Origins of choice-related activity in mouse somatosensory cortex

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During perceptual decisions about faint or ambiguous sensory stimuli, even identical stimuli can produce different choices. Spike trains from sensory cortex neurons can predict trial-to-trial variability in choice. Choice-related spiking is widely studied as a way to link cortical activity to perception, but its origins remain unclear. Using imaging and electrophysiology, we found that mouse primary somatosensory cortex neurons showed robust choice-related activity during a tactile detection task. Spike trains from primary mechanoreceptive neurons did not predict choices about identical stimuli. Spike trains from thalamic relay neurons showed highly transient, weak choice-related activity. Intracellular recordings in cortex revealed a prolonged choice-related depolarization in most neurons that was not accounted for by feed-forward thalamic input. Top-down axons projecting from secondary to primary somatosensory cortex signaled choice. An intracellular measure of stimulus sensitivity determined which neurons converted choice-related depolarization into choice-related spiking. Our results reveal how choice-related spiking emerges across neural circuits and within single neurons.

For decades it has been known that the spike trains of sensory cortex neurons can predict trial-to-trial variability in perceptual decisions about identical stimuli1–3. Correlations between sensory neuron spike trains and behavioral choices are often quantified using ‘choice probability’4 or ‘detect probability’5, the probability with which an ideal observer could predict behavioral choice on the basis of neural activity evoked by identical stimuli. Choice-related activity (choice or detect probability >0.5) has been observed in multiple sensory brain areas, including monkey extrastriate 1,2,4,6–10 and primary 11,12 cortex. Choice-related variability in cortical spiking could reflect variability in the primary sensory afferents16, could accumulate in a feed-forward manner17 as activity propagates from the sensory periphery to cortex, or could reflect non–stimulus-driven, ‘top-down’ influences on sensory cortex8. The contributions of these and other sources of variability to cortical responses remain poorly understood.

To address this problem, we developed a preparation to study choice-related activity during tactile detection in mice. We recorded from the primary mechanoreceptive afferent neurons, which transduce tactile stimuli into action potentials; thalamus neurons that provide the main feed-forward drive to cortex; and primary somatosensory (S1, barrel) cortex neurons. Choice-related variability in spike rate was absent in the primary afferents and was weak and transient in thalamic relay neurons. Intracellular recordings in cortex showed a prolonged choice-related depolarization in most neurons. Choice-related activity was present in top-down axons projecting from secondary somatosensory cortex to S1. An intracellular measure of stimulus sensitivity (the touch reversal potential)18–21 determined which neurons converted choice-related depolarization into choice-related spiking.

RESULTS

We trained mice in a simple head-fixed, ‘go/no-go’ tactile detection task (Fig. 1a,b). Each trial began with an auditory cue to alert the mice to the time of possible stimulus onset (Fig. 1b). This cue was intended to eliminate ambiguity about the time at which the stimulus could arrive. A single whisker was deflected with a sinusoidal waveform (0.5 s, 20 or 40 Hz; Online Methods) on 50% of trials (‘go’ trials). On the other 50% of trials (‘no-go’), the whisker was not deflected. Lick responses occurring during a response window (Fig. 1b) determined trial outcome (Fig. 1c). Task performance varied with the strength (angular speed) of the whisker stimulus (Fig. 1d). Spiking responses increased monotonically for these whisker stimuli in the primary afferents, thalamus and S1 (Supplementary Fig. 1a). When we randomly interleaved trials with stimulation of three whiskers rather than one whisker, performance increased even as the single-whisker curve began to saturate (Fig. 1d). Thus, tasks performed with a single whisker may be perceptually demanding for mice, even with relatively strong stimulation19,22,23. Strong multi-whisker stimulation led to high performance (~85% correct), indicating that performance was limited by the stimulus rather than task engagement (Fig. 1d).

For subsequent experiments, we used a stimulus strength (single whisker, ~500–900° s−1)24 that yielded a mixture of detection successes (‘hits’) and failures (‘misses’). Performance with this stimulus was limited by the stimulus rather than task engagement (Fig. 1d).

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and correct no-go (correct-rejection) trials (Fig. 1c), over periods before the typical reaction times (Supplementary Fig. 1). Because whisking produces self-generated tactile input that can affect detection behavior\(^2\)\(^3\), we limited analysis to periods of passive stimulation (Online Methods). Performance was abolished by reversibly silencing somatosensory cortex using optogenetic stimulation of GABAergic neurons\(^1\)\(^9\)\(^2\)\(^2\)\(^3\) (Supplementary Fig. 2).

**Choice-related activity in mouse somatosensory cortex**

We used two-photon calcium imaging to record spiking-related activity\(^2\)\(^6\) of cortical layer 2/3 neurons in the C2 whisker and surrounding columns of S1 (Fig. 1e). Correct-rejection trials (with no stimulus applied) showed no evoked activity (Fig. 1f,g). Hit and miss trials (with identical C2 whisker stimuli applied) both showed robust evoked activity, but evoked responses were larger on hit than miss trials (Fig. 1f,g: 0.018 ± 0.002 versus 0.013 ± 0.002 ∆F/F\(^0\)). Kolmogorov–Smirnov test, P < 1 × 10\(^{-3}\), n = 1,746 neurons in 6 mice).

**No choice-related spiking in primary afferent neurons**

We sought to trace the origins of the choice-related activity we observed in the primary somatosensory cortex. We began at the earliest possible stage: the primary mechanoreceptive afferents that transduce mechanical stimuli into action potentials. These neurons innervate the whisker follicle, have cell bodies located in the trigeminal ganglion and send projections to somatosensory brainstem nuclei. Trigeminal ganglion neurons have single-whisker receptive fields\(^2\)\(^7\). We made extracellular recordings from single trigeminal ganglion neurons while mice performed the detection task using the single whisker in the neuron's receptive field (Fig. 2). We analyzed spike rates during a baseline period before the time of possible stimulus onset and during a window shortly after stimulus onset but before the typical reaction time on go trials. Trigeminal ganglion neurons had low baseline spike rates that did not differ between hit and miss trials (0.77 ± 0.20 versus 0.62 ± 0.28 Hz (median ± interquartile range), P = 0.55, two-tailed sign test, n = 17; Fig. 2c,e), indicating that pre-stimulus activity in primary afferents was not a significant factor in perceptual outcome. Neurons were strongly driven by the whisker stimulus during both hit and miss trials (Fig. 2b,c). There was no
difference in evoked rate between the two trial types (82.5 ± 9.2 versus 79.2 ± 9.9 Hz, \( P = 0.38 \); Fig. 2c,d).

Choice probability\(^1\) is the probability with which an ideal observer could predict the choice of the animal (here corresponding to a hit or a miss), given the neural response. In the context of detection tasks, the same quantity is often referred to as detect probability (DP)\(^4,5\); we adopt this convention here. We asked how well an ideal observer could predict the choice of the animal given the trigeminal ganglion spike trains. We also calculated stimulus probability (SP, Online Methods), which is calculated similarly but quantifies how well an ideal observer could categorize the sensory stimulus (in our case, presence or absence of stimulus) on the basis of the neural response. Trigeminal ganglion neurons robustly signaled the presence or absence of the stimulus (SP calculated over the ‘evoked’ window: 0.94 ± 0.02 (mean ± s.e.m.), \( P < 1 \times 10^{-3} \), two-tailed sign test, \( n = 15 \) recordings). (Online Methods and

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**Figure 3** Transient choice-related activity in VPM thalamus. (a) Extracellular recording in the VPM. Image adapted from the Allen Mouse Brain Atlas, Allen Institute for Brain Science (http://mouse.brain-map.org). (b) Example traces for hit (blue), miss (black) and correct rejection (red) trials. Arrows indicate stimulus onset; blue circles indicate licking. (c) Top, mean peristimulus spike time histograms (2-ms bins; ±s.e.m.) for \( n = 17 \) recordings. Bottom, mean of differences between hit and miss histograms for each neuron (purple; mean ± 95% confidence interval (CI)). Gray traces indicate individual recordings; arrows indicate stimulus onset. (d) Evoked AP rate for hit trials and miss trials during a transient (10-ms) window at the peak of the response (\( P = 0.0065 \), two-tailed Wilcoxon signed rank test, \( n = 17 \)). (e) Evoked AP rate after the peak of the response for hit and miss trials (\( P = 0.63 \), two-tailed sign test, \( n = 17 \)). (f) Pre-stimulus AP rate for hit and miss trials (\( P = 0.14 \), two-tailed sign test, \( n = 17 \)). (g) Mean time course of DP (black) and SP (gray) across all VPM recordings. Arrow indicates stimulus onset.

