tuning curves**

We next examined whether enrichment affected receptive field sharpness for individual neurons. In order to compare whisker response magnitudes across ROIs and imaging fields, in which different levels of GCaMP expression can lead to different spike-to-fluorescence transfer functions [19], we normalized whisker responses by z-scoring each cell’s median whisker responses to its mean baseline activity during blank stimuli (methods). We quantified receptive field structure by ranking each cell’s baseline-normalized whisker responses from largest to smallest. In L2/3, enrichment sharpened receptive fields by increasing the average response to the strongest 3 whiskers while reducing responses to the weakest 4 whiskers, compared to control cells (Fig. 3A). When columnar whisker responses were tracked separately, we found that enrichment
increased the CW response and best 2 SW responses (Fig. 3B). This demonstrates that the magnified responses to the best 3 whiskers in EN mice reflected in part an enhanced response to the CW (Fig. 3B). These effects were also evident in the bulk distributions of responses to the CWs, SWs, and BWs (Fig. 3E).

In L4, whisker receptive fields were also sharpened in EN cells compared to CT cells. However, unlike in L2/3, enrichment sharpened receptive fields by decreasing responses to most SWs, while CW responses were unchanged relative to CT cells (Fig. 3B, 3F). In particular, responses to the best SW were largely reduced in EN compared to CT (Fig. 3B). Thus, enrichment sharpens receptive fields in L4 specifically for the CW by reducing responses to SWs.

Previous research has found that enrichment applied in adult rats shrinks whisker receptive fields in L2/3, but not L4 [3]. In contrast, we found that juvenile enrichment in mice expands L2/3 receptive fields: 74% of whisker-responsive ROIs in EN mice responded to two or more whiskers above baseline, compared to 63% in CT (Fig. 3C) (p=0.01864, 2-tailed t-test). Additionally, a greater proportion of EN L2/3 cells were equivalently tuned to more than one whisker (65%) than in CT (53%, p=0.0393, 2-tailed t-test) (Fig. 3d). Spontaneous activity was also reduced in EN compared to CT (Fig. 3E) (EN: 0.082 ± 0.001, CT: 0.092 ± 0.002; p<0.00001, permutation test). This reduction in spontaneous activity could partially account for the increased receptive field size in EN, because we considered a whisker to be part of a cell’s receptive field if it elicited a response significantly greater than baseline activity; however, the increased RF size does not reflect a uniform shift in gain across all whiskers, since responses to the 4 worst ranked whiskers were reduced in EN compared to CT.

In L4, in both EN and CT the majority of cells were tuned to a single whisker, rather than multiple equal best whiskers (Fig. 3D) (CT, 64.5%; EN, 84.0%; p=0.1111, permutation test). However, in contrast to L2/3, RFs (e.g. the number of whiskers evoking significant responses) in EN L4 cells were on average smaller than in CT: 72.4% of EN cells were responsive to a single whisker, compared to 33.9% in controls (Fig. 3C) (p<0.0001, permutation test). Unlike in L2/3, there was no difference in spontaneous ΔF/F (Fig. 3E). These findings suggest that enrichment has opposite effects on receptive field size in L2/3 and L4, but the net effects of these changes in both layers lead to sharpening.

**Enrichment sharpens the point representation of a single whisker in L2/3, but not L4**

Whisker map topography is best assessed by quantifying the point representation, i.e. the spatial distribution of activity elicited in S1 by deflection of one whisker. Stimulation of one whisker evoked a spatial peak of activity in L2/3 of S1, with the maximum response at the center of that whisker’s anatomical column, and the mean response magnitude to each whisker falling off sharply with increasing distance from the column center. In CT mice, the mean baseline-normalized whisker response within 91 µm of a column center was 0.36 ±0.03, decreasing to 0.14 ±0.01 for cells between 455 and 546
µm from the column center (Fig. 4A). For comparison, the average anatomical column radius (defined between midpoint of septa in L4) was 147 ± 11 µm. In EN, whisker responses also decayed with distance from the center of a column, but were significantly greater than in CT near the center of a column: within 91 µm of a column center, the mean baseline-normalized response was 0.47 ± 0.02. Responses were larger in EN compared to CT within 364 µm of a column center (Fig. 4A) (p<0.0002 at all bins <364 µm), but equivalent at greater distances. Thus, enrichment sharpened single-whisker point representations by increasing responses to a whisker within and closely surrounding its anatomically corresponding column. This effect was also evident from 2d maps of mean whisker responses in cells clustered by position relative to an orientation-normalized reference column (Fig. 4B). This is consistent with the increased CW and best two SW responses observed for individual neurons (Fig. 3).

Whisker responses in L4 at the column center were greater than in L2/3, and they fell off more rapidly with distance. Due to differences in spatial sampling with respect to columns in L2/3 and L4, we quantified the point representation in L4 over slightly smaller distance bins. In CT mice, the mean response within 62 µm of the column center was 0.62 ±0.08 and decreased to 0.04 ± 0.01 between 496 and 558 µm from the column center (Fig. 4C). However, unlike in L2/3, enrichment did not change the peak or spatial profile of the point representation in L4 (Fig. 4C). The 2d spatial profile of whisker responses was largely equivalent in EN and CT, suggesting that enrichment did not affect the point representation of single whiskers in L4 (Fig. 4D). Thus, enrichment sharpens the point representations of individual whiskers in L2/3, but not L4, of barrel cortex by enhancing responses to each whisker within and surrounding its corresponding column.

