Sensory and decision-related activity propagate in a cortical feedback loop during touch perception

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The brain transforms physical sensory stimuli into meaningful perceptions. In animals making choices about sensory stimuli, neuronal activity in successive cortical stages reflects a progression from sensation to decision. Feedforward and feedback pathways connecting cortical areas are critical for this transformation. However, the computational functions of these pathways are poorly understood because pathway-specific activity has rarely been monitored during a perceptual task. Using cellular-resolution, pathway-specific imaging, we measured neuronal activity across primary (S1) and secondary (S2) somatosensory cortices of mice performing a tactile detection task. S1 encoded the stimulus better than S2, while S2 activity more strongly reflected perceptual choice. S1 neurons projecting to S2 fed forward activity that predicted choice. Activity encoding touch and choice propagated in an S1–S2 loop along feedforward and feedback axons. Our results suggest that sensory inputs converge into a perceptual outcome as feedforward computations are reinforced in a feedback loop.

Perceptual decisions involve propagation and transformations of sensory signals across multiple hierarchically organized cortical areas. Feedback projections are a ubiquitous feature of cortical organization and are implicated in numerous functions, such as contextual modulation of perception, attention, sensory expectation, and perceptual learning. Feedback to primary sensory cortical areas is even hypothesized as essential for sensory awareness. Sensory cortex feedforward and feedback connections form recurrent neural networks. Recurrent networks can exhibit complex dynamics and perform sophisticated computations, such as by forming content-addressable memory networks for pattern completion, amplification of input signals, and the maintenance of neural activity over timescales longer than permitted by the biophysics of individual neurons.

How feedforward and feedback cortical dynamics mediate the transformation from raw sensory input to actionable interpretations of the sensory world (that is, to perceptual decisions) is not understood. This is largely due to the difficulty of using traditional methods to measure neural activity within defined synaptic pathways during behavior. Progress requires theoretical work on recurrent networks to be embodied in specific circuitry. In vivo two-photon imaging, combined with strategies to mark axons or neurons by their projection patterns, allows the activity of specific cortico-cortical pathways to be monitored during behavior. Here we used a combination of pathway-specific imaging and optogenetics to investigate the perception-related dynamics of a recurrent network between primary and secondary somatosensory cortex.

RESULTS

We trained mice to perform a head-fixed tactile detection task in which they reported by licking or withholding licking whether a single whisker received a brief sinusoidal deflection (20 Hz, 0.5 s, ~800 degrees s⁻¹ peak speed; Fig. 1). Trial outcomes comprised a mixture of successful detections (‘hits’) and failed detections (‘misses’) following stimulus delivery, as well as correct responses (‘correct rejection’) and incorrect responses (‘false alarms’) in the absence of the stimulus (Fig. 1c and Supplementary Fig. 1). The relationship between touch perception and responses across large-scale populations of cortical neurons remains poorly understood, even for S1. Anatomy and physiology in anesthetized or narcotized rodents suggest that mouse S2 is a higher, or more integrative, cortical area than S1. However, responses of rodent S2 neurons during tactile behavior are nearly entirely unexplored (but see refs. 24,25). We thus began by mapping responses to stimulation of a single whisker across whisker representation areas of S1 and S2, in separate mice, as they performed the detection task.

Mapping activity in S1 and S2 during tactile detection

We used in vivo two-photon imaging of GCaMP6 genetically encoded calcium indicators to measure spiking-related fluorescence signals from the cell bodies of layer 2/3 (L2/3) neurons. For our single-whisker stimuli, we found that 37.0 ± 2.3% (mean ± s.e.m. across mice) of neurons in S1 and 23.6 ± 4.1% of neurons in S2 gave task-related responses (‘responsive’ neurons; Online Methods, Fig. 1h). Thus, behaviorally relevant whisker stimulation is represented robustly in whisker regions of both S1 and S2. We limited subsequent analyses to responsive neurons, except where noted, and used responses occurring in a time window (0.25 s after stimulus onset) preceding the typical reaction times (Fig. 2a,b). The end of this window precedes 96.5% of reaction times (Supplementary Fig. 1).

Overall, responses of individual neurons to the whisker stimulus tended to be larger in S1 than in S2 (S1: hit: 0.036 ± 0.006 ΔF/F₀; miss: 0.024 ± 0.006 ΔF/F₀; S2: hit: 0.029 ± 0.016 ΔF/F₀; miss: 0.015 ± 0.008 ΔF/F₀; mean ± s.e.m. across mice; z = 3.19, P = 0.0014, for comparison of hits; z = 7.46, P = 8.38 × 10⁻¹⁴ for comparison of misses; 1,370 neurons in S1; 607 neurons in S2; Wilcoxon rank sum tests; Fig. 2a,b), suggesting a more robust representation of the tactile stimulus in S1.

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Touch-evoked activity in both S1 and S2 predicted the subsequent perceptual choice of the mouse. Responses on hit trials were larger than on miss trials in both S1 and S2 (hit – miss mean ± [95% confidence interval, CI] for evoked $\Delta F/F_0$: S1: 0.013 ± [0.008, 0.019] $\Delta F/F_0$; S2: 0.012 ± [0.010, 0.023] $\Delta F/F_0$). Thus, activity in each area predicted whether the mouse would succeed or fail to detect an identical stimulus. In the absence of a whisker stimulus, activity was higher on false alarm trials than on correct rejections in S2 (false alarm – correct rejection $\Delta F/F_0$; S2: 0.006 ± [0.009, 0.011] $\Delta F/F_0$). To quantify modulation of neuronal activity by behavioral choice, we normalized responses of individual neurons to the mean hit response, in order to account for overall differences in evoked activity between the two areas. We observed a smaller normalized miss response in S2 (Fig. 2c). Thus, individual neurons were more modulated by choice in S2 than in S1.