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**Figure 4** Brief cortical response to transient VPM activity. (a) Simultaneous optogenetic stimulation and extracellular recording in VPM (right) and fluorescence image (left) of channelrhodopsin-2 expression (white) in VPM (dashed line). Image (right) adapted from the Allen Mouse Brain Atlas, Allen Institute for Brain Science (http://mouse.brain-map.org). (b) Example spike rasters from a VPM recording with whisker stimulation alone (black) or whisker stimulation plus weak (dark blue) or strong (light blue) photostimulation. Arrows indicate onset of whisker stimulus; bolts indicate photostimulation. Responses to light alone are shown toward the end of the rasters (delivered 1.5 s after the first pulse). (c) Mean peristimulus spike time histograms (4-ms bins; ±s.e.m.) for whisker stimulus alone (\( n = 12 \) recordings), whisker stimulus plus weak photostimulation (\( n = 12 \)) and whisker stimulus plus strong photostimulation (\( n = 9 \)). Arrow indicates onset of whisker stimulus followed by light pulse (delay of 0–4 ms; Online Methods). Inset, magnified view of peak response. (d) Evoked action potential (AP) rate in a transient (10-ms) window at the peak of the whisker-evoked response is higher for whisker stimulation (W) plus photostimulation (single bolt, weak light: \( P = 0.039 \), two-tailed sign test, \( n = 12 \); double bolts, strong light: \( P = 0.039 \), two-tailed sign test, \( n = 9 \)) than for whisker stimulation alone. (e) Evoked AP rate after the peak of the whisker-evoked response showed no differences between whisker stimulus alone and whisker plus photostimulation (weak light: \( P = 0.57 \), \( n = 12 \); strong light: \( P = 1.0 \), two-tailed sign test, \( n = 9 \)). (f) Pre-stimulus AP rate is similar for trials with whisker stimulus alone vs. whisker stimulus plus photostimulation (weak light: \( P = 0.34 \), \( n = 12 \); strong light: \( P = 0.57 \), \( n = 9 \)). (g) Simultaneous optogenetic stimulation (bolt) in VPM and cell-attached recording in S1. (h) Example spike rasters from an S1 recording. Conventions as in b. (i) Mean peristimulus spike time histograms (4-ms bins; ±s.e.m.) for \( n = 15 \) recordings. Arrow indicates onset of whisker stimulus followed by light pulse (4-ms delay; Online Methods). (j) Evoked AP rate for whisker stimulus alone and whisker stimulus plus photostimulation (weak light: \( P = 0.61 \), two-tailed sign test, \( n = 15 \); strong light: \( P = 1 \times 10^{-3} \), \( n = 15 \)). (k) Evoked AP rate after the peak of the whisker-evoked response showed no differences between whisker stimulus alone and whisker stimulus plus photostimulation (weak light: \( P = 0.54 \), \( n = 15 \); strong light: \( P = 0.42 \), two-tailed sign test, \( n = 15 \)). (l) Pre-stimulus AP rate is similar for trials with whisker stimulus alone and whisker stimulus plus photostimulation (weak light: \( P = 0.71 \), \( n = 15 \); strong light: \( P = 0.36 \), \( n = 15 \)). (m) Behavioral hit (top) and false alarm (FA, bottom) rates for three mice (designated by color) during experiments in g–l. NS, no stimulus. Data are mean performance ± bootstrap s.e.m. from 5–6 sessions per mouse. n.s., \( P > 0.05 \); *\( P < 0.05 \); **\( P < 0.001 \).
Figure 5 Choice-related membrane potential dynamics in S1 cortex. (a) Top, intracellular (whole-cell) recording in primary somatosensory (barrel) cortex. Bottom, example membrane potential (V_m) traces for hit (blue) and miss (black) trials. Image adapted from the Allen Mouse Brain Atlas, Allen Institute for Brain Science (http://mouse.brain-map.org/). (b) Removing spikes from V_m traces. Top, example raw V_m traces; action potentials (APs; shown truncated) are evident in the hit and miss trials. Bottom, V_m traces after median filtering and smoothing to eliminate APs. Arrows indicate stimulus onset for hit and miss traces. (c) Top, mean V_m change after AP removal (s.e.m.; n = 22 neurons) for hit (blue), miss (black) and correct rejection (red) trials. Bottom, mean of differences between mean V_m on hits and mean V_m on misses (purple; mean ± 95% confidence interval (CI); n = 22 neurons). Gray traces indicate individual recordings. (d) Left, stimulus-evoked ΔV_m is larger on hit trials than on miss trials (P < 1 × 10^{-3}, two-tailed sign test, n = 22). Right, histogram of the mean difference in ΔV_m between hits and misses for each neuron (arrow indicates mean at 1.4 mV). (e) Pre-stimulus membrane potential dynamics are similar for hit and miss trials, both mean V_m (P = 0.13, n = 22) and s.d. of V_m (P = 0.64, two-tailed Wilcoxon signed-rank test). (f) DP (left) and SP (right) computed from ΔV_m. Horizontal bars and asterisks indicate means ± 95% confidence interval. Dashed gray lines indicate chance level. (g) Mean time course of DP (black) and SP (gray) across V_m recordings (n = 22). VPM DP (blue) is shown for comparison (same data as in Fig. 3g). (h) Extracting AP times from V_m traces. Top, example V_m traces from an S1 neuron for hits, misses and correct rejections. Bottom, AP rasters obtained from the example traces. (i) Mean time course of DP and SP calculated using AP times instead of V_m, across the top one-third of neurons ranked by DP (n = 7). (j) Left, DP calculated for each neuron using AP rate or evoked change in V_m. Right, SP calculated for each neuron using AP rate or evoked change in V_m. Neurons that did not spike had AP detect and stimulus probability values set to 0.5. Purple symbols represent neurons included in traces in i, n.s., P > 0.05; *** P < 0.001. H, hit; M, miss; CR, correct rejection.

Fig. 2f) but not the subsequent perceptual choice of the animal (DP over the evoked window: 0.52 ± 0.02, P = 0.41 (Fig. 2f).

Transient, weak choice-related spiking in thalamus

Choice-related activity was present in primary somatosensory cortex (Fig. 1), but not in the primary mechanoreceptive afferents (Fig. 2). Does choice-related activity emerge en route to cortex? Whisker touch information arrives to the cortex largely along thalamocortical axons from the ventral posteromedial nucleus (VPM) of the thalamus. We recorded extracellular multi-unit spiking activity (typically ~2–5 neurons) from regions of VPM that represent the whisked used to solve the detection task (Fig. 3a,b and Supplementary Fig. 3).

Baseline VPM spike rate was similar for hits and misses (20.0 ± 2.6 versus 18.7 ± 2.6 Hz, P = 0.14, two-tailed sign test, n = 17; Fig. 3c,f), indicating that at the thalamic level, pre-stimulus spike rate was not a crucial determinant of perceptual choice. On hit and miss trials, thalamic activity increased rapidly after stimulus onset (latency from onset of whisker stimulation: 5–6 ms) and peaked early (~10–12 ms). VPM activity showed dramatic adaptation during the stimulus28 (Fig. 3c). Spike rates on hit trials were slightly higher than on miss trials but only during a transient (~10–ms) window around the peak of the response (162.7 ± 21.0 versus 125.2 ± 26.0 Hz, P = 0.0065; Fig. 3c,d). This transient difference was also apparent in a measure of thalamic synchrony29,30 (Supplementary Fig. 3). Shortly after the peak response, spike rates on hits and misses were no longer different (51.4 ± 7.5 versus 50.1 ± 7.7 Hz, measured in a 100-ms window starting 15 ms after stimulus onset, P = 0.63, two-tailed sign test; Fig. 3e). Spike rates on hits and misses did not differ during any other period in the trial (we did not analyze periods after the typical reaction time).

To quantify the ability of an ideal observer to predict the mouse’s choice and the stimulus from VPM activity, we calculated DP and SP (Fig. 3g). DP measured by spike rate over a brief window at the peak of the response (from 5–14 ms after stimulus onset) was significantly higher than chance (0.6 ± 0.3, P = 0.0086, two-tailed Wilcoxon signed rank test of DP = 0.5). This choice-related activity was fleeting, and disappeared immediately after the first peak in stimulus-evoked activity (mean DP from 15–114 ms after stimulus onset: 0.51 ± 0.03, P = 0.59). SP peaked early (at peak: 0.85 ± 0.03, P < 1 × 10^{-3}), but remained above chance for the duration of the stimulus (data not shown). Thus, VPM shows a weak, highly transient difference in stimulus-evoked spike rate that predicts choice.