**Enrichment strengthens functional column boundaries in L2/3**

Anatomical columns are prominent in whisker S1, but in the published salt-and-pepper maps, there is no functional boundary that occurs at specific, spatial column boundaries [7],[27]. To test whether enrichment may drive functional segregation at anatomical column boundaries, we examined two measures of functional similarity between pairs of neurons – signal correlations, which are a measure of tuning similarity, and noise correlations, which measure trial-to-trial variability in activity that is independent of the stimulus, and which are thought to reflect shared functional synaptic input [28,29]. We compared correlation strengths for simultaneously imaged pairs within columns versus pairs across column boundaries (Fig. 5A,B,D). In L2/3 of control mice, signal correlation between neuron pairs located within the same anatomical column fell off modestly as a function of distance between neurons, consistent with the known preference for different SWs at different edges of a column [27]. Signal correlations were modestly lower on average for pairs across columns, and also decreased with distance between neurons. Enrichment modestly increased signal correlations within a column, particularly for cells located far apart within the column (CT,0.637 ± 0.004; EN, 0.659 ± 0.002; p<0.0001, permutation test). Enrichment powerfully reduced signal correlations across columns, even for cells located just ~100 µm apart (CT,0.541 ± 0.004; EN, 0.425 ± 0.004;
p<0.0001, permutation test). As a result, enrichment strongly enhanced the columnar organization of whisker tuning, by making whisker tuning within each column more homogeneous and distinct from other columns.

Noise correlations also exhibited different spatial structures for intra-columnar pairs versus cross-columnar pairs (Fig. 5A,C,E). In both EN and CT columns, noise correlation strength decreased with increasing inter-cell distance, but were modestly reduced across distances in EN (CT, 0.334 ± 0.002; EN, 0.307 ± 0.001; p<0.0001, permutation test). In contrast, cross-column noise correlation strengths in EN pairs were much lower than their intra-column counterparts across distances, while CT cross-column noise correlations were equivalent to within-column pairs (CT, 0.327 ± 0.002; EN, 0.255 ± 0.001; p<0.0001, permutation test). Thus, in addition to reducing tuning similarity between columns, enrichment also reduced trial-by-trial noise correlations in activity between neighboring neurons, especially across columns.

These results indicate that location relative to a column influences tuning and activity correlations in L2/3 neurons, and that enrichment enhances this spatial structure. To confirm this, we measured each neuron’s mean signal and noise correlations to populations of neurons based on position relative to a column. For each ROI, we quantified its mean signal and noise correlations to all ROIs located within each column and compared this to mean correlations to all ROIs located outside of each column. In both EN and CT, neurons had higher mean signal correlations with neurons located in the same column compared to neurons outside of the column (Fig. 5F,H). However, in EN, mean signal correlations were much lower to neurons outside of a column than in CT. Mean noise correlations exhibited a similar relationship with location relative to a column (Fig. 5G,I): in both groups, mean correlation values were higher for neurons located within the same column compared to neurons located outside of the reference neuron’s column. In EN, however, mean noise correlations were lower than CT for both within-column neurons and out-of-column neurons (CT in column: 0.354 ± 0.013, out of column: 0.301 ± 0.001; EN in column: 0.314 ± 0.007, out of column 0.260 ± 0.004; in column p<0.0001, out of column p<0.0001). These findings reinforce the conclusion that enrichment enhances functional boundaries between columns in L2/3, both by increasing tuning correlations within columns and decreasing noise correlations, especially between cells located across column boundaries.

In L4, very few simultaneously-imaged pairs of ROIs were located across column boundaries, so we restricted our analysis to pairs within columns (Fig. 6A). In contrast to L2/3, signal correlations did not decrease significantly with increasing distance between pairs of co-columnar cells (Fig. 6B). Across inter-ROI distances, signal correlations were higher in EN compared to CT (CT, 0.681 ± 0.011; EN, 0.774 ± 0.005; p<0.0001, permutation test). (Fig. 6B,D). Noise correlations decreased with increased inter-ROI distance in both EN and CT, and in contrast to L2/3, noise correlations were much larger in EN compared to CT (CT, 0.241 ± 0.006; EN, 0.356 ± 0.002; p<0.0001, permutation test). (Fig. 6B,E). Thus, unlike in L2/3, enrichment increased both signal and noise correlations within column boundaries in L4. This suggests that the
enrichment-induced changes we observed in L2/3 and L4 may result from distinct circuit mechanisms.

**Enrichment improves population coding of columnar whisker deflections**

The effects of enrichment that we observed indicated that whisker representations were strongly altered within S1; however, it is unclear whether these changes substantially impact the stimulus information present in S1 on single trials. To test whether enrichment impacts coding of whisker deflections, we constructed two neural population decoders that predicted detection of whisker deflections and discrimination of whisker identity from single-trial ΔF/F. Because our dataset sampled L2/3 much more densely than L4, we limited the decoding analysis to L2/3. To detect whisker deflections (e.g. to discriminate a whisker deflection from spontaneous activity), each ROI was modeled by a set of 9 independent classifiers that predicted the probability of a given whisker being deflected based on evoked ΔF/F on single trials selected at random from every trial for that whisker. Each classifier was trained by logistic regression on a random subset of trials of a single whisker deflection and blank trials, and the output was the probability that a whisker was deflected on a single trial (Fig. 7A). Performance was quantified as the percent correct predictions on all stimulus and blank trials. On average, single-ROI decoders from EN mice were slightly better than CT at predicting deflection of their CWs (Fig. 7B; EN mean proportion correct: 0.65 ± 0.004, CT mean proportion correct: 0.63 ± 0.005; p=0.004, permutation test of difference in means). This effect is likely due to the reduced spontaneous activity and increased CW responses that we observed in L2/3 with enrichment, and reinforces the conclusion that signal-to-noise for whisker detection is improved in L2/3.