Trials-by-trial coding by single neurons and populations

Ideal observer analysis is frequently used to correlate the trial-by-trial activity of single sensory cortex neurons with stimuli and perceptual choices. To investigate stimulus and choice encoding by single neurons, we first calculated stimulus probability (SP). SP gives the probabil- ity with which an ideal observer could correctly categorize the stimulus condition (present versus absent) of a trial on the basis of the response of a single neuron (Online Methods). We also calculated detect probability (DP), which is mathematically identical to choice probability but often renamed in the context of detection tasks. DP is the probability with which an ideal observer could correctly categorize the behavioral choice of the mouse on a single trial (in our case, lick versus no-lick) on the basis of the response of a neuron. We observed robust (above chance level, >0.5) SP and DP in S2 as well as S1 (Fig. 2d). S1 trended toward having a higher fraction of neurons with significant SP ('SP neurons', defined as neurons whose 95% confidence interval for SP did not include 0.5; 49.7 ± 3.1% of responsive neurons in S1 versus 32.2 ± 3.4% of responsive neurons in S2; mean ± s.e.m. across mice; $P = 0.24$, permutation test; Supplementary Fig. 2). The distribution of SP showed larger values overall in S1 than in S2 (Fig. 2d). The distribution of DP was similar in the two cortical areas, despite the weaker stimulus representation in S2 (Fig. 2d). The ratio of DP to SP for neurons in S2 was higher than for neurons in S1 (Fig. 2d), consistent with a greater choice-related modulation of S2 responses.

Choice-related activity is thought to depend on correlations among neuronal responses, which in turn reflect cortical topography. However, while detect (or choice) probability is frequently quantified, its cellular-resolution organization within cortex is unknown. We therefore mapped SP and DP across S1 and S2 in separate mice (Fig. 2e–j). First, we tested whether SP neurons and DP neurons (defined as a neuron with 95% confidence interval for DP not including 0.5) were clustered. In S1, pairwise distances among SP neurons were smaller than pairwise distances among all neurons (median distances: 243 μm versus 281 μm; Fig. 2g). Clustering of SP neurons is expected as a result of the somatotopic organization of primary somatosensory cortex in anesthetized mice. By contrast, in S2, neither SP nor DP neurons formed obvious clusters (Fig. 2h). We next examined how SP and DP were distributed as a function of distance from the center of the somatotopic representation of the stimulated whisker. In S1, this somatotopic representation corresponds to the stimulated whisker's barrel column. We estimated the center of...
Figure 2  Coding of stimulus and choice in S1 and S2. (a) Activity (mean ± s.e.m. ΔF/F_0 across mice) averaged across hit (H; blue), miss (M; black), false alarm (FA; green) and correct rejection (CR; red) trials from S1 imaging sessions (12 sessions, 5 mice, 274 ± 52 neurons per mouse; mean ± s.e.m.). Evoked ΔF/F_0 responses on hits were larger than on misses (t_{1,369} = 16.79, P = 1.17 × 10^{-5}; 1,370 neurons; paired t-test). Cyan shading, first 0.25 s after stimulus (stim) onset, which preceded 96.6% of first licks. Dashed line, last time point before median first-lick time. (b) Same as a for S2 (11 sessions, 5 mice, 103 ± 26 neurons per mouse). Evoked ΔF/F_0 responses on hits were larger than on misses (t_{606} = 20.32, P = 2.22 × 10^{-70}; 607 neurons; paired t-test). In S2, evoked ΔF/F_0 responses on false alarms were larger than on correct rejections (t_{606} = 4.07, P = 5.33 × 10^{-5}; paired t-test). (c) Mean evoked ΔF/F_0 responses normalized to hits across individual neurons in S1 and S2 (mean ± s.e.m. across mice; circles show individual mice). For both S1 and S2 neurons, responses on misses were smaller than on hits (miss/hit ratio: S1: 0.57 ± 0.04; S2: 0.32 ± 0.05; z = 18.84, **P = 3.76 × 10^{-79}; 607 neurons; Wilcoxon sign rank tests), with stronger modulation for S2 compared with S1 (**P = 7.46, **P = 8.38 × 10^{-14}; Wilcoxon rank sum test). (d) Cumulative histograms (means across mice) of SP (left), DP (middle) and DP/SP ratio (right) for all (responsive and nonresponsive) neurons in S1 (brown) and S2 (orange). DP/SP ratio was higher in S2 than in S1 (medians: 1.01 versus 0.97; D = 0.093, **P = 1.96 × 10^{-7}; 2,490 S1 and 1,471 S2 neurons; Kolmogorov-Smirnov test); SP was higher in S1 than in S2 (medians: 0.55 versus 0.52; D = 0.063, **P = 0.0013; Kolmogorov-Smirnov test) while DP was similar (D = 0.044, P = 0.056; Kolmogorov-Smirnov test). (S1: 12 sessions, 5 mice, 498 ± 74 neurons per mouse; S2: 11 sessions, 5 mice, 294 ± 47 neurons per mouse). (e) Maps from one mouse showing distributions of stimulus- and decision-encoding neurons in S1. White neurons are those whose SP or DP 95% confidence intervals included 0.5. Responsive and nonresponsive neurons are included. Black plus signs mark the center of the somatotopic column of the stimulated whisker. (f) Same as a for S2. (g) Cumulative histograms of pairwise distances among SP neurons (black), DP neurons (gray), and all neurons (dashed blue; responsive and nonresponsive) in S1. Both SP and DP neurons had smaller pairwise distances among themselves compared with all neurons (both **P < 5 × 10^{-5}; 26,898 SP, 12,387 DP, and 431,962 all-neuron pairs; bootstrap). (h) Same as a for S2. (i) Mean SP (black) and DP (gray) values averaged across all neurons (8 bins of 50 µm ± s.e.m.) as a function of distance from the center of the somatotopic column representing the stimulated whisker in S1. SP and DP both decreased with distance from the column center (SP: −0.028 per 100 µm; DP: −0.017 per 100 µm; test of zero slope: F_{1,74} = 54.84, P = 7.11 × 10^{-12}; difference in slopes for SP and DP: F_{1,74} = 3.58, P = 0.062; 78 binned values total from 5 mice; ANCOVA). (j) Same as i for S2 (test of zero slope: F_{1,54} = 3.11, P = 0.083; difference in slopes for SP and DP: F_{1,54} = 0.92, P = 0.342; 58 values from 5 mice). (k) Performance of a classifier (mean ± s.e.m. across mice) in decoding the stimulus condition from population activity at each time point reached higher levels for S1 (84 ± 7% correct by 0.25 s after stimulus (stim) onset, and 89 ± 5% by the median reaction time of 0.52 s) compared with S2 (68 ± 8% correct by 0.25 s, 82 ± 4% by 0.52 s; performance diverged by 0.32 s: U = 76, P = 0.045; 7 S1 and 9 S2 sessions; one-tailed Wilcoxon rank sum test). (l) Mean pairwise noise correlations between each responsive neuron and other responsive neurons (top), each DP neuron and other DP neurons (middle), or each SP neuron and other SP neurons (bottom). Noise correlations were higher in S2 (all **P < 5 × 10^{-5}; S1: 1,370 responsive, 536 SP and 338 DP neurons; S2: 607 responsive, 287 SP and 259 DP neurons; permutation tests).
~400 μm from the center of the somatotopic representation (Fig. 2j). Thus, choice-related activity was clustered across somatosensory cortex, but clustering depended on cortical area.