What is the cortical impact of this transient, choice-related difference in thalamic spiking? First, we optogenetically approximated the spiking obtained on hit and miss trials (Fig. 3) by implanting an ‘optrode’ in the VPM of mice expressing channelrhodopsin-2 in VPM (Fig. 4a–f, Online Methods). We paired whisker deflection with VPM photostimulation (after a brief delay of 0–4 ms from onset of whisker deflection) by implanting an ‘optrode’ in the VPM of mice expressing channelrhodopsin-2 in VPM (Fig. 4a–f, Online Methods). We paired whisker deflection with VPM photostimulation (after a brief delay of 0–4 ms from onset of whisker deflection) by implanting an ‘optrode’ in the VPM of mice expressing channelrhodopsin-2 in VPM (Fig. 4a–f, Online Methods). We paired whisker deflection with VPM photostimulation (after a brief delay of 0–4 ms from onset of whisker deflection) by implanting an ‘optrode’ in the VPM of mice expressing channelrhodopsin-2 in VPM (Fig. 4a–f, Online Methods). We paired whisker deflection with VPM photostimulation (after a brief delay of 0–4 ms from onset of whisker deflection) by implanting an ‘optrode’ in the VPM of mice expressing channelrhodopsin-2 in VPM (Fig. 4a–f, Online Methods). We paired whisker deflection with VPM photostimulation (after a brief delay of 0–4 ms from onset of whisker deflection) by implanting an ‘optrode’ in the VPM of mice expressing channelrhodopsin-2 in VPM (Fig. 4a–f, Online Methods). We paired whisker deflection with VPM photostimulation (after a brief delay of 0–4 ms from onset of whisker deflection) by implanting an ‘optrode’ in the VPM of mice expressing channelrhodopsin-2 in VPM (Fig. 4a–f, Online Methods).
n = 9; Fig. 4c–d). Spike rates in a window immediately after the peak of the whisker-evoked response were identical (whisker alone versus whisker + weak light: 82.5 ± 10.3 versus 78.5 ± 9.7 Hz, P = 0.57; n = 12; versus whisker + strong light: 79.0 ± 13.7 versus 83.9 ± 16.6 Hz, P = 1.0, two-tailed sign test, n = 9; Fig. 4e). Thus, photostimulation approximated the transient difference in thalamic spiking we observed previously between hits and misses (Figs. 3c and 4c).

Next, we performed single-unit (loose-seal cell-attached) recordings in the somatotopically aligned region of S1 (in the cortical column representing both the whisker used to solve the task and the stimulated region of VPM; Online Methods) while mice with an optrode in VPM performed the detection task (Fig. 4g–m). Photostimulation in VPM led to a brief increase in S1 spike rate (whisker-alone versus whisker + weak light: 15.1 ± 5.8 versus 22.9 ± 8.1 Hz, P = 0.61, two-tailed sign test, n = 15; versus whisker + strong light: 15.1 ± 5.8 versus 57.2 ± 11.8 Hz, P < 1 × 10−3, n = 15; Fig. 4i,j). The increase in S1 spiking was brief (~30 ms), even with strong photostimulation (in a window immediately after the peak whisker-evoked response: whisker-alone versus whisker + weak light: 9.7 ± 3.7 versus 9.1 ± 3.9 Hz, P = 0.54, n = 15; versus whisker + strong light: 9.7 ± 3.7 versus 2.6 ± 4.8 Hz, P = 0.42, two-tailed sign test, n = 15; Fig. 4k and Supplementary Fig. 4). For strong but not weak photostimulation, the increase in spiking was followed by a trend toward modest inhibition (Fig. 4k). Strong stimulation produced a decrease in hit rate (Fig. 4m). A decreased hit rate in the presence of late inhibition is consistent with a finding that the late cortical response to whisker deflection determines behavioral detection19.

Together, our photostimulation experiments show that a transient increase in thalamic relay neuron spiking, such as that differentiating hits from misses, produces a transient increase in cortical spiking but not in hit rate.

**Prolonged choice-related depolarization in cortex**

Intracellular recording, recently made possible in task-performing animals3,9,32,33, reveals the subthreshold membrane potential (V_m) dynamics that govern spiking. To investigate the transformation of thalamic and other synaptic inputs into choice-related spiking, we made intracellular (whole-cell) recordings from non–fast-spiking cortical neurons across all layers (n = 22) during our detection task (Fig. 5a; Supplementary Fig. 5). We used intrinsic signal imaging (Online Methods) to target recordings to the cortical column representing the whisker used to solve the task.

Our whisker stimuli depolarized the membrane potential of nearly all recorded neurons (21 of 22). These V_m responses occurred at short latency (~7 ms on average). We analyzed V_m on hit, miss and correct rejection trials after removing spikes from the raw V_m traces (Fig. 5b). Depolarization was larger on average for hits than for misses (Fig. 5c). This difference emerged rapidly after stimulus onset (within approximately 30 ms; initial V_m slopes were greater on hits versus misses: 0.53 ± 0.08 versus 0.44 ± 0.10 mV/ms; hit – miss difference: 0.10 ± 0.03 mV/ms, P = 0.0049) (Fig. 5c and Supplementary Fig. 5b) and persisted for the remainder of the trial (Supplementary Fig. 5). The stimulus-evoked change in membrane potential (ΔV_m) (Fig. 5c) was larger on hits than on misses (5.7 ± 0.75 versus 4.3 ± 0.72 mV, P < 1 × 10−3, two-tailed sign test, n = 22; Fig. 5d). Approximately 90% (20 of 22) of neurons showed larger ΔV_m on hit trials. In contrast, we observed no difference in hit versus miss trials in pre-stimulus V_m mean or s.d. (mean: −59.6 ± 1.3 versus −59.1 ± 1.3 mV, P = 0.13; s.d.: 2.4 ± 0.21 versus 2.5 ± 0.23 mV, P = 0.64; Fig. 5e). Thus, successful detection is associated with a widespread difference in stimulus-evoked depolarization19.

How well do membrane potential dynamics predict the choice of the mouse on a trial-by-trial basis? We again calculated DP on the basis of ΔV_m rather than spike rate. The majority of neurons (15 of 22) showed choice-related membrane potential dynamics (DP over evoked window: 0.58 ± 0.03, P = 0.04, two-tailed sign test, n = 22 neurons; 95% confidence interval: (0.52, 0.65); Fig. 5f). ΔV_m also strongly discriminated stimulus presence from absence (SP: 0.83 ± 0.03, P < 1 × 10−3, two-tailed sign test; Fig. 5f). SP rose above chance level rapidly following stimulus onset (within 8 ms), peaked early (15 ms) and remained above chance level for the remainder of the trial (Fig. 5g and Supplementary Fig. 5). DP rose following stimulus onset (within ~10–12 ms) and persisted above chance level for the remainder of the trial (Fig. 5g and Supplementary Fig. 5).

Comparison of VPM spike rate and cortical V_m on hits and misses showed distinct time courses, with a transient difference between hit and miss trials in VPM and a prolonged difference in V_m (Supplementary Fig. 6). Similarly, detect probabilities calculated.
separately from VPM spike rate (Fig. 3g) and cortical Vₘ showed notable differences in dynamics (Fig. 5g). In thalamus, DP showed a brief ‘bump’ immediately after stimulus onset that rapidly disappeared (Fig. 5g). In cortical Vₘ, DP increased more slowly and persisted for the duration of the trial (Fig. 5g). This difference in time course was not explained by convolving the VPM activity with an exponential kernel chosen to simulate the passive membrane time constant of cortical neurons (Supplementary Fig. 6). Moreover, the prolonged hit-versus-miss difference in ∆Vₘ (Fig. 5c) contrasted sharply with the transient increase in S1 spiking produced by optogenetic microstimulation in VPM (Fig. 4i,j).

To examine the relationship between choice-related depolarization and choice-related spiking in individual cortical neurons, we obtained spike times from our Vₘ recordings (Fig. 5h). We calculated DP on the basis of spike rate for the same neurons in which we had previously calculated DP on the basis of Vₘ (Fig. 5i,j). Across the neurons that spiked during our recordings (19 of 22; our methods allowed us to sample rarely spiking neurons)³¹, mean spike rate DP was 0.54 ± 0.02 (P = 0.21, two-tailed sign test of DP = 0.5). Spike rate DP and SP both rose after stimulus onset in a subset of neurons (Fig. 5i; the precise time courses of spike rate and subthreshold detect probabilities can differ owing to factors such spike rate adaptation). Comparison of detect probabilities calculated from spike rate and membrane potential did not reveal a clear relationship (Fig. 5j). Nearly all neurons (21 of 22) discriminated the stimulus condition (present versus absent) in their membrane potential (SP > 0.5), but only a subset did so in their spiking (Fig. 5j).