To make predictions from populations of ROIs, probabilities were summed across single-ROI decoders, and the trial type (whisker deflection or blank) was predicted by the highest probability. Ensembles of 2-30 ROIs were selected at random from simultaneously-imaged ROIs (e.g., within imaging fields). Performance improved with increasing ensemble size in both CT and EN. For ensembles of 1 and 2 ROIs, performance was better in CT than EN (p<0.001 for 1 and 2 ROI ensembles; permutation test of difference in means) though performance was greater in EN for all ensemble sizes >3. (Fig. 7C; p<0.001 for ensembles of 3,5,10,15,20,25, and 30 ROIs; permutation test of difference in means). This suggests that smaller ensembles in EN can encode the same information about whisker detection as larger ensembles in CT.

We measured detection performance as a function of ROI location relative to the column for a given whisker. Single ROI decoder performance for each whisker was highest for ROIs closest to the corresponding column center, and decreased with distance. Performance was slightly higher in EN within 91 µm of a column center (EN proportion correct: 0.66 ± 0.004, CT: 0.63 ± 0.01; p=0.046, 2-way ANOVA), but otherwise equivalent to CT. Performance reached a minimum in both groups ~600 µm from column center (Fig. 7D; EN minimum proportion correct: 0.51 ± 0.005, CT: 0.51 ±
To assess population decoding performance with columnar topography, we constructed a spatially-clustered version of the population decoder in which ROIs were clustered into ensembles based on position within an imaging field. For each imaging field, ROIs were clustered by k-means clustering. We varied the number of clusters within each imaging field from 2 to the total number of responsive ROIs in the field in order to sample a range of ensemble sizes. We then binned performance by distance from the centroid of the ensemble to the reference column center. As with single-ROI decoders, performance was higher in both groups near the center of a column and fell with distance. Performance was greater in EN within 154 µm from a column center (EN maximum proportion correct: 0.72, CT: 0.66; p=2 * 10^{-6} for comparisons of ensemble performance within 77 µm of column center and at 77:154 µm, 2-way ANOVA) and fell off more sharply with distance than in CT (Fig. 7E; EN proportion correct at 308:385 µm: 0.58, CT: 0.61; p=2 * 10^{-6}, 2-way ANOVA). The altered spatial profile of detection performance of these decoders reinforces our conclusion that point representations were spatially sharpened following enrichment, and suggests that the topography of populations codes for whiskers are affected on individual trials.

We constructed a second decoder to examine whether enrichment affected discrimination of individual whiskers from population activity in S1. As with the detection decoder, individual ROIs were modeled independently by 9 logistic regression-based classifiers. Rather than discriminating a specific whisker from spontaneous activity, each classifier was trained to discriminate a single whisker from the 8 other whiskers. Each logistic function was trained on a random subset of deflections of a single whisker and deflections of the other 8 whiskers combined into one group. Thus, the output of each logistic function was the probability that a specific whisker was deflected versus all other whiskers on a test trial, and the output of each classifier was 9 probabilities (one for each whisker) for a given test trial, normalized to sum to 1. For population decoding, probabilities were summed across all ROIs in a decoding ensemble, and the prediction was the whisker with the highest probability (Fig. 7F). Discrimination decoders were identical to detection decoders in all other aspects.

We quantified single-ROI decoder performance on discrimination of the CW. Performance was equivalent in EN and CT, and fewer ROIs performed significantly above chance compared to performance on detection (Fig. 7G; EN mean performance 0.56 ± 0.003, CT mean performance 0.55 ± 0.004; p=0.38, permutation test of difference in means). Similar to detection performance, population discrimination performance increased in both groups with increasing ensemble size when ROIs were grouped randomly within imaging fields. For ensembles >2 ROIs, EN performed better than CT (Fig. 7H; p=0.0001 for all ensemble sizes >2, permutation test of difference in means). As with the spatially distributed detection decoder, this effect suggests that smaller ensembles in EN can encode the same information for discriminating whiskers as larger ensembles in CT. However, we did not see this effect at the single cell level.
Performance of both single ROI decoders and spatially-clustered ensemble decoders was highest for each whisker near the center of the corresponding column, and decreased sharply with distance until approaching chance performance (0.5) around 500 µm from column center (Fig. 7I, J). For single-ROI decoders, performance was equivalent in EN and CT at all distance bins (Fig. 7I; p>0.05 for all comparisons, 2-way ANOVA). Ensemble decoders within 77 µm from column center performed slightly better in EN compared to CT (EN proportion correct: 0.61, CT proportion correct: 0.59; p=2.3 × 10⁻⁶, 2-way ANOVA). In EN, performance increased above minimum performance (0.51 for ensembles at 539:616 µm) for ensembles >693 µm from column center (Fig. 7J, p<0.001 for all bins >616 µm compared to minimum performance, 2-way ANOVA). Performance was also better in EN compared to CT for ensembles >692 µm from column center (p=2 × 10⁻⁶ for all comparisons, 2-way ANOVA). Thus, ensemble discrimination performance was slightly improved for individual whiskers for some decoding ensemble positions. However, this effect was smaller than the improved performance of the spatially-clustered detection decoder, suggesting that the altered topography we observe has a stronger impact on the organization of population codes for whisker detection on single trials compared with whisker discriminating.