Sensory and motor variables can be encoded not just by single neurons but also by activity patterns across neural populations. We quantified how well large populations of neurons in S1 and S2 encoded the whisker stimulus and the perceptual choice of the mouse using a machine learning classifier (Random Forests31,32; Online Methods). The classifier attempted to decode the stimulus condition (present versus absent) and the behavioral choice (lick versus no-lick) from the simultaneous activity of all responsive neurons, at each time point within a trial. Performance of the classifier in decoding the stimulus condition (‘SP’), followed by stimulus onset for both S1 and S2, and reached higher levels in S1 than in S2 (Fig. 2k). Classifier performance in decoding the choice of the mouse (‘DP’) followed the stimulus onset in both S1 and S2, but reached somewhat higher levels in S2 (Fig. 2i). Thus, decoding of tactile stimuli from population responses was better in S1, but decoding of choice was slightly better in S2.

Choice-related activity is thought to depend on trial-to-trial correlations in the responses of pairs of neurons24,30 (noise correlations33; Online Methods). Consistent with stronger encoding of choice, S2 showed higher average noise correlations than S1 (0.19 ± 0.05 versus 0.09 ± 0.01; mean ± s.e.m. across mice; Fig. 2m). Stronger noise correlations in S2 were especially prominent in pairs of DP neurons (that is, pairs of neurons that both encoded choice; Fig. 2m).

To ensure that the differences in touch-related dynamics we observed between S1 and S2 were not due to differences among animals, we monitored S1 and S2 simultaneously in individual mice (11 sessions total from 4 mice; Supplementary Fig. 3; Online Methods). Responses on hits were larger than on misses in both S1 and S2 (Supplementary Fig. 3e). The miss/hit response ratio was smaller in S2 than in S1 (Supplementary Fig. 3i). At the population level, decoding of choice was slightly better in S2 than in S1 (Supplementary Fig. 3k). These results are consistent with S2 responses depending to a greater degree on choice.

Two results from simultaneous S1 and S2 imaging experiments suggest that perceptual detection may be associated with coordination of activity between S1 and S2. First, pairs of simultaneously recorded S1 and S2 neurons showed slightly higher noise correlations on hit than on miss trials (Supplementary Fig. 3g,h). Second, population decoding of choice was superior when using the pooled sets of S1 and S2 neurons, compared with decoding from either set alone (Supplementary Fig. 3k), implying that choice was encoded in at least a partly nonredundant manner across S1 and S2.

Feedforward propagation of task-related activity

To what degree can the strong encoding of choice in S2 be attributed to feedforward inputs from S1? To quantify feedforward propagation of activity from S1 to S2, we labeled S2-projecting (S2p) neurons in S1 using injections into S2 of cholera toxin subunit B, a retrograde tracer, conjugated to fluorescent dye14-17,34 (Fig. 3a,b). We then identified these S2p neurons among the larger set of GCaMP6s-expressing neurons using in vivo imaging (8 sessions total from 3 mice; Fig. 3c). Unlabeled neurons presumably comprised both neurons that did not project to S2 and false-negative neurons that did project to S2 but were not labeled. Responses on hit trials were larger than on miss trials for both S2p neurons (hit – miss mean ± [95% CI]: 0.019 ± [0.005, 0.040] ΔF/RF, Fig. 3d,f) and unlabeled neurons (hit – miss mean ± [95% CI]: 0.010 ± [0.002, 0.017] ΔF/RF, Fig. 3e,f). However, S2p neurons showed slightly larger choice-related modulations (Fig. 3f; see also ref. 17).

Ideal observer analysis showed higher values of both SP (Fig. 3g) and DP (Fig. 3h) among S2p neurons than among unlabeled neurons. At the population-coding level, classifier performance in decoding the stimulus condition from S2p neurons reached a higher level compared...
with unlabeled neurons (Fig. 3i). Classifier performance in decoding choice trended higher with S2p neurons than with unlabeled neurons (Fig. 3j). Moreover, noise correlations were stronger among pairs of S2p neurons than among pairs of unlabeled neurons (0.14 ± 0.04 versus 0.10 ± 0.02; mean ± s.e.m. across sessions; Fig. 3k). Thus, S2p neurons showed a more coordinated response and stronger association with perceptual choice.

A cortico-cortical loop for task-related activity

S1 and S2 are strongly and bidirectionally interconnected by long-range axonal projections. Recurrent loops in cortical circuits can serve many computational functions, including amplifying and prolonging stimulus evoked activity. To determine whether S1 and S2 could act in concert as a feedback loop during sensory decision making, we quantified stimulus- and choice-related activity propagating along feed-forward (S1→S2) and feedback (S2→S1) axonal pathways (4 of 10 sessions of the S2→S1 data are from previously published experiments and are reanalyzed here in greater detail). We expressed GCaMP6s in axons using adeno-associated virus (AAV) injections into either S1 (to monitor S1→S2 axons; Fig. 4a,b) or S2 (for S2→S1 axons, Fig. 4c,d). We then imaged axonal activity during behavior in S2 (S1→S2 axons, imaged in L2/3; Fig. 4b) or in S1 (S2→S1 axons, imaged in L1, a site of major top-down input to the dendrites of L2/3 neurons; Fig. 4d and Supplementary Fig. 4).