Top-down feedback of choice-related activity to S1
We observed prolonged choice-related depolarization in most S1 neurons (Fig. 5c,d). Choice-related differences in the rapid, feedforward response to whisker stimulation were too transient to explain this prolonged choice-related depolarization (Figs. 3 and 4 and Supplementary Figs. 4 and 6). The late phase of this cortical response may be relevant for the perceptual decision.¹⁹ To investigate sources of choice-related activity other than feed-forward drive, we used two-photon calcium imaging during behavior to monitor top-down axons projecting from the secondary somatosensory (S2) cortex to layer 1 of S1 (Fig. 6a,b). Inputs to layer 1 are thought to provide state-dependent modulation of cortical activity.⁳⁵

Whisker stimulation produced activity-dependent increases in axonal fluorescence (Fig. 6c,d). These top-down inputs showed enhanced responses on hits as compared with misses (Fig. 6d) before the earliest reaction times (Fig. 6e; 0.027 ± 0.003 versus 0.015 ± 0.003 ΔF/F₀, P = 0.0078, Kolmogorov–Smirnov test, n = 167 axons from 4 mice). Activity in these axons predicted choice on a trial-by-trial basis (Fig. 6f; DP, mean: 0.529 ± 0.005; 95% confidence interval: (0.516, 0.534); median: 0.528). Thus, S1 receives top-down input from S2 that predicts perceptual choice.

Vₘ stimulus sensitivity predicts choice-related spiking
Not all sensory cortex neurons show choice-related activity.¹ This neuron-to-neuron variability is poorly understood.³³,⁶ We analyzed our intracellular recordings in more detail to understand what determines whether a neuron converts a widespread choice-related depolarization (Fig. 5c,d) into choice-related spiking.

First, we asked whether three biophysical quantities that affect neuronal excitability could explain which neurons showed choice-related spiking: (i) the membrane potential at which a spike is initiated (Vₘ), (ii) the resting Vₘ (Vₘ-rest) and (iii) Vₘ - Vₘ-spike, which sets the amplitude of depolarization necessary to cause a spike. None of these simple measures of excitability correlated with spike rate DP (Fig. 7a).

Next, for each neuron we calculated the whisker stimulation reversal potential (Vₘrest) (Fig. 7b). Vₘ-rest, is the membrane potential toward which a stimulus drives a neuron. If Vₘ-rest exceeds spike threshold (Vₘ-rest > Vₘ-spike), the stimulus will drive the neuron to spike. Vₘ-rest is thus an intracellular measure of stimulus sensitivity.³³,⁶,²⁰

As expected, there was a strong correlation between spike rate SP (a measure of stimulus sensitivity) and Vₘ-rest - Vₘ-spike (Fig. 7c) (R² = 0.6, P = 0.001). Notably, we found that Vₘ-rest - Vₘ-spike also strongly predicted spike rate DP (R² = 0.6, P = 0.001 (Fig. 7c); the effect was mainly...
due to $V_{rev}$: $R^2 = 0.44$, $P = 0.0096$ (Supplementary Fig. 7)). The subthreshold stimulus sensitivity of a neuron, therefore, accounts for its spike rate DP.

If spike rate DP and SP are both determined by subthreshold stimulus sensitivity ($V_{rev} - V_{spike}$), they should be correlated\(^1\).\(^6\).\(^10\).\(^12\).\(^37\).\(^38\). To test this prediction, we calculated DP and SP for 1,746 layer 2/3 neurons from our two-photon imaging data set. DP and SP were weakly but significantly correlated (n = 1,746 neurons in 6 mice; $R^2 = 0.14$, $P < 1 \times 10^{-3}$) (Fig. 7d and Supplementary Fig. 8).

Together, these results show that the stimulus sensitivity of a neuron determines whether it will convert a widespread choice-related depolarization into choice-related spiking.

**DISCUSSION**

We traced perceptual choice-related variability in spike rate across the early somatosensory system of mice from primary afferents to cortex. Choice-related variability in spike rate was absent in the primary afferents and was weak and transient in the thalamus. In contrast, choice-related activity was prominent in the cortex. The transient choice-related differences in VPM thalamic spiking could not account for the prolonged choice-related activity in the cortex. Top-down axons projecting from secondary to primary somatosensory cortex signaled upcoming choice. Within single cortical neurons, an intracellular measure of stimulus sensitivity governed the mapping of a widespread choice-related depolarization into spiking.

Microneurography studies in humans\(^16\) and a recent study in monkeys\(^39\) have demonstrated the power of combining psychophysics with single-unit recording from primary afferents. We have adapted this approach to the mouse, a genetically tractable organism, by recording from primary afferents during a perceptual task. In humans, receptor type and location shape the ability to perceive spikes from primary afferents\(^16\). Our approach permits monitoring and manipulating genetically defined classes of receptor neuron to assay their impact on perceptual behavior.

What are the contributions of feed-forward, feedback and local circuit processing to cortical choice-related activity? Here we found a significant but transient DP in thalamic relay neurons of VPM, which provide the main feed-forward drive to whisker S1. Though choice (and detect) probability has been studied almost entirely in the cortex, a previous study reported significant choice probabilities in subcortical structures\(^6\). The brief time course of DP in VPM, however, argues against the possibility that cortical choice-related activity is simply inherited in a passive feed-forward manner from VPM. Moreover, pairing optogenetic stimulation in VPM with S1 recordings showed that a transient difference in feed-forward thalamic spiking could not by itself generate a sustained difference in S1 activity (for example, via local processing within the cortical column).

We observed choice-related activity in a feedback circuit comprising axons projecting from S2 to S1. This supports a prior finding of top-down contributions to choice probability in monkey visual cortex\(^8\) and is consistent with the stronger choice-related activity in S2 than in S1 of monkeys\(^14\). Additional long-range inputs to S1—such as neuromodulatory\(^40\) axons, projections from the higher-order posteromedial thalamic nucleus\(^41\) or top-down projections from other motor-sensory areas\(^14\).\(^42\).\(^43\) may also have a role. We found that mouse S1 neurons showed robust choice-related activity. In contrast, during a tactile detection task in monkeys, choice probability exceeds chance levels only in areas downstream of S1 (refs. 14,44). Because of the greater number and specialization of somatosensory areas in primates, features of neural activity present in mouse S1 may first emerge at downstream areas in primate.

The extent to which different sensory brain areas show choice-related activity, and the role of task features, is an active topic of investigation\(^9\).\(^11\).\(^12\).\(^44\).\(^45\).

We applied ideal-observer analysis based on spike rate (over tens to hundreds of milliseconds) to recordings from primary afferents, thalamus and cortex. We did this to understand variability in cortical spike rate, which has been widely observed to predict choice\(^1\).\(^2\).\(^4\).\(^6\).\(^11\).\(^13\).\(^14\). However, the relevant features of neural activity can differ across areas\(^30\) and even in S1 may encompass features other than spike rate\(^24\). Indeed, we found that hits and misses differed slightly in a measure of thalamic synchrony\(^29\).\(^30\). Future work should compare alternative approaches to decode choice on the basis of multiple features of neural activity. In addition, computational work suggests that choice probability is shaped by patterns of interneuronal correlations, which may differ across perceptual tasks\(^3\).\(^7\).\(^36\).\(^46\).\(^47\). Future work is required to understand whether our findings during tactile detection generalize to other tasks, such as the discrimination of similar stimuli.

We found no clear effect of pre-stimulus neural activity on detection in primary afferents, thalamus or cortex. However, we quantified pre-stimulus activity using only simple measures. More sophisticated analyses, such as those based on population decoding\(^48\).\(^49\) or precise knowledge of network oscillations\(^50\), might reveal an influence of pre-stimulus brain state on perceptual choice. In addition, we aimed to reduce variation in pre-stimulus behavioral state by alerting the mice to the time of possible stimulus arrival on every trial (via an auditory cue). This may have encouraged heightened levels of vigilance and/or affected neuromodulatory\(^46\).\(^50\) systems that can alter neural variability.

Neurons with higher choice probability tend to be more sensitive to the stimulus (for example, they may have lower ‘neurometric’ thresholds)\(^1\).\(^6\).\(^10\).\(^12\).\(^37\). This is intriguing because it suggests that more sensitive neurons contribute more to perceptual decisions. However, computational\(^46\) and theoretical work\(^38\).\(^47\) has shown that multiple factors, including both read-out strategies\(^47\) and interneuronal correlations, can shape choice probability\(^3\).\(^7\).\(^36\). Here we provide a mechanistic view of the correlation between choice probability and stimulus sensitivity at the level of membrane potential in single cortical neurons. Specifically, we show that the subthreshold stimulus sensitivity of a neuron explains both its spike rate stimulus sensitivity and its DP.

Our results trace the emergence of choice-related activity as sensory information is transformed from primary afferents to cortex, and within individual cortical neurons. Choice-related variability in neural activity emerged largely at the cortical level. Choice-related activity was relatively weak along the rapid feed-forward pathway but was prominent in a major feedback pathway to S1 cortex. A widespread, subthreshold choice signal was transformed into spiking in a subset of cortical neurons according to their stimulus sensitivities. Our work offers a key step toward mechanistic dissection of correlations between sensory cortex activity and perceptual choice.