2.4 Discussion

Whisker tuning is heterogeneous among excitatory cells in both L2/3 and L4

Previous research has shown that tuning for whiskers is heterogeneous at the sub-columnar scale in L2/3, with as few as 25% of cells tuned for their anatomical columnar whisker [8], [7]. These studies did not restrict imaging by cell type, so the “salt-and-pepper” tuning that was observed could have been due to differences in tuning between excitatory and inhibitory cell types. We limited our study to PYR cells. Our estimate of the somatotopically-tuned proportion of PYRs in L2/3 was higher than previously-reported non-cell type-specific estimates, but we did observe a substantial proportion of PYRs tuned to non-columnar whiskers. L2/3 PYRs tend to respond to multiple whiskers [27], and we found that 65% of ROIs in L2/3 were tuned to more than one EBW. To adjust for this, we considered ROIs for which the CW was an EBW to be CW-tuned. With this adjusted measurement, we found that a higher percentage of ROIs were CW-tuned. Thus, even when analyzing purely PYR cell tuning, and even using the EBW-based analysis, salt-and-pepper organization exists in L2/3, with 35% of PYR cells in each column not tuned for the CW.

In V1, orientation tuning exhibits equally heterogeneous salt-and-pepper organization in both L2/3 and L4 [30]. However, in A1, tonotopic organization appears to be more topographic and locally homogeneous in L4, becoming more salt-and-pepper in L2/3 [31]. In S1, highly topographic projections from VPM thalamic barreloids to the corresponding L4 barrel suggest that L4 may exhibit more somatotopic precision.
Consistent with this, extracellular spike recording in L4 suggests strong somatotopy (e.g., [32],[33]). Our imaging data from L4 excitatory neurons in Scnn1a-Cre mice revealed that salt-and-pepper heterogeneity exists in L4, with 25% of neurons tuned for a non-CW whisker. This proportion is higher than estimates from extracellular spike recordings. However, we found that tuning was much more topographically precise in L4 compared to L2/3, with the proportion of CW-tuned ROIs falling off more sharply with distance. As with L2/3, our estimate of the proportion of somatotopically-tuned cells increased when we took into account cells that were equivalently tuned to multiple whiskers, though many fewer cells were tuned (or even responsive to) multiple whiskers.

Our estimates of CW tuning might be different from extracellular recording estimates due to less (or differently) biased sampling, since single-unit extracellular recordings tend to be biased toward the most active cells. In addition, the prominence of FS units in L4 extracellular recordings [34] means that tuning estimates may be biased toward FS cells, which average synaptic input across nearby PYR cells [35] and thus may have more consistent columnar tuning. This difference may also be due to the stimulus we used. Most studies of whisker receptive fields have used single deflections of (e.g. [36]), but we used trains of 5 deflections to increase the number of spikes per trial to increase the probability that responses could be detected with GCaMP6s and measured evoked activity over a relatively long time post stimulus. With increasing time post whisker deflection, excitation spreads laterally across columns [37]. Thus, L4 responses to non-columnar whiskers may reflect longer latency activity than what has typically been included in receptive field measurements made with extracellular spike recordings.

**Enrichment decreases tuning heterogeneity in the salt-and-pepper map**

Somatotopy in S1 develops in an experience-dependent manner and changes with manipulations of input to the whiskers. In juvenile rodents, depriving input to subsets of whiskers drives robust changes in somatotopy in L2/3 [14]. Previous enrichment studies have found diverse effects in sensory responses, tuning, and maps in S1, especially concerning the extent of overlap in representations of the whiskers and digits. One study [2] found that enrichment in young adult rats led to a finer-grained map of cutaneous input to the forepaw. Enrichment studies in adult barrel cortex have found less overlapping representations of the whiskers following enrichment [3], while others have found larger areas responding to multiple whiskers [38]. These studies mapped somatotopy using multi-unit extracellular electrode recordings or intrinsic signal optical imaging, both of which report activity of populations of neurons. Thus, it was unclear whether enrichment/naturalistic sensory experience during juvenile development would affect the salt-and-pepper map for whisker tuning that we and others have observed. Enrichment could potentially lead to a sharper whisker map with more distinct columnar boundaries, or a smoother map with a more gradual gradient between whisker representations, as has been observed for tuning in S1 for object position across the rostro-caudal axis of the whisker pad [39].
We examined the effects of enrichment on the whisker map at single-neuron resolution in young adult mice. In L2/3 in enriched mice, the proportion of PYRs for which the CW evoked the largest response of the 9 we tested was slightly increased. However, we only observed this effect near column centers (Fig. S1). When we took into account ROIs for which the CW was an EBW, the increased proportion of CW-tuned ROIs was larger and extended to a larger proportion of the column. We did not expect to observe similar experience-dependent changes in L4, consistent with the smaller capacity for plasticity in L4 following whisker deprivation [26], and consistent with the lack of receptive field plasticity in L4 observed after adult enrichment [3]. However, we observed an increase in tuning precision in L4 that was similar to that observed in L2/3 (Fig. 2). Thus, early enrichment increased tuning precision in both L2/3 and L4.

**Enrichment spatially sharpens whisker representations and increases signal to noise in L2/3**

Enrichment sharpened whisker representations in several ways in L2/3. First, enrichment sharpened single-cell receptive fields by increasing the mean response to the CW and to the strongest SW, while reducing responses to the weakest SWs. This sharpened overall tuning around the CW, but slightly increased the number of response-evoking whiskers. In addition, enrichment reduced spontaneous calcium events in L2/3.