Axonal activity increased following stimulus onset, to higher levels on hits compared with misses in both S1→S2 axons (hit – miss mean ± [95% CI]: 0.29 ± [0.07, 0.50]; P = 0.001; 160 axons; paired t-test). Conventions as in Fig. 2a. (f) Same as e for S2→S1 axons (10 sessions, 4 mice, 440 axons). Responses on hits were larger than on misses (0.031 ± 0.004 ΔF/F versus 0.016 ± 0.002 ΔF/F; t(439) = 3.13, P = 0.002; paired t-test). A subset of S2→S1 data in f,g,i are reanalyzed from ref. 38. (g) Mean evoked ΔF/F0 responses normalized to hits across individual axons (mean ± s.e.m. across mice; circles show individual mice). For both S1→S2 and S2→S1 axons, responses on misses were smaller than on hits (miss/hit ratio: S1→S2: 0.61 ± 0.10; S = 99; *P = 0.003; 160 axons; S2→S1: 0.29 ± 0.11; S = 313. **P = 1.15 × 10−18; 440 axons; sign tests), with stronger modulation for S2→S1 axons (z = 3.82, **P = 1.33 × 10−4; Wilcoxon rank sum test). (h) Performance of a classifier (mean ± s.e.m. across mice) in decoding the stimulus condition from population activity reached a higher level for S1→S2 compared with S2→S1 axons (82 ± 2% versus 66 ± 6% at 0.28 s after stimulus onset; z = 2.05, P = 0.044; 7 S1→S2 and 10 S2→S1 sessions; one-tailed Wilcoxon rank sum test). Conventions as in Fig. 2k. (i) Same as h for decoding choice. (j) Schematic of feedforward and feedback propagation of task-related activity (dashed: hypothetical functional pathways). (k) Top, optogenetic silencing experiment. In mice expressing channelrhodopsin-2 in GABAergic neurons, a 473-nm laser was directed over the C2 column (in S1), the whisker region of S2 or a control area (within S1 barrel cortex but ~1 mm from the C2 column). Photostimulation was randomly delivered on 30–40% of all behavioral trials. Bottom, example electrophysiology traces showing responses of an S1 neuron to whisker stimulation with and without laser illumination. (l) Cell-attached electrophysiology recordings were targeted to the C2 column in awake mice (16 neurons in 2 mice). The C2 whisker was stimulated in the presence or absence of laser illumination directed to the recording site (centered over the C2 column in S1), S2 or the control area. Silencing was quantified as the ratio of whisker-evoked spike count in the presence versus absence of illumination. S1 C2 area illumination produced stronger silencing of whisker-evoked responses than illumination of either S2 or the control area. Silencing was similar for S2 and the control area. Vertical axis is broken to accommodate one outlier. (m) Task performance (mean ± s.e.m. across 4 mice) was reduced by illuminating either S1 (from 74 ± 1.7% to 70 ± 1.8%; 10 S1 sessions; Wilcoxon rank sum test) or the control area (from 77 ± 2.0% to 75 ± 4.6%; correct), to a similar degree (S1 versus S2 reductions: z = −0.81, P = 0.421; 12 S1 and 15 S2 sessions; Wilcoxon rank sum test). Illuminating the control area caused a much smaller drop in performance (from 75 ± 2.6% to 70 ± 1.8% correct; S2 versus control area reductions: z = −3.27, P = 0.001; 12 control area sessions; Wilcoxon rank sum test). Gray lines, performance of individual mice averaged across sessions for interleaved trials with (bolts) and without (no bolts) illumination. Black symbols: mean ± s.e.m. across mice.
similar levels based on S1→S2 axons or S2→S1 axons (Fig. 4l). Thus, activity encoding both the sensory stimulus and the upcoming perceptual choice propagated along feedforward and feedback cortico-cortical axons between S1 and S2. Our results are consistent with a model in which touch-evoked activity propagates in a feedback loop between S1 and S2 during formation of a perceptual choice, with ‘readout’ by downstream circuits occurring at least in part from S2 neurons (Fig. 4j). This model predicts that silencing S2 should impair task performance. However, S1 and S2 comprise densely interconnected networks,35–37, and the spatial resolution of cortical silencing is inherently too coarse to silence the two areas independently (for a description of the spatial resolution of cortical silencing, see ref. 39). Therefore, to test for a causal role of S2 in task performance, we devised a strategy based on comparing the behavioral impact of partially silencing S2 and S1 to different degrees. We directed a laser to S1 (centered over the cortical column representing the C2 whisker, which was used to solve the task), to S2, or to a nearby control area (at stereotactic coordinates within the S1 barrel field but ~1 mm away from the C2 column) to optogenetically silence regions of cortex by photostimulation of GABAergic neurons expressing channelrhodopsin-2 (Fig. 4k). First, we recorded whisker-stimulus-evoked spikes from S1 neurons using loose-seal cell-attached recordings while directing the laser beam to S1, S2, or the control area. Silencing S2 or the control area led to decreases in S1 stimulus responses to 61% and 46% of baseline responses measured without photostimulation, respectively (Fig. 4l). Direct silencing of S1 by illumination of the recording site reduced responses further to 29% of baseline (Fig. 4l). Thus, direct silencing of somatosensory cortex yielded a 1.5-fold to 2-fold greater reduction in spiking than indirect silencing (via illumination of a nearby area). Although silencing S2 or the control area reduced activity to similar levels in S1 (Fig. 4l), the behavioral impact of silencing S2 was markedly greater (Fig. 4m and Supplementary Fig. 5). In other words, task performance was more effectively disrupted by silencing S2 than by silencing a region of barrel cortex representing whiskers not used in the task, despite similar indirect effects on S1 activity. Moreover, direct silencing of either S2 or S1 decreased performance to similar levels (Fig. 4m). We conclude that the behavioral impact of reducing activity in either S1 or S2 was similar, indicating causal roles for both areas in tactile detection.