**METHODS**

Methods and any associated references are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS
H.Y., S.E.K. and D.H.O. planned the project. H.Y., S.E.K. and K.S.S. performed experiments. H.Y. and D.H.O. built the apparatus. All authors analyzed data. H.Y., S.E.K. and D.H.O. wrote the paper with comments from K.S.S.

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Mice. Mice were C57BL/6N Hsd (Harlan) and were adults (>8 weeks) except where specified. We report 22 whole-cell recordings from S1 (barrel) cortex in 17 males with ages ranging 7–12 weeks; 17 extracellular recordings in VPM during behavior from 4 males; 48 total recordings with optogenetic VPM stimulation from mice obtained by crossing Scnn1a-Tg3-Cre51 (Jackson Labs: 009613; B6;C3-Tg(Scnn1a-cre)Ai32(Rosa)/Mmum) mice with Ai32 (ref. 52) (Jackson Labs: 012569; B6;129S-Gt(Rosa)26Sortm1(CAG-COP4*H134R/EYFP)litter (Jackson Labs) with Ai32 mice on a mixed background: 12 from VPM in 3 females, 21 from S1 of awake mice in 2 males, and 15 from S1 during behavior in 1 male and 2 females; 17 extracellular recordings in trigeminal ganglion (TG) during behavior from 6 males; neuronal stimulus-response curves from 16 loose-seal cell-attached recordings in S1 in 2 females, 15 extracellular recordings in VPM in 4 females, 9 extracellular recordings in TG in 2 males; two-photon calcium imaging data from L2/3 neurons in 6 males; axon imaging data from 4 males; muscimol silencing experiments in 2 males; optogenetic silencing experiments in 2 females obtained by crossing PV-IRES-Cre53(Jackson Labs: 008069; B6;129P2-Pvataltm1(cre)Arbr) with Ai32 mice on a mixed background; behavior experiments from 6 adult C57BL/6N Hsd males and 1 adult male obtained by crossing TH-Cre (Jackson Labs: 086001) with Ai32 mice on a mixed background. Mice were housed in a vivarium with reverse light-dark cycle (12 h each phase). Experiments occurred during the dark phase. Mice were house in groups of up to 5 before the start of water restriction, after which mice were housed singly. All procedures were in accordance with protocols approved by the Johns Hopkins University Animal Care and Use Committee.

Surgery for electrophysiology. Titanium head posts were implanted for head fixation22. Briefly, mice were anesthetized (1–2% isoflurane in O2; Surgivet) and mounted in a stereotaxic apparatus (David Kopf Instruments). Body temperature was maintained with a thermal blanket (Harvard Apparatus). Scalp and periosteum over the dorsal surface of the skull were removed. The skull surface was scored with a dental drill and the head post was affixed using cyanoacrylate mounted in a stereotaxic apparatus (David Kopf Instruments). Body temperature was monitored in A31 mice (ref. 52) (Jackson Labs: 012569; B6;129S-Gt(Rosa)26Sortm1(CAG-COP4*H134R/EYFP)litter) mice on a mixed background: behavior experiments from 6 adult C57BL/6N Hsd males and 1 adult male obtained by crossing TH-Cre (Jackson Labs: 086001) with Ai32 mice on a mixed background. Mice were housed in a vivarium with reverse light-dark cycle (12 h each phase). Experiments occurred during the dark phase. Mice were house in groups of up to 5 before the start of water restriction, after which mice were housed singly. All procedures were in accordance with protocols approved by the Johns Hopkins University Animal Care and Use Committee.

Intrinsic signal imaging. After recovery from head post surgery (>24 h), mice were anesthetized with light isoflurane (0.5–1%) and chlorprothixene (0.02 ml of 0.36 mg/ml IM). Intrinsic signal imaging (ISI) was performed as described22. In most cases, the target whisker was right C2. In rare cases C2 was missing at the time of ISI, and D2 or C3 was substituted. ISI was performed through the skull, typically with the skull covered by a thin layer of cyanoacrylate adhesive and in some cases also by a thin layer of clear nail polish (Electro-Microscopy Sciences) to reduce glare.

Behavioral task. Behavioral apparatus was controlled by BControl software (C. Brody, Princeton University). For 7–10 d before training, mice received 1 ml per day 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 satiated. Additional water was given if mice consumed <0.3 ml. In the first 1–2 sessions, mice received a drop of water (~6 µl) each time the tongue contacted a ‘lickport’ tube placed near their snouts. In subsequent sessions, mice were operantly conditioned to lick at the lickport in response to a passive whisker deflection. The target whisker (on the right side of the face for S1 and VPM recordings, on the left side for TG recordings) was threaded into a glass pipette attached to a piezo actuator (Piezo Systems), with ~3–5 mm at the base exposed. In S1 experiments, all whiskers except the target whisker were trimmed to near the base. In VPM and TG experiments, all whiskers were shortened. For training, on go trials, the whisker was deflected for 1.5 s with a 40-Hz sinusoidal deflection (rostral to caudal, peak angular speed ~800 deg/s). A ‘hit’ trial occurred when mice licked within a response window, and a drop of water was delivered (~6 µl). The response window was defined as 0.2–2 s after onset of whisker stimulation. The initial 0.2 s after stimulus onset was a ‘grace period’ in which licks had no consequence. On go trials, if mice did not lick within the 1.8-s response window, the trial was scored as a ‘miss’ and no reward or punishment was delivered. Go trials were randomly mixed with no-go trials, in which the whisker was not deflected. No more than three consecutive trials of the same type were allowed. On no-go trials, if mice licked within the response window, it was scored as a ‘false alarm’, and mice were punished with a 3–5-s time-out. If mice licked during the time-out, an additional time-out was triggered. A ‘correct rejection’ occurred when mice withheld licking during the response window. Correct rejections were not rewarded. After performance reached >65% of trials correct, the duration of the whisker stimulus was shortened to 0.5 s. After performance reached >65% correct with the shorter stimulus, 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 speaker separate 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 two consecutive days (0.5 s, ~800 deg/s stimulus). Typically, mice were trained one session per day for 1–2 weeks to reach this criterion. The behavioral task for two-photon imaging sessions used a 20 Hz (0.5 s) sinusoidal whisker deflection instead of 40 Hz. One additional step was implemented for the mice trained for VPM and TG recordings. In later training sessions, the target whisker was selected arbitrarily among the large caudal vibrissae to ensure that mice could generalize the task to other whiskers.

For the single-vs-three whisker psychometric curve experiments (Fig. 1d), three mice were trained initially with single whisker deflections (rostral-caudal, peak angular speed ~800 deg/s). Later, a second piezo stimulator was introduced and mice were tested using deflections of either a single whisker (C3) or three whiskers (C1, C2, C3 deflected simultaneously), with four different angular speeds ranging ~300–1,200 deg/s. In total, there were nine trial types (eight go types: single- and three-whisker deflection, each with four speeds, and one no-go type). Thus, each session had eight hit rates and one false alarm rate. Trials were selected randomly from among these nine types, subject to the constraint that no-go trials were 50% of all trials. Performance with stronger, multi-whisker stimulation (Fig. 1d) was assayed in a separate experiment after training on the single-whisker task. The piezo stimulator was positioned in a vertical configuration such that the glass pipette was in contact with multiple (3–5) whiskers. The auditory cue was omitted. On go trials, multiple whiskers were deflected for 1.5 s at 40 Hz (rostral-caudal, ~2,200 deg/s).

Mice sometimes began a behavior session with impulsive licks (false alarm rate >0.4 and/or miss rate <0.1), or, in cases where mice had to wait to start the task after a prolonged search for an electrophysiological recording, a low hit rate (<0.16). These blocks of initial trials were excluded from analysis (49 ± 29, mean ± s.d.). We focused our analyses on hit, miss and correct-rejection trials for the following reasons: (i) false alarm trials in mice can be due in part to impulsive licking, and (ii) we had few false alarm trials to work with in our data set (after excluding any trials at the beginning of the session that reflected impulsive licking). Analysis of this limited data set confirmed that responses on false alarm trials were larger than those on correct rejections (Supplementary Fig. 9).

Trigeminal ganglion recording. Under isoflurane (1–2%), a craniotomy of 1 mm × 2 mm (medial-lateral, anterior-posterior) was made over the left hemisphere, centered at 1 mm posterior to bregma and 1 mm lateral to midline. Mice were transferred to the behavior-recording apparatus and lightly anesthetized (0.5–1.5% isoflurane). A tungsten microelectrode (1.0 or 2.0 MΩ nominal impedance, parylene-coated, WPI) was advanced vertically through the brain (MP-225, Sutter) at the speed of 50–100 µm/s until reaching the depth of 5.3 mm, then slowly advanced (~10 µm/s) while ipsilateral whiskers were stimulated manually to find whisker-responsive neurons in TG. Evoked spikes were monitored via oscilloscope (Tektronix) and audio monitor (A-M Systems). The craniotomy was typically covered with 2% agarose (Sigma-Aldrich) in cortex buffer (in mM: 125 NaCl, 5 KCl, 10 Glucose, 10 HEPES, 2 CaCl2, 2 MgSO4, pH 7.4) to reduce brain movement. After successful identification of whisker responses in TG, the penetration site was marked as a reference for future recordings.