Second, enrichment spatially sharpened whisker point representations in L2/3. Whisker deflections are coded and communicated to downstream targets by populations of neurons, rather than single cells [40],[27],[41]. To examine how enrichment impacts the spatial organization of these population codes, we measured the point representations – the spatial extent and density of responsive neurons – of single whiskers. In L2/3, we found that enrichment had a strong impact on the spatial spread and magnitude of whisker responses. Responses to each whisker were strongest near the center of its anatomical column, and fell off gradually with cortical distance in both EN and CT. In EN, responses to each whisker were substantially larger than in CT both within and surrounding the boundaries of the corresponding column (Fig. 4). This is likely the same effect observed in in the mean tuning curves in L2/3 (Fig. 3) and contributes to the increased tuning for the CW at the center of each column in EN (Fig. 2).

Enrichment had different impacts in L4. Single-neuron receptive fields in L4 were effectively sharpened around the CW following enrichment, but this occurred by a different mechanism than in L2/3. Responses to most whiskers were globally decreased while CW responses remained unchanged, on average (Fig. 3). This reduced the number of strongly responsive whiskers for each cell. Despite the decrease in most whisker responses, spontaneous activity was not reduced in L4. This suggests that sharpened tuning in L2/3 reflects functional changes in recurrent, top-down, or POm input to L2/3, rather than inherited responses from L4. Alternately, it could reflect strengthening of L4-L2/3 intracolumnar synapses.
Also in contrast to L2/3, enrichment did not impact point representations in L4. This is consistent with previous studies demonstrating less receptive field and map plasticity in L4 than L2/3 following whisker deprivation and overstimulation/enrichment [26][3]; however, this contrasts with the altered single neuron tuning profile we observed (Fig. 3A,B). Thus, the increased tuning for CWs near column centers in L4 did not result in spatially sharper population-level single-whisker representations. L4 responses to each whisker were strongest near the center of its anatomical barrel and fell of much more sharply with distance compared to L2/3. This agrees with previous studies of map topography in L2/3 and L4 [42].

**Enrichment enhances functional column boundaries in L2/3**

Do anatomical column boundaries represent sharp functional boundaries? Map topography estimated from single unit extracellular recordings suggests that column boundaries represent sharp edges between whisker-specific representations [32], [33]. But cellular-resolution imaging studies of tuning in non-enriched mice suggest gradual gradients of tuning with small or no discontinuities at column boundaries [27], [7],[8]. To ask whether enrichment might generate functional discontinuities or sharp gradients at column boundaries, we examined signal and noise correlations, which are important measures of information coding and structure of neural ensembles [29]. Both types of correlation can provide insight into the information capacity of neuronal populations, and thus are also useful in understanding how distributed populations of neurons represent whisker information.

In L2/3 CT mice, both signal and noise correlations fell off gradually with distance between a neuron pair, as expected in cortical circuits [43], [44]. Column boundaries were essentially indistinguishable from the spatial profiles of pairwise correlations: signal and noise correlation spatial profiles were almost equivalent for pairs located within and across column boundaries. In EN mice however, column boundaries had much more impact on correlations. Signal correlations between co-columnar pairs were modestly increased within columns, and signal correlations between cross-columnar pairs were substantially decreased. This indicates that receptive fields varied more across columns than within columns following enrichment. Because whisker tuning in L2/3 is thought to primarily reflect feedforward L4-L2/3 input and horizontal L2/3-L2/3 circuits [42], the decrease in signal correlations across columns is likely to represent improved functional segregation of L4-L2/3 and L2/3-L2/3 circuits relative to anatomical column boundaries.

Enrichment reduced noise correlations within columns, but a strikingly larger decrease was observed across columns. This suggests that neuronal activity in L2/3 was overall more independent following enrichment, but that activity correlations were especially decreased across column boundaries. Because noise correlations are generally detrimental for sensory coding [29], the overall reduction in noise correlations is likely to
improve whisker sensory coding for downstream areas. In addition, because noise correlations are likely to substantially reflect shared activity received within common synaptic networks [28], the reduction of noise correlations between columns suggests potentially reduced cross-columnar excitatory circuits, or increased cross-columnar inhibition, after enrichment.

In L4, enrichment increased signal correlation for neuron pairs within a barrel, similar to L2/3. However, enrichment also greatly increased within-barrel noise correlations. The parsimonious explanation for these two findings is that enrichment promoted development of recurrent excitatory connections within each barrel, thus enhancing locally generated activity and increasing tuning similarity. Cross-columnar correlations could not be examined because imaging field size in L4 was small, so that simultaneous imaging in multiple columns occurred only rarely.

**Enrichment improves population coding of whisker deflection in L2/3**

Most of our observations of changes in population codes depend on average measures of responses to whiskers over many iterations. However, responses to stimuli/sensory detection and discrimination of whisker deflections and an animal’s behavior in response to stimuli occur on single trials. In S1, responses to whisker deflections are highly variable from trial-to-trial, despite relative stability in average coding [24],[25]. Thus, average measures of sensory coding may obscure how sensory input is coded in real time. We created neural decoders to examine the effects of enrichment on trial-by-trial detection and discrimination of whisker deflections by L2/3 neurons and ensembles. Decoding performance for detecting the CW was better in EN mice in single-ROI decoders, indicating that single L2/3 PYR cells can better discriminate the deflection of the CW above spontaneous activity. This corresponds well with the increased representation of CWs we observed among PYRs in L2/3, as well as the reduction in spontaneous activity. Essentially, signal-to-noise is improved at single-cell level for the CW. This increased performance was also observed for each ROI’s BW.