DISCUSSION

Our results show that as mice attempted to detect a faint whisker stimulus, larger responses within a cortico-cortical loop predicted perceptual choice (successful detection) on a trial-by-trial basis. Notably, ‘late’ stimulus responses in S1 have been linked to perceptual detection in both rodents17,40 and primates3. These late responses occur at delays longer than the times for which feedforward activity can be sustained cell-autonomously by individual cortical neurons. We show here that touch-related activity propagates in specific pathways that form a direct loop between S1 and S2 (activity may also propagate between S1 and S2 indirectly41). Propagation in a loop can create circuit dynamics that act to amplify and sustain sensory activity, such as those of a Hebbian assembly. Although future work with electrophysiology will be required to define temporal dynamics, our work is consistent with the hypothesis that perception-related5,17,46 late sensory responses in S1 reflect reverberation of activity10 among cortical areas42.

Overall, patterns of activity in S1 and S2 had obvious similarities and subtle but intriguing contrasts. In both S1 and S2, activity encoded the stimulus and the perceptual choice. Neurons in both S1 and S2 propagated stimulus- and choice-related activity to the other area. However, neurons in each area also showed key differences in their response properties (see also refs. 24,25). S2 activity was more associated with perceptual outcome than S1, whereas S1 activity better encoded the stimulus. While sensory stimuli are usually analog in nature, perception is often all-or-none. Neuronal representations of sensory stimuli must therefore converge into states representing discrete perceptions, perhaps via attractor dynamics. We found that trial-to-trial noise correlations (coordinated variability not explained by the stimulus) among pairs of S2 neurons were higher than among pairs of S1 neurons, consistent with a model in which S2 neurons encoded the binary perceptual outcome of each trial. Recent computational modeling has shown that top-down feedback of choice-related activity (similar to what we observed between S2 and S1) may be critical for the development of neuronal responses that reflect perceptual outcome43.

Recently, a small number of studies14–17,25,34,44 have examined in vivo responses of S1 neurons that project to S2 and have shown that these neurons have distinct intrinsic and task-related response properties. Here we found that L2/3 neurons that project from S1 to S2 tended to show higher choice-related activity15,17 and stronger noise correlations than other L2/3 neurons. These characteristics were similar to those of L2/3 neurons located in S2, suggesting a partly feedforward inheritance of response properties45,46 and even choice-related activity (compare ref. 47).

A limitation of our work is that we focused analysis of L2/3 neurons on the relatively small fractions in S1 (~37%) and S2 (~24%) that were task responsive (Fig. 1h). Studies across a wide variety of experimental contexts have revealed sparse activity patterns among cortical L2/3 neurons, but the nature of this sparseness remains poorly understood (reviewed in ref. 48). While we made no attempt to optimize our stimulus for the studied neurons, even systematic exploration of subthreshold whisker receptive fields has shown that activity is sparse among L2/3 neurons in S1 over the course of an experimental session49. The degree to which responses of L2/3 neurons change during learning of tactile tasks remains an active area of research13,17,50. Intriguingly, a recent study using a single-whisker tactile detection task found that detection-related activity emerged with learning specifically in S1 neurons that project to S2 (ref. 17).

We examined cortico-cortical dynamics during detection of passive touch in head-fixed animals, but S1–S2 interactions could depend on motor-sensory context. A recent study24 used electrophysiology to quantify S1 and S2 spiking as rodents moved freely to interact with and identify textured surfaces. This study found that stimuli and choice were encoded via both the rate and timing of spikes across the S1→S2 network24. A second recent study25 imaged activity in S1 and S2, including activity from neurons that project in each direction between these areas, and found behavior-dependent coordination of S1 and S2 activity during texture discrimination. Together, our results suggest that the transformation from raw sensory input to perception occurs via feedforward computations that are reinforced through feedback in a cortico-cortical loop.

METHODS

Methods, including statements of data availability and any associated accession codes and references, are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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Feedforward and feedback synaptic pathways shape how neural activity evolves across cortical areas, but they are difficult to monitor using traditional methods during behavior. The authors use pathway-specific and cellular-resolution microscopy to quantify sensory and decision-related neural activity both within and propagating between two cortical areas critical for touch perception.
ONLINE METHODS

Mice. All procedures were in accordance with protocols approved by the Johns Hopkins University Animal Care and Use Committee. We report calcium imaging experiments from 16 male C57BL/6N (Harlan) mice and from 2 male and 2 female C57BL/6J Thyl-GCaMP6s Gt(ROSA)26Sor tm32(CAG-COP4*H134R/EYFP)Hze/J (Jackson Labs) mice\(^1\), with ages ranging from 9 to 16 weeks. We report optogenetic silencing experiments from 4 females and 1 male obtained by crossing PV-IRES-Cre (Jackson Labs) 080069; B6;129P2-Pou5f1tm1(Cre)Zemb/J\(^2\) with Ai32 (Jackson Labs: 012569; B6;129S-Gt(Rosa)26Sor tm32(CAG-COP4*H134R/EYFP)Hze/J\(^3\)) on a mixed background, with ages ranging from 10 to 12 weeks. Mice were housed in a vivarium with reverse light-dark cycle (12 h each phase). Experiments occurred during the dark phase. Mice were housed in groups of up to five before the start of water restriction, after which mice were housed singly. Assignments of mice to experimental conditions and analyses are detailed in Supplementary Figure 6.