During subsequent behavioral sessions, mice were first lightly anesthetized (0.5–1.5% isoflurane). A tungsten microelectrode (1.0 or 2.0 MΩ nominal, WPI) was advanced to the previously mapped region. After locating a single unit and determining its single whisker receptive field, the whisker was inserted into the whisker stimulator in its resting position. Test stimuli identical to those used during
the behavioral task were applied with the whisker stimulator. Neurons that could be manually stimulated but could not be made to spike with the rostral-caudal whisker stimulation were not recorded. If piezo stimulation evoked spikes, isoflurane was turned off, and the animal was allowed to recover for at least 15–30 min. After recovery, the behavioral task was initiated. Electrophysiological signals were amplified (1,000×; filtered at 300–3,000 Hz (DAM80, WPI) and acquired at 20 kHz with Ephus (http://www.ephus.org). During the recording, electrophysiological signals were monitored and, if necessary, the electrode was slightly adjusted to maintain recording quality. After each session, the head-post well was covered with silicone elastomer and a thin layer of dental acrylic. We found that recording quality remained acceptable over tens of electrode penetrations across 3–4 d.

Data preprocessing. Voltage recordings were filtered between 300–3,000 Hz in software. A threshold for spike detection was manually set for each trial. Spike sorting to obtain single units was performed in MClust-4.0 (ref. 54). Trials with large movement artifacts, as determined by large amplitude voltage fluctuations occurring before stimulus onset, were rejected. In all, we obtained 45 ± 21 (mean ± s.d.) trials per single unit for analysis.

VPM recording. Under isoflurane (1–2%), a cranioectomy (1–1 mm diameter) was made over the left hemisphere, centered at 1.8 mm posterior to bregma, 1.5 mm lateral to midline55. Under light isoflurane (0.5–1.5%), a tungsten microelectrode (1.0 MΩ nominal impedance, WPI) was advanced vertically through the brain at a speed of 50–100 µm/s until reaching the depth of 2.5 mm, then slowly advanced at ~10 µm/s. Contra-lateral whiskers were manually deflected after each 30–50-µm advance. Evoked spikes were monitored using an oscilloscope (Tektronix) and audio monitor (A-M Systems). We used the somatotopic organization of VPM (including the whiskers, other facial regions, teeth and microvibrissae), and the presence of other structures (lateral geniculate nucleus) as a guide during mapping. If no whisker-evoked activity was identified by a depth of 4 mm, the electrode was withdrawn and reentered at a neighboring site. This procedure was repeated until clear whisker-evoked responses could be identified around a depth of 3 mm. The cranioectomy was typically covered with agarose (2% in cortex buffer) to reduce brain movement. This mapping process took 1–4 h. After successfully locating a recording site, a map of brain surface vasculature was manually drawn and used to locate penetration sites for future recordings.

During subsequent behavioral sessions, mice were placed in the behavior apparatus and lightly anesthetized (0.5–1.5% isoflurane). A tungsten microelectrode (1–2 MΩ nominal, WPI) was advanced to the previously mapped region. The electrode was then slightly moved along its tract while whiskers were manually deflected in order to identify the ‘principal’ whisker producing the strongest response. At this point, the principal whisker was inserted into the whisker stimulator, isoflurane was turned off, and the mouse was allowed to recover from anesthesia. During recovery, test stimuli identical to those used during the behavioral task were applied and evoked spiking was monitored to detect any movement-related signal degradation (decrease in spike height). In these cases, careful advancement or withdrawal of the electrode usually recovered the signal. Otherwise, a new recording was sought. After 15–30 min (during which time the mouse fully recovered from anesthesia), the behavioral task was initiated. Electrophysiological signals were amplified 1,000× or 10,000× and filtered 300–3,000 Hz (DAM80, WPI) and acquired at 20 kHz with Ephus. During the recording, electrophysiological signals were monitored and, when necessary, the electrode was slightly adjusted to maintain recording quality. If quality (amplitude of evoked spikes) degraded too severely to be recovered within 2–3 go trials, behavioral trials were paused until high-quality multi-units were regained. After each session, the head-post well was covered with silicone elastomer and a thin layer of dental acrylic. We found that recording quality remained acceptable over tens of electrode penetrations across >1 week. To aid recording site identification, a microelectrode coated with Dil (Life Technologies) was lowered into the brain following the same trajectory used for recording. The brain was later perfused (PBS flush followed by 4% PFA), post-fixed overnight (4% PFA), sectioned coronally at 100 µm thickness on a vibratome (Microm), and processed for cytochrome oxidase staining. Bright-field and fluorescence images (BX-41 microscope, Olympus) were acquired (QImaging), and Dil tracts were used to verify VPM targeting (Supplementary Fig. 3).

Data preprocessing. Voltage recordings were filtered 300–3,000 Hz in software. Traces were inspected visually and included for analysis if spike height was >4–5 times the s.d. of baseline noise. The threshold for spike detection was set manually to be at least 4–5 s.d. of baseline noise, and was constant across all trials in a recording session. Detected spikes were not sorted and were presumably multi-units. Trials with large movement artifacts were rejected, as determined by large amplitude voltage fluctuations occurring before stimulus onset. In all, we obtained 63 ± 26 (mean ± s.d.) trials per multi-unit recording for analysis. Interpretation of multi-unit activity. DP calculations for VPM were based on multi-unit (2–5 neurons) rather than single-unit activity as in our primary afferent and cortical recordings (see Supplementary Fig. 3c–g for results from 7 single units). Our VPM DP calculations may therefore differ from the detect probabilities expressed by single neurons. This would not change our conclusion that VPM shows significant but highly transient choice-related activity.

VPM optogenetic stimulation with recordings. Optrodes were implanted in Scnna1a-Tg3-Cre;Ai32 mice. Optrodes were made by gluing a tungsten microelectrode (2.0 MΩ nominal impedance, Parylene-coated, WPI) to a multimode optic fiber (105 µm core diameter, 0.22 NA, Thorlabs), with the microelectrode tip extending ~100 µm beyond the tip of optic fiber. VPM was located as described above using a normal (non-optrode) tungsten microelectrode. This microeolecetrode was then withdrawn and an optrode was used to probe the target site and surrounding area to relocate VPM. The optrode was coupled to a 470-nm LED (M470F1, Thorlabs) with high-power driver (LEDD1B, Thorlabs). The maximal output from the optrode was 170–230 µW before implantation. Targeting was considered successful when (i) clear spiking was driven by deflection of a principal whisker (usually C2), and (ii) photostimulation (50–100 ms pulses at full power) yielded spikes with similar amplitude as those evoked by principal whisker stimulation.

To record VPM activity evoked by photostimulation with and without whisker stimulation in awake mice, isoflurane was withdrawn and the principal whisker was inserted into the piezo stimulator. While recording with the optrode in VPM, three types of stimuli were delivered on separate trials: (i) whisker alone, (ii) whisker plus ‘weak’ photostimulation (40–60 µW, 1 ms) or (iii) whisker plus ‘strong’ photostimulation (170–230 µW, 1 ms). The light pulse was delivered at 0, 3 or 4 ms following the onset of the whisker stimulus (rostral-caudal, peak angular speed ~1,200 deg/s). A second light pulse was given 1.5 s after the first pulse to determine the effect of light stimulation alone. On the basis of calculations56 (http://www.optogenetics.calc) of light intensity vs. distance from fiber tip, we estimate an excitation volume of roughly 1–3 thalamic barroids. Data preprocessing was as described above for VPM.

To record S1 responses to VPM stimulation in awake (Supplementary Fig. 4) or task-performing (Fig. 4g–m) mice, the exposed brain was first covered with agarose (2% in cortex buffer) and dental cement was used to secure the optrode in place. The skull over barrel cortex was kept clear of dental cement. The next day, a cranioectomy (~200 µm diameter) was made over the cortical barrel column (identified using ISI) corresponding to the principal whisker of the VPM optrode recording (identified using whisker stimulation and photostimulation). Glass pipettes and recording procedures were identical to those described below for whole-cell recording steps before break-in, except that pipettes were filled with cortex buffer, and seal resistance was ~20–50 MΩ. For recordings (current clamp mode, I = 0) in awake non-performing mice, trials of the three types used in VPM recordings were delivered with equal probability. For recordings in performing mice, the three types of go trials (whisker alone; whisker + weak photostimulation; whisker + strong photostimulation) comprised 60–70% of all trials. The remaining trials were of three no-go types (no stimulus; weak photostimulation alone; strong photostimulation alone). The light pulse (1 ms duration) was delivered 4 ms after the onset time of the whisker stimulus (awake mice: rostral-caudal, peak angular speed ~1,200 deg/s; task-performing mice: rostral-caudal, peak angular speed ~800 deg/s). Silent cells51 that spiked very sparsely or did not respond to whisker stimulation were avoided during the process of establishing the recording.