Information about whisker deflections in S1 is likely communicated to downstream areas by ensembles of neurons [45]. We measured detection performance by randomly-chosen ensembles of increasing size, hypothesizing that performance would be higher in EN ensembles of smaller sizes compared to CT. In other words, the same information could be encoded by smaller numbers of ROIs in EN mice, given the increased signal/noise in single ROIs and decreased pairwise noise correlations, which can limit information in a population. Because ROIs were diversely tuned, we expected that random populations of ROIs would be able to detect deflections of multiple whiskers above spontaneous activity, so we measured performance as the mean percent correct across all whiskers. Performance was improved in EN over CT for all ensemble sizes larger than 3. This increased performance in EN could be due to the overall decrease in noise correlations we observed.
Our observations of increased somatotopy, spatially-sharpened point representations, and enhanced columnar structure in signal and noise correlations suggests that after enrichment, information about individual whiskers is encoded in a more discrete map. We confirmed these effects on single-trial coding by measuring detection performance as a function of location of decoding ensembles relative to each column. Both single ROIs and spatially-clustered ensembles of varying size performed best at detecting deflections of whiskers corresponding to the closest column center. Performance was better in EN than CT for both single ROIs and clustered ensembles within ~150 µm of the reference whisker’s corresponding column center, in agreement with the sharpened point representation we observed.

Single ROI decoders on average performed equally on discriminating the CW in EN and CT, with large peaks around chance performance. We expected performance on discrimination between 9 whiskers to be lower compared to detection because the majority of ROIs in both EN and CT were responsive and/or tuned to multiple whiskers; thus, confusion between whiskers is more likely. The increased responses to CWs that we observed in EN did not translate into improved performance on CW discrimination. This is likely due to the parallel increases in responses to the best 3 SWs, which could result in more confusion of the CW with these SWs. Despite the equivalent performance of single ROI decoders, mean discrimination performance by randomly-selected ensembles was greater in EN for all ensemble sizes we tested >1.

As with detection, discrimination performance was highest for single cells and ROIs near the centers of columns, but decayed more sharply with distance, approached chance performance, then increased again ~750 µm. Performance was higher in EN ensembles within 91 µm of column centers (less than the radius of a column, 150 µm) and at distances >750 µm (which would correspond to ~2.5 average column widths) from column center. The increased discrimination performance at greater distances likely reflects the performance of ensembles located more than one column or arc away from the reference column. Discriminating whiskers spaced more than one arc or column away from each other is most likely easier than discriminating directly adjacent whiskers, since many ROIs respond similarly to their CW and one or more adjacent SWs. The increased performance in EN could be due to the decreased responses we observed for each ROI’s “worst” SWs (Fig. 2A,B).

**Conclusion**

Tactile enrichment during early adulthood leads to robust changes in the structure of the whisker map in both L2/3 and L4 of S1. We found that whisker representations were spatially sharpened at both the single cell level in L2/3 and L4, and the population level in L2/3, and that pairwise correlations in tuning and trial-by-trial responses to whiskers were more discretely structured according to column boundaries. Additionally, neural decoding models based on the L2/3 neurons we imaged more accurately predicted whisker input in EN mice, and the spatial profile of decoder performance was well
aligned with the increased somatotopic precision we observed near anatomical column centers. Combined, these effects show that environmental enrichment in juvenile mice robustly enhances functional columnar organization in S1.