Intrinsic signal imaging. After recovery from headpost surgery (>24 h) or head-post surgery plus GCaMP6 injection and cranial window implantation (7–9 d), mice were lightly anesthetized with isoflurane (0.5–1%) and chloroprocaine (0.02 ml of 0.36 mg ml\(^{-1}\), intramuscular). Intrinsic signal imaging (ISI) was performed as described\(^4\). In all virus-injected mice, the target whisker was right C2. ISI was performed through the cranial window or a clear skull cap\(^5\). Whisker S2 could be identified as a region of decreased reflectance clearly delineated from whisker S1. Sound from the piezo stimulator is a potential source of movement during ISI mapping of regions in the vicinity of S2. As a control experiment to distinguish auditory responses from tactile responses, ISI was occasionally performed without threading the target whisker into the stimulator, which otherwise remained in a nearly identical position. Areas responsive under this condition were considered auditory areas and were distinct from whisker S2. In Thyl1-GCaMP6s mice used for simultaneous S1 and S2 imaging, responses to several different whiskers (right B2, B3, C2, C3) were mapped through the cranial window after recovery from surgery. We selected a target whisker for behavioral training on the basis of the criterion that the target whisker’s responsive regions in both S1 and S2 could be covered by the same two-photon field of view. As a result, the B3 whisker was used in 2 mice, B2 in 1 mouse, and C3 in 1 mouse. We further validated S1 and S2 identification using wide-field fluorescence imaging under the same field of view used for ISI. Taking advantage of homogeneous expression of GCaMP6s across cortex, we identified regions showing evoked fluorescence in response to the same whisker stimulation protocol used for ISI. In all cases, wide-field imaging yielded two clearly separated regions (corresponding to S1 and S2) that matched those identified by ISI.

Behavioral task. Mice were trained to perform a tactile detection task while head-fixed. Behavioral apparatus was controlled by BControl software (C. Brody, Princeton University). For 7–10 d before training, mice received 1 ml d\(^{-1}\) of water. On training days, mice were weighed before and after each training session to determine water consumed. Mice were allowed to perform the task until they licked during the timeout, an additional timeout was triggered. A correct rejection occurred when mice withheld licking during the response window. Correct rejections were not rewarded. After performance reached >65% correct, a 0.1 s auditory cue (8 kHz tone, ~80 dB SPL) was introduced starting 1 s before stimulus onset. During all sessions, ambient white noise (cut off at 40 kHz, ~80 dB SPL) was played through a separate speaker to mask any other potential auditory cues associated with movement of the piezo stimulator. Mice were considered trained when performance reached >70% correct for at least 2 consecutive days. Typically, mice were trained one session per day for 7–10 d to reach this criterion. Trials with premature licks occurring close to stimulus onset (~0.51 s to +0.12 s) were excluded from subsequent analysis. Only 2% of trials had reaction times occurring after the end of this premature lick window but before the end of the grace period. There was a modest trend toward higher pre-stimulus AF\(_F0\) on miss trials in S1 (Supplementary Fig. 7), which could be due to occasional pre-stimulus whisker motion. We considered the possibility that self-generated whisker or tongue movements early in the trial could partly account for the different responses we observed in hit versus miss trials. We used high-speed video to monitor whisker and tongue motion and thereby to obtain a set of trials in which we could confirm negligible motion (Supplementary Fig. 8). We observed a robust hit versus miss difference in evoked AF\(_F0\) for this subset of trials (Supplementary Fig. 8).

Two-photon calcium imaging of layer 2/3 somata. A circular craniotomy was made over the left barrel cortex (2.5 mm diameter; center relative to bregma: lateral, 3.5 mm; caudal, 1.3 mm) of P40–50 mice. The dura was left intact. GCaMP6s or, in the case of one animal used in S1 cell body calcium imaging\(^6\), GCaMP6f, was expressed under the human synapsin-1 (SYN1) promoter following infection with recombinant adeno-associated virus (serotype 2/1, Syn.GCaMP6s.WPRE.SV40, or Syn.GCaMP6f.WPRE.SV40, University of Pennsylvania Gene Therapy Program Vector Core). Injections were made at 4–6 sites within the craniotomy (30–50 nl per site; depth, 250–300 μm; rate, ~1 nl s\(^{-1}\)) using a glass pipette (30–50 μm diameter). After virus injection, the craniotomy was covered with an imaging window made by gluing together two pieces of microscope cover glass\(^7\). The smaller piece (fisher; number 2 thickness) was fitted into the craniotomy and the larger piece (number 1.5 thickness) was glued to the bone surrounding the craniotomy\(^8\). Intrinsic signal imaging was used to localize a barrel column or whisker S2 within the area of the cranial window (7–9 d after window implantation). All whiskers on the right side of the snout except the relevant one (a row C whisker, except as noted below for simultaneous S1 and S2 imaging) were trimmed after the intrinsic signal imaging. Mice were then water restricted for 2 weeks before training. Imaging was started 3–5 weeks after surgery.

For simultaneous imaging of S1 and S2, Thyl1-GCaMP6s mice were prepared using the same method as described above except that a row B whisker (B2 or B3) was used for 3 of 4 mice and C3 whisker for 1 mouse. S1 and S2 were identified using intrinsic signal imaging and wide-field imaging of evoked GCaMP6s fluorescence as described above.

Images were acquired on a custom two-photon microscope (http://open.wiki.janelia.org/wiki/display/sharedesigns/MIMMS) equipped with a resonant scanning module (Thorlabs), GaAsP photomultiplier tubes (Hamamatsu) and a 16× 0.8 NA microscope objective (Nikon). GCaMP6s was excited at 1,000 nm (40–60 mW at specimen) with a Ti:sapphire laser (Chameleon Ultra II, Coherent). Imaging fields were restricted to areas where GCaMP6s expression overlapped with the desired barrel columns or the identified whisker area of S2. For S2 imaging sessions, we rotated mice ≤5 degrees from the sagittal plane to enable access of the microscope objective to the cranial window. Mice exhibited no signs of discomfort. We did not rotate mice for simultaneous imaging of S1 and S2 sessions because the image whisker representation in S2 was located relatively medially. The field of view ranged from 760 μm × 790 μm to 440 μm × 485 μm (440 × 512 pixels; pixel size, 1.72 μm × 1.55 μm to 1.0 μm × 0.94 μm). Images were acquired continuously at 30 Hz using ScanImage\(^9\), 4.2 (http://www.scanimage.org/). A movie, corresponding to a single trial, consisted of 140 image frames. In 2 sessions from 1 mouse, images were acquired at 15 Hz and movies comprised 65 frames.