Whole-cell recording. Under isoflurane (1–1.5%), the skull above the C2 column, previously localized via intrinsic signal imaging, was thinned with a dental drill. A cranioectomy (~200 µm diameter) was made by removing a small piece of the thinned bone with a tungsten needle (Fine Science Tools). The mouse was transferred to the behavior apparatus and allowed to recover from anesthesia. Borosilicate glass pipettes (1.5 mm OD, 0.86 mm ID; Harvard Apparatus) were pulled (P-97, Sutter) to have a long shank and were 4–7 MΩ when filled with
solution containing (in mM): 135 potassium gluconate, 4 KCl, 10 sodium phosphocreatine, 4 ATP magnesium salt, 0.3 GTP sodium salt hydrate, 10 HEPES, 3 mg/ml biocytin (pH 7.3 with KOH). Electrophysiological signals (Multiclamp 700B, Molecular Devices) were filtered at 10 kHz and acquired at 20 kHz using Ephys. Voltage clamp mode was used to search for neurons. Square wave voltage pulses (50 ms, −5 mV, 5 Hz) were applied to the electrode tip to monitor resistance. Positive pressure of ~3 p.s.i. was applied as the pipette tip approached the cortical surface at an angle of 27 deg relative to vertical. A sudden change of pipette resistance indicated contact. Micromanipulator (MP-225, Sutter) depth reading of the cortical surface was recorded, and the pipette was quickly retracted by −200 µm. Agarose (2%) in cortex buffer was applied to cover the craniotomy. The pipette was then quickly advanced through the dura (if necessary) to −200 µm below the surface (~100 µm/s). Pressure was then reduced to 0.3–0.5 p.s.i., and the pipette was advanced slowly (~10 µm/s) to search for neurons. When an abrupt increase in pipette resistance (30–50%) was observed, positive pressure was released. Sometimes negative pressure was applied (<0.2 p.s.i.) to facilitate seal formation. Before break-in, pipette capacitance and offset were compensated. Break-in was performed when resistance >1 GΩ and stable. Break-in was accomplished by applying a slow ramp of negative pressure (up to 1 p.s.i.) repetitively. Brief voltage pulses (Multiclamp ‘zap’ function) were occasionally used to assist. After successful break-in, the recording mode was switched to current clamp (I = 0), any negative pressure was released, and the behavioral session was initiated. Traces of raw membrane potential were acquired for each behavioral trial, synchronized via triggers from the behavior control software. Liquid junction potential was not corrected. The recording was terminated when the recorded cell was lost, when membrane potential became depolarized above −40 mV or when the mouse stopped performing the task. Typically each mouse could be recorded from once per day over 2–3 d, with a typical yield of one cell per animal.

Rejection criteria. Recorded trials were rejected for analysis if resting Vm was above ~45 mV, or resting Vm increased by more than ~10 mV from the value measured in the first trial. A test pulse (50 pA current injection, 0.2 s) was delivered at the end of each trial. Mean total resistance to ground (Rtotal) was calculated by subtracting mean Vm over a baseline window (0.5–0.1 s before test pulse onset) from mean Vm in a window during the test pulse (0.05–0.15 s after onset). Recording sessions were excluded from further analysis if Rtotal exceeded 300 MΩ. Rtotal for accepted recordings (n = 22) was 201.9 ± 44.3 Ω (mean ± s.d.). In all, we obtained 37 ± 22 (mean ± s.d.) trials per neuron for analysis.

Spike removal and resting potential estimate. To remove spikes, Vm was first median filtered then smoothed using a 4–10 ms (depending on spike width) moving average. Resting membrane potential (Vrest) was calculated as mean Vm in a window 0.1–0.5 s after trial onset (~0.1 s before auditory cue) across all accepted trials.

Spike detection and spike threshold estimate. Spikes were detected offline using a threshold (10–30 mV) applied to band-pass–filtered (100–3,000 Hz) moving average. Resting membrane potential (Vrest) was calculated as mean Vm in a window 0.1–0.5 s after trial onset (~0.1 s before auditory cue) across all accepted trials.

PSP slope estimate. PSP slopes were estimated by fitting a line to Vm over an approximately 10-ms window containing the most linear and steepest Vm within the first 30 ms after stimulus onset. In most cases, the slope was estimated within the first 20 ms.

Reversal potential. The evoked PSP (Vm following the stimulus) was estimated in a 10- to 20-ms window starting typically 10–20 ms after stimulus onset, and was plotted against pre-stimulus Vm (mean over a 20-ms window preceding stimulus onset). A line was fitted to these x,y pairs using linear regression. Vrev was the fitted value of pre-stimulus Vm (extrapolated past the last x value in some cases) at which the evoked PSP was 0. We attempted to calculate Vmrev in the 19 of 22 neurons in which action potentials occurred (where we could determine Vmpeak). Out of these, 5 of 19 showed poor linear fits (R2 = 0.15 ± 0.11 (mean ± s.d.), which can occur owing to insufficient numbers of trials such that observations do not span an adequate range of pre-stimulus Vm values. Linear fits were good (R2: 0.69 ± 0.19 (mean ± s.d.) and allowed estimation of Vmrev in the remaining 14 of 19 neurons. Vmrev did not differ between hit and miss trials (~5.4 ± 4.9 vs. −53.9 ± 8.0 mV (mean ± s.d.), P = 0.49).

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; posterior, 1.3 mm) of P40–50 mice. The dura was left intact. GCaMP6s was expressed under the human synapsin-1 promoter following injection with recombinant adeno-associated virus (serotype 2/1, Syn.GCaMP6s.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; ~1 nl per second). After virus injection, the craniotomy was covered with an imaging window made by gluing together two pieces of microscope cover glass. The smaller piece (Fisher; #2 thickness) was fitted into the craniotomy and the larger piece (~1.5 thickness) was glued to the bone surrounding the craniotomy. To localize a barrel column within the cranial window, intrinsic signal imaging was performed through the window 1 week after surgery. All whiskers on the right side of the snout except the relevant one (a row C whisker) 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. To minimize active whisking, botulinum toxin A (BOTOX, Allergan) was prepared in PBS at 1 ‘mouse unit’ (MU) per microliter, and 0.5 MU was injected to the right whisker pad of trained mice using a 1-µl syringe (Hamilton). Mice fully recovered from the toxin treatment after ~7 d.

Images were acquired on a custom two-photon microscope (http://openwiki.janelia.org/wiki/display/sharedesigns/MIMMS) equipped with a resonant scanning module (Thorlabs), GaAsP photomultiplier tube (Hamamatsu) and a 16x:0.8 numerical aperture (NA) microscope objective (Nikon). GCaMP6s was excited at ~1000 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. The field of view was 760 µm × 790 µm (440 × 512 pixels; pixel size, 1.72 µm × 1.55 µm). Images were acquired continuously at 15 Hz using ScanImage 4.1/4.2. A movie, corresponding to a single trial, consisted of 65 image frames.

Two-photon calcium imaging of S2-to-S1 axons. Adeno-associated virus (serotype 2/1, Syn.GCaMP6s.WPRE.SV40) was injected into S2 (relative to bregma: lateral, 4.3 mm; posterior, 1.3 mm) at 2 depths (250 µm and 350 µm; 30–40 nl each; −1 nl per second), and covered with a cranial window. 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 excluded. Imaging planes were from layer 1 of S1 (70–100 µm from pial surface). 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 layer 2/3 somata: data analysis. A line-by-line correction algorithm was used to correct for brain motion. For each behavioral trial, we used five consecutive frames with a minimum of luminance changes to generate an average reference image. Each line was registered to the reference image by maximizing the line-by-line Pearson correlation. Regions of interest (ROIs) corresponding to individual neurons were manually selected with the help of maximum intensity and s.d. projections across movie frames. For each ROI, the time series of raw fluorescence was estimated by averaging all pixels within the ROI. Neuronal signal surrounding each ROI was estimated by averaging all pixels, excluding those from neighboring ROIs, within a 2-pixel-wide ring that starts at 2 pixels away from the border of the ROI. This neuronal signal was subtracted from the raw fluorescence time series to yield the corrected fluorescence time series: F(t) = Fraw(t) − τ × Fneurop(t), with τ = 0.7 (refs. 26,58). ∆F/F0 was calculated from this corrected fluorescence signal as (F(F0−F)/F0) where F0 was the mean F over 4 baseline frames immediately preceding the time of stimulus onset for each trial. Evoked ∆F/F0 was calculated as the mean ∆F/F0 over 3 frames following the stimulus onset time and before the answerlick (~100–300 ms after stimulus onset). For presentation in Figures 1B and 6C, we subtracted a baseline comprising the mean ∆F/F0 of the 10 frames preceding the stimulus.