2.5 References


Figure 1. In vivo 2-photon calcium imaging of pyramidal neuron responses to whisker deflections in L2/3 and L4 barrel cortex. (A) Left: schematic of experimental design. Right, examples of toys used for enrichment. (B) Left: schematic of whisker stimulus train. Middle: schematic of imaging setup. Right: Schematic of barrel cortex. Barrels that correspond anatomically to stimulated whiskers, which were targeted for imaging, are shaded in gray. (C) Example ΔF/F traces from 3 co-columnar ROIs imaged simultaneously. Shaded bars indicate whisker stimulations. Top: identity of whisker deflected for each stimulus. (D) Top: responses to 9 whiskers on each trial for the same ROIs shown in C. Within each square, rows show baseline-subtracted responses to each trial for the indicated whisker, aligned to stimulus (0 sec). Gray scale indicates ΔF/F. Bottom, left of each panel: Peri-stimulus average ΔF/F traces for each the ROIs shown above. Responses were aligned to stimulus onset, baseline-subtracted, and averaged across iterations separately for each whisker from -0.5 sec to 3 sec surrounding stimulus onset. Black, blue, and magenta traces indicate average responses to whiskers evoking the largest, second largest, and third largest response, respectively. All other whisker responses are shown in gray. Cyan vertical bars indicate timing of individual whisker deflections. Bottom right of each panel, median responses to each whisker. Grayscale indicates median ΔF/F across trials, averaged over 1 second following stimulus onset on each trial. (E) Histological recovery of imaging field location with respect to barrels. Left, middle: Cytochrome oxidase-stained tangential sections showing superficial blood vessels and optical lesion (left) and barrels in layer 4 (middle). Right: example time-averaged 2p movie in L2/3, with outlines of barrel projections indicated. White squares in left, middle panels indicate XY-position of example imaging field.
Figure 1. In vivo 2-photon calcium imaging of pyramidal neuron responses to whisker deflections in L2/3 and L4 barrel cortex.
Figure 2. Enrichment increases the proportions of somatotopically-tuned ROIs located near barrel-column centers. (A) Whisker preference in L2/3 pyramidal cells is heterogeneous on the sub-columnar level. Example time-averaged imaging field from a control mouse with whisker-responsive ROIs color-coded to indicate whisker preference. White lines indicate projections of barrels onto L2/3 imaging field. For ROIs located within columns, columnar whisker is indicated as “best whisker” if it evoked a median response statistically equivalent to the whisker that evoked the absolute largest median response. Otherwise, and for ROIs located outside of barrel columns, the color-coded best whisker is the whisker that evoked the absolute largest median response. (B) Percentage of all responsive ROIs tuned to a reference whisker, binned by distance from the anatomically-corresponding column center. ROIs were considered tuned if the whisker evoked a response statistically equivalent to the ROI’s absolute best whisker. Blue refers to L4 ROIs imaged in control animals; black, L2/3 control ROIs. Inset: schematic of average barrel column radius. (C) Axes as described in B, comparing tuning in enriched (EN) and control (CT) animals in L2/3 (upper row) and L4 (lower row). Left Y-axis denotes percent of ROIs in each bin for which the reference whisker is statistically equivalent to BW. Right Y-axis refers to number of ROIs located in each distance bin with respect to each whisker, shown by red and black dashed lines for EN and CT, respectively. (D) Mean percent of ROIs in each column for which the CW is equivalent to the BW. Top, L2/3; Bottom, L4. Black circles denote individual columns; sizes of circles indicate the number of responsive ROIs in the column. Red circles denote means across columns; red lines indicate standard error of the mean. (E) All whisker-responsive ROIs color-coded by tuning and plotted by XY position in um relative to reference column center. Blue indicates ROIs statistically tuned to the reference whisker, yellow indicates ROIs not tuned to this whisker. White or black circles indicate the perimeter of an average barrel; position (0,0) is the center of the circle. Upper row: L2/3; Lower row: L4. Left plots: CT; Right plots: EN.
Figure 2. Enrichment increases the proportions of somatotopically-tuned ROIs located near barrel column centers.
**Figure 3. Enrichment sharpens whisker tuning curves.** (A) Average response to each whisker for EN and CT ROIs, ranked individually for each ROI from largest to smallest response. Whisker rank 1 indicates best whisker. Upper, L2/3; lower, L2/3. (B) Average responses to CW, and averaged ranked responses to surround whiskers, ranked from largest to smallest response for each ROI. Rank 1 indicates best surround whisker. Color coding and panel arrangement as in G. (C) Distributions of receptive field size in Enriched (red) and Control (black) ROIs. X axis denotes number of whiskers out of 9 tested that evoke a response above median spontaneous activity in each ROI. Upper, L2/3; lower, L4. (D) Distributions of number of best whiskers (e.g. whiskers evoking a median response statistically equal to the absolute maximum median response). Color coding as in E. Upper, L2/3; lower, L4. (E) Cumulative distributions of spontaneous ΔF/F in whisker-responsive ROIs. Upper: L2/3; lower: L4. (F) Left: cumulative distribution of L2/3 ROI normalized responses to columnar whisker (CW) deflection. Middle: normalized responses to all surround whiskers (SW). Right: distributions of normalized response of each ROI to its best whisker (BW). Upper row: L2/3; lower row: L4.
Figure 3. Enrichment sharpens whisker tuning curves.
Figure 4. Enrichment sharpens point representations of single whiskers in L2/3, but not L4, by increasing pyramidal cell responses to the columnar whisker. (A) Solid lines denote L2/3 population mean response to individual whiskers (left Y-axis) by distance of ROIs to corresponding barrel column centers. Red and black indicate EN and CT, respectively. Responses are averaged over ~30 µm bins. Shaded areas indicate standard error of the mean (SEM). Dashed lines denote number of ROIs in each distance bin (right Y-axis). (B) Mean whisker response by ROI position relative to the corresponding column. ROIs were grouped by k-means clustering of positions relative to the center of a column for each of the 9 columns corresponding to stimulated whiskers. Each color-coded polygon represents one cluster of ROIs; color-code depicts the mean response of ROIs in the cluster to the reference whisker whose corresponding column is centered at (0,0). Black dashed circles depict location and size of an average column. Left, L2/3 ROIs from control mice; right, enriched. (C,D) Plots as in A and B, for L4 data.