Retrograde labeling of S2-projecting neurons in S1. A subset of mice that were used for S1 soma imaging experiments were injected post hoc with a retrograde tracer, CTB-Alexa555 (5 μg\(^{-1}\) in PBS, Invitrogen), in S2 localized by intrinsic signal imaging. The post hoc injection occurred shortly after conclusion of behavioral experiments. A small hole was drilled through the
glass cranial window. One hundred nanoliters of CTB-Alexa555 was injected (depth, 400–500 μm; rate, ~1 nl s⁻¹) through the hole via a glass pipette (30–50 μm). The injection site was sealed with dental cement. Seven to 10 d after the injection, the labeled cells were examined under the two-photon microscope. To localize co-labeling, GCaMP6P-labeled cells and CTB-Alexa555-labeled cells were excited at 940 nm or 1,020 nm, respectively (40–60 mW at specimen). Fluorescence emission was separated using a 568-nm dichroic (FS680-DI01-35.5x0.2, Semrock), passed through green (ET525/70m-2p, Chroma) and red (FF01-625/90-30-D, Semrock) channel filters before detection with two GaAsP photomultiplier tubes (Hamamatsu).

Two-photon calcium imaging of axons. A subset of data for S2→S1 axons (4 of 10 sessions) comes from published experiments 25,26 and is analyzed here in greater detail. Adeno-associated virus (serotype 2/1, Syn.GCaMP6.WPRE.SV40) was injected into the C2 barrel column (identified by ISI) for S1 axon imaging at two depths (250 μm and 350 μm; 30–40 nl each; ~1 nl s⁻¹) and the site was covered with a cranial window. For S2→S1 axon imaging, intrinsic signal imaging was performed through the window. GCaMP6s expression was examined under a wide-field fluorescence microscope, and mice showing excessive cell body fluorescence outside the ISI-localized S2 region were not used for experiments. Imaging planes were from layer 2/3 of S2 (150–250 μm from pial surface) for S1→S2 axon imaging or layer 1 of S1 (70–100 μm from pial surface) for S2→S1 axon imaging. The field of view was 100 μm × 108 μm (440 × 512 pixels; pixel size, 0.23 μm × 0.21 μm). Images were acquired continuously at 30 Hz using ScanImage 4.2. A movie, corresponding to a single trial, consisted of 140 image frames.

Optogenetic silencing. PV-IRES-Cre;Ai32 mice were implanted with a clear skull cap 39, or a coverglass over the left barrel cortex following a circular craniotomy. Light from a 473-nm laser (MBL-III-473-100, Ultralasers) was passed through an acousto-optic modulator (MT510-A3-VIS, QuantPhaTech), focused into a multimode optical fiber, recollimated, and directed onto the targeted cortical area. The beam at the skull or cranial window had an approximately Gaussian profile with a full width at half maximum of 600 μm. Photostimulation was randomly delivered on 30–40% of all trials. Photostimulation comprised a train of 5 ms pulses at 100 Hz delivered from −300 ms to +2,200 ms relative to the time of whisker stimulus onset for Go trials. The same time window was used for NoGo trials. Average power at the brain surface was ~3 mW for glass-implanted animals (n = 2) or ~7 mW for those with a clear skull cap 39 (n = 2). A visual masking flash (2 ms pulses at 10 Hz) was delivered for the duration of every trial via a 470-nm LED (7007-PB000-D, LEDDynamics) placed near the eyes. For behavioral experiments, the laser was steered over the C2 column in S1 (localized with ISI), whisker S2, or a control area in separate sessions within the same animal. The control area was separated from the C2 column and S2 illumination sites by ~1 mm, but at stereotactic coordinates still within whisker S1 (4 mm lateral, 0.3 mm caudal to bregma). Cell-attached recordings for S1 axon imaging. The field of view was 100 μm × 108 μm (440 × 512 pixels; pixel size, 0.23 μm × 0.21 μm). Images were acquired continuously at 30 Hz using ScanImage 4.2. A movie, corresponding to a single trial, consisted of 140 image frames.

Two-photon calcium imaging of axons: data analysis. Analysis procedures were as described above except that neuropil subtraction was not implemented. To distinguish ROIs that belong to the same axon from those that belong to different axons, we used a correlation-based method (adapted from ref. 13) to build clusters of highly correlated ROIs (Supplementary Fig. 4). Briefly, varicosities that were clearly part of the same axon were identified by visual inspection (8 sessions). Correlation coefficients of these varicosities were calculated from ΔFt/Fo concatenated from trials over an entire session and were compared to all pairwise correlation coefficients. ΔFt/Fo was calculated as (Ft – Fo)/Fo, where Fo was the 15th percentile of F. Correlation coefficients of varicosities from the same axon were clearly distinct from the rest and showed values larger than 0.7 on average. We selected all sets of two varicosities with correlation coefficient >0.7. These N pairs of varicosities were randomly ordered into a N-tuple, P = (P1, P2, …, PM). We then started a clusterization process by initializing N clusters, Ci = (Ci1, Ci2, …, Cin CiM), where the ith cluster Ci contained the two varicosities from Pi. Next, Pi was tested for inclusion in Ci, if Pi ∩ Ci ≠ ∅, then the elements of Pi were added to Ci, Pi was then tested for inclusion in Ci+1 and Cj, and so on. That is, for all j ≥ i, the elements of Pi were added to Cj if Pi ∩ Cj ≠ ∅. At the end of this process, C contained overlapping clusters. Finally, we reduced each cluster to its unique elements and selected for subsequent analysis only those clusters having no overlap with previous clusters. That is, Ci was selected if and only if Ci ∩ Cj = ∅ for all j < i. For subsequent analysis, ΔFt/Fo was calculated as (Ft – Fo)/Fo, where Fo was the mean F over 8 baseline frames immediately preceding the time of possible stimulus onset on each trial. The ΔFt/Fo for each putative axon was calculated as the mean ΔFt/Fo from all responsive varicosities within a cluster.