Two-photon calcium imaging of S2-to-S1 axons: data analysis. 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. 34) to build clusters of highly correlated ROIs. Only responsive ROIs were included in the clustering.
and subsequent analysis. To determine whether a ROI was ‘responsive’, an evoked $\Delta F/F_0$ value was calculated using the mean $F$ over 8 frames immediately preceding the stimulus onset time as $F_0$, and the mean $F over 20 frames immediately following the stimulus onset time as the post-stimulus response. A Wilcoxon signed rank test (for samples with absolute value skew <0.6) or a sign test (absolute value skew >0.6) was performed on these evoked $\Delta F/F_0$ values. If the resulting $P$ value was <0.01, the neuron was considered responsive. Analysis procedures subsequent to clustering were as described above for L2/3 somata, except that neuronal subtraction was not performed, $F_0$ was calculated using 8 baseline frames (as imaging was at 30 Hz rather than 15 Hz), and evoked $\Delta F/F_0$ was calculated as the mean $\Delta F/F_0$ over 5 frames following the stimulus onset time and before the answer lick (from ~117 to 283 ms after stimulus onset). The $\Delta F/F_0$ for each putative axon was calculated as the mean $\Delta F/F_0$ of all ROIs within a cluster.

**Optogenetic and pharmacological silencing.** PV-IRES-Cre;Ai32 mice were implanted with a clear skull cap$^{33}$. Light from a 473 nm laser (MBL-III-473-100, Ultralasers) was passed through an acousto-optic modulator (MTS110-A3-VIS, QuantaTech), focused into a multimode optical fiber, recombined and directed onto the C2 column (targeted using ISL). The beam at the skull had an approximately Gaussian profile with full width–half-maximum (FWHM) of 600 µm. Photostimulation was randomly delivered on 25–35% of all trials. Photostimulation comprised a train of 5-ms pulses at 100 Hz delivered from ~3 mW relative to the time of whisker stimulus onset. Average power on the brain surface was ~3 mW. 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, LED dynamics) placed near the eyes.

Muscimol hydrobromide (Sigma-Aldrich) was dissolved in cortex buffer at 5 µg/µl and stored at ~20 °C. For injection, mice were anesthetized with isoflurane and kept on a thermal blanket. A cranialotomy was made as described for whole-cell recordings. Muscimol was injected into the C2 column (identified by intrinsic signal imaging) in different sessions as follows: 100 nl at 500 µm (n = 2 sessions total in 2 mice), 50 nl at 350 and 700 µm (total 100 nl, n = 1). V1 injection was performed at 3 mm posterior to bregma and 2 mm lateral to midline with 150 nl at 350 and 700 µm (total 300 nl, n = 2 sessions total in 2 mice) and 300 nl at 500 µm (n = 1). Injection was performed at the speed of ~1 nl/s. After injection, the pipette was left in place for 3 min. Mice were allowed 1.5–3 h to recover before initiating the behavioral session.

**Stimulus-response curves for TG, VPM and S1 in awake mice.** Recordings were obtained as described above for TG, VPM and S1, except that S1 recordings were made with cortex-buffer filled pipettes in loose-seal cell-attached$^{31}$ rather than whole-cell mode. Whisker stimuli (0.5 s, rostral-caudal sinusoidal deflections) of four different amplitudes (~300, 600, 900 and 1,200 deg/s peak angular speeds) were randomly delivered with equal probability. Windows and procedures for calculating evoked responses were identical to those used for TG, VPM and S1 recordings acquired during task performance (described below).

**Licking artifact removal and licking trial exclusion.** We used an electrical method of detecting tongue contact with the lickport. Contact could cause brief artifacts in electrophysiology traces. In $V_\text{m}$ analysis, this artifact was already removed by median filtering and smoothing (see above). For spike rate analysis (whole-cell and extracellular recordings), the raw voltage samples from ~3.75 to ~3.75 ms, centered at the lick, were replaced by the mean value over ~7.5 to ~3.75 ms and ~3.75 to ~7.5 ms relative to the lick. Licking artifacts had negligible impact on our analyses because (i) we limited analyses to periods before the earliest reaction times (occurring ~0.2 s after stimulus onset) and (ii) trials with ‘premature’ licks occurring close to stimulus onset (in a window from ~0.583 to 0.12 s relative to the time of possible stimulus onset for electrophysiology, and ~0.5 s to +0.12 s for calcium imaging) were excluded from subsequent analysis.

**Elimination of whisking artifacts.** Multiple experimental strategies and results ensure that periods of spontaneous whisking did not significantly affect our results. First, as described above for the two-photon imaging of S1 neurons, we used BOTOX to eliminate the ability of mice to whisk. We obtained similar results in separate experiments without BOTOX (data not shown). Second, we observed that periods of whisking in our trained mice were nearly always accompanied by movement artifacts in our metal microelectrode recordings. Epochs with movement artifacts were excluded as described above. Third, although whisker movements would cause a difference in neural activity, we observed no differences in pre-stimulus baseline activity in any of our recordings (Figs. 2–5). Fourth, any differences in mechanical input that affect brain activity must first cause spiking in the mechanoreceptive primary afferent neurons. We recorded from these primary afferents and observed no differences between hits and misses (Fig. 2).

**Anesthesia and task performance.** As detailed above, TG, VPM and S1 whole-cell recording sessions involved use of anesthesia before task performance. Our two-photon sessions, however, did not involve anesthesia. The behavioral performance levels obtained in these anesthesia-free sessions (73 ±3% correct, n = 10 sessions) did not differ from those obtained in sessions involving anesthesia (74 ±8% (mean ± s.d. correct, n = 56 sessions; P = 0.78, Wilcoxon rank-sum test). Thus, anesthesia did not have a noticeable impact on performance.

**Data analysis: windows for measurement of baseline and evoked activity.** For all electrophysiological recordings, pre-stimulus baseline activity was calculated using a 200-ms window ending 5 ms before stimulus onset. Post-stimulus activity was calculated in a 100 ms window starting 5 ms after stimulus onset for $V_\text{m}$ and a 100-ms window starting 7 ms after stimulus onset for spike rate in whole-cell recordings, a 10-ms window starting 5 ms after stimulus onset for VPM peak response calculation, a 100-ms window starting 15 ms after stimulus onset to calculate VPM after peak response, and a 27-ms window starting 2 ms after stimulus onset for TG recordings. The window length of 27 ms was chosen to include the first two peaks of the TG-evoked response. Because some TG neurons we recorded were direction selective (data not shown), the first two peaks capture the initial response of all neurons. Our conclusions are similar if we include only the first peak. For VPM recordings combined with photostimulation, we used the same peak and after peak windows as described above for VPM. For cell-attached recordings in S1 combined with VPM photostimulation, post-stimulus activity was calculated in a 30-ms window starting 5 ms after stimulus onset for peak response and a 100 ms window starting 35 ms after stimulus onset for after peak response. For VPM and S1, evoked action potential (AP) rate (or $V_\text{m}$) was defined as the difference in AP rate (or $V_\text{m}$) between the post- and pre-stimulus windows. Because baseline TG spike rates were extremely low (medians for hit and miss trials: 0.77 and 0.62 Hz, respectively) compared with rates during the stimulus (hit and miss medians: 76.6 and 62.7 Hz), evoked AP rate was calculated simply as the AP rate during the post-stimulus window. For TG and S1, results were similar across a range of post-stimulus window sizes (Supplementary Fig. 10).

**Receiver operating characteristic analysis.** We used receiver operating characteristic (ROC) analysis to calculate DP (mathematically identical to choice probability!), but typically renamed in the context of detection tasks) and SP. For detect-probability calculation, go trials were split into hits and misses. A decision variable (DV) was assigned for each trial on the basis of the neural response. DP was then calculated (using MATLAB ‘perfcurve’) as the area under the ROC curve for discrimination on the basis of DV. SP was calculated by the same method, except that trials were split into go and no-go rather than hits and misses. For $V_\text{m}$ analyses, the DV was $\Delta V_\text{m}$. For spike rate analyses the DV was evoked AP rate (defined previously). For two-photon calcium imaging, the DV was evoked $\Delta F/F_0$ (defined previously). DP and SP time series were calculated in a 5-ms moving window with 1-ms step size.

**Statistics.** Data analyses were conducted in MATLAB. Mice of appropriate genotypes were assigned to experimental groups arbitrarily, without randomization or blinding. A summary of experimental groups is given in Supplementary Table 1. No statistical methods were used to predetermine sample sizes. Data are reported as mean ± s.e.m. unless otherwise noted. Statistical tests were by two-tailed Wilcoxon signed rank test unless otherwise noted (MATLAB ‘signrank’). Assumptions of the Wilcoxon signed rank test were verified by