Figure 4. Enrichment sharpens point representations of single whiskers in L2/3, but not L4, by increasing pyramidal cell responses to the columnar whisker.
Figure 5. Enrichment alters the spatial structure of signal and noise correlations along column boundaries in L2/3. (A) Schematics depicting spatial relationship of L2/3 ROI pairs for which correlations were measured in B-D. Left: co-columnar ROI pairs; right: cross-column ROI pairs. (B) Pairwise signal correlations by distance in L2/3. Left, pairs within columns; right, cross-column pairs. Solid lines depict mean signal correlation (left Y-axis) for ROIs by inter-ROI distance. Shaded areas represent SEM. Black and red correspond to control and enriched, respectively. Dashed lines indicate number of ROI pairs in each inter-ROI distance bin (right Y-axis). (C) Axes as described in B, for pairwise noise correlations. Left: in-column pairs; right: cross-column pairs. (D-E) Mean signal (D) and noise (E) correlation coefficients for L2/3 ROI pairs, grouped as indicated. (F) Mean signal correlation to ROIs within a reference column by position. Each polygon corresponds to a cluster of ROIs positioned relative to the column centered at (0,0), which is located at the center of the dashed circle. Color scale refers to the mean signal correlation of ROIs in each cluster to each ROI located within the reference column. Black dashed line indicates the radius of an average column. Left, control; right, enriched. (G) Plots as described in A, but color scale indicates mean noise correlation of ROIs in each cluster to ROIs in the reference column. (H) Mean signal correlation coefficient of all ROIs in a reference column to ROIs within the column and ROIs outside of the column. Black, control; red, enriched. (I) Mean noise correlation coefficient of ROIs located outside of a reference column to all ROIs within the column, and to all ROIs located outside of the column.
Figure 5. Enrichment alters the spatial structure of signal and noise correlations along column boundaries in L2/3.
Figure 6. Enrichment increases signal and noise correlations within columns in L4. (A) Schematic of L4 in-barrel ROI pairs. (B) Mean signal correlation by inter-ROI distance for L4 within-column pairs. Axes as described for 5B. (C) Mean noise correlation by inter-ROI distance for L4 within-column pairs. Axes as described for 5C. (D) Mean signal correlations for within-barrel L4 ROI pairs. (E) Mean noise correlations for within-barrel L4 ROI pairs.
Figure 6. Enrichment increases pairwise signal and noise correlations within barrels in L4.
Figure 7. Enrichment improves population coding of columnar whisker deflections. (A) Structure of detection decoders. (B) Distributions of performance of single-ROI decoders on discrimination of columnar whisker stimuli from blank trials. (C) Detection decoder performance improves with increasing number of ROIs in the decoding ensemble. ROIs in each ensemble were randomly spatially distributed. Error bars indicate standard error of the mean. (D) Performance of single ROI decoders on detection of whisker deflections by distance of ROI to column center corresponding to reference whisker. Shading indicates standard error of the mean. Dashed lines indicate number of ROIs in each distance bin. (E) Same axis as D for clustered population decoders. Simultaneously-imaged ROIs were grouped into decoding ensembles by k-means clustering of X-Y positions. Ensemble sizes ranged from 2 to 40. Distance bins refer to distance between reference column center and the centroid of each cluster. (F) Structure of discrimination decoders. (G–J) As described for B–E for discrimination decoder performance.
Figure 7. Enrichment improves population coding of columnar whisker deflections.
Supplementary Figure 1. Measurements of somatotopy based on absolute best whisker. (A) Whisker preference in L2/3 pyramidal cells is heterogeneous on the sub-columnar level. Example time-averaged imaging field with whisker-responsive ROIs color-coded to indicate whisker preference. White lines indicate projections of barrels onto L2/3 imaging field. ROIs are color-coded based on best whisker (e.g. the whisker that evoked the absolute largest median response). (B) Percentage of all responsive ROIs tuned to a reference whisker, binned by distance from the anatomically-corresponding column center. ROIs were considered tuned if the whisker evoked the absolute largest median response. Blue refers to L4 ROIs imaged in control animals; black, L2/3 control ROIs. (C) Axes as described in B, comparing tuning in enriched (EN) and control (CT) animals in L2/3 (upper row) and L4 (lower row). Left Y-axis denotes percent of ROIs for which the reference whisker evokes the largest median response. Red, enriched; black, control. Right Y-axis refers to number of ROIs located in each distance bin with respect to each whisker, shown by red and black dashed lines for EN and CT, respectively. (D) Mean percent of ROIs in each column for which the CW was the BW. Top, L2/3; Bottom, L4. Black circles denote individual columns; sizes of circles indicate the number of responsive ROIs in the column. Red circles denote means across columns; red lines indicate standard error of the mean.
Supplementary Figure 1. Measurements of somatotopy based on absolute best whisker.
Supplementary Figure 2. Non-normalized whisker tuning curves and evoked response distributions. (A) Average response to each whisker for EN and CT ROIs, ranked individually for each ROI from largest to smallest response. Whisker rank 1 indicates best whisker. Upper, L2/3; lower, L2/3. (B) Average responses to CW, and averaged ranked responses to surround whiskers, ranked from largest to smallest response for each ROI. Rank 1 indicates best surround whisker. Color coding and panel arrangement as in A. (C) Left: cumulative distribution of L2/3 ROI median ΔF/F responses to columnar whisker (CW) deflection. Middle: median ΔF/F evoked by surround whiskers (SW). Right: distributions of median ΔF/F response of each ROI to its best whisker (BW). (D) As in C, for L4 ROIs.
Supplementary Figure 2. Whisker tuning curves and evoked response distributions in L2/3 and L4 based on non-normalized dF/F.
Supplementary Figure 3. Non-normalized point representations of single whisker in L2/3 and L4. (A) Solid lines denote L2/3 population mean response to individual whiskers (left Y-axis) by distance of ROIs to corresponding barrel column centers. Responses of individual ROIs were computed as median evoked ΔF/F and not normalized to spontaneous activity. Red and black indicate EN and CT, respectively. Responses are averaged over ~30 um bins. Shaded areas indicate standard error of the mean (SEM). Dashed lines denote number of ROIs in each distance bin (right Y-axis). (B) As in A, for L4 data.
Supplementary Figure 3. Non-normalized point representations of single whiskers in L2/3 and L4.
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Table 1. Mouse information.