Single-neuron ideal observer analysis. We used receiver operating characteristic (ROC) analysis to calculate the detect probability (identical to “choice probability” 25) and stimulus probability. A decision variable (DV) was assigned for each trial on the basis of the neural response. DV was the evoked ΔFt/Fo as defined above. Trials were grouped by the mouse’s choice (lick versus no-lick, for detect probability) or by stimulus condition (present versus absent, for stimulus probability). An ROC curve was obtained by systematically varying the criterion value across the full range of DV (using MATLAB “perfcurve”). The area under the ROC curve (AUC) represents the performance of an ideal observer in categorizing trials based on the DV. Detect probability was the AUC for discriminating choice. Stimulus probability was the AUC for discriminating the stimulus condition. A 95% confidence interval for each AUC was obtained by bootstrap (MATLAB “perfcurve”).

Noise correlation analysis. Noise correlations 33 were quantified as Pearson’s correlation coefficient between evoked ΔFt/Fo values for a pair of neurons across all Go trials. The values were averaged across all pairs to obtain the mean noise correlation for a neuron.

Population decoding analysis. We used Random Forests ensemble classifiers 31 (via MATLAB “TreeBagger” class with 300 trees and minimum leaf size of 1) to discriminate the stimulus condition (Go trials versus NoGo trials) or choice (hits and false alarms versus misses and correct rejections) on the basis of the vector of ΔFt/Fo for all responsive neurons at a given time point (image frame) during the
trial. A separate classifier was trained on each time point. Classifier performance was quantified as the area under the ROC curve for classification of out-of-bag observations. We denote the performance in decoding the stimulus condition based on population responses as SPpop by analogy to our ideal observer ‘stimulus probability’ quantity for single neurons. Similarly, we denote performance on decoding choice as DPpop. In Figure 3, data from 1 mouse were acquired at 15 Hz whereas data from 2 mice were acquired at 30 Hz. We accommodated the slower imaging speed for this 1 mouse as follows. For display in Figure 3d, we combined 15 Hz and 30 Hz movies by excluding every other frame of the 30 Hz movies. Random Forest classification was performed using all frames (65 frames for 15 Hz, 140 frames for 30 Hz). For Figure 3i, decoding performance curves were combined across different imaging speeds by excluding every other frame of the 30 Hz movies. The image frame at which decoder performance diverged was defined as the first frame at which a one-tailed Wilcoxon rank sum test (Figs. 2j,k and 4h,i) or a one-tailed Wilcoxon sign rank test (Fig. 3i,j) reached \( P < 0.05 \). Statistical tests for population decoders were one-tailed and performed across sessions rather than mice because (i) each analysis began with a hypothesis for the sign of any difference, and (ii) each behavioral session yielded one decoder performance curve.

**Data analysis: pooling across sessions and mice.** Our convention in the text and figures is to report variability across mice. Unless otherwise noted, we used two strategies to combine data from multiple behavioral sessions for an individual mouse. For cumulative histograms in Figure 2d, we pooled ROIs across sessions to obtain histograms for each mouse, and then averaged these histograms. For mean \( AF/F_0 \) time series in Figures 2a,b, 3d,e and 4e,f, bar graphs and scatter plots in Figures 1h, 2c, 3f and 4g, plots of SP or DP versus distance in Figure 2i,j, and population decoder time series in Figures 2k,l, 3i,j and 4h,i, we averaged values across sessions for each mouse. Cumulative histograms in Figures 2g,h,m and 3g,h,k were obtained by pooling all data across mice and sessions. The number of monitored or responsive neurons could vary from session to session for a given mouse. For brevity in the text and figure legends, therefore, we report the number of neurons obtained on average across sessions for each mouse. We also report the total number of neurons or observations used for each statistical test.

**Statistics.** We report data as mean ± s.e.m. unless otherwise noted. Statistical tests were two-tailed except where noted. We made no adjustments for multiple comparisons. We chose statistical tests in the following order of decreasing preference: (i) parametric tests when appropriate (paired and unpaired \( t \)-tests); (ii) rank- and sign-based nonparametric tests (Wilcoxon signed rank, Wilcoxon rank sum, sign test); Kolmogorov-Smirnov tests; (iii) randomization tests (permutation and bootstrap). Prior to using \( t \)-tests, we assessed normality using quantile–quantile plots. Prior to using Wilcoxon signed rank tests, we confirmed symmetry of the tested sample about its median (defined as absolute value skewness < 0.6); otherwise we used a sign test.

To test whether the median pairwise distance (Fig. 2g) among SP neurons, \( D_{SP} \), was the same as the median distance among all neurons, \( D_{all} \), we used a bootstrap method. The test statistic was the observed difference in median distances, \( Y = D_{all} - D_{SP} \). A bootstrap replicate, \( Y^* \), was obtained from each of 20,000 bootstrap samples. Each replicate was calculated as the difference between \( D_{all} \) and the median of \( N_0 \) distances sampled with replacement from the set of distances among all neurons, where \( N_0 \) was the number of distances among SP neurons. The \( P \)-value was calculated as the fraction of \( Y^* \) values more extreme than \( Y \). We used the same procedure for DP neurons.

Unless otherwise noted, we calculated confidence intervals using a non-parametric multistage bootstrap method that simulates the data generation process and incorporates both variability among neurons for a given mouse and variability among mice. We calculated the confidence interval for statistic \( Y \) (for example, a mean hit – miss difference across mice) as follows, where \( Y \) was calculated using data from \( N \) mice, and where \( N_0 \) is the number of neurons obtained from the \( m \)th mouse (we first pooled neurons collected in separate sessions for a mouse). First, mice were sampled randomly with replacement to obtain a set of \( N \) primary sampling units (PSUs). Next, \( N_0 \) neurons were sampled with replacement for each PSU. A bootstrap replicate, \( Y^* \), was calculated for each of 50,000 such bootstrap samples. The 95% confidence interval for \( Y \) was calculated as the 2.5th and 97.5th percentile values of \( Y^* \).

We assigned mice of appropriate genotypes to experimental groups arbitrarily, without randomization or blinding. We did not use statistical methods to pre-determine sample sizes. Sample sizes are similar to those reported in the field.

**A Supplementary Methods Checklist is available.**

**Data availability.** The data that support the findings of this study are available from the corresponding author upon request.

**Code availability.** Data analyses were conducted in MATLAB using scripts available from the corresponding author upon request.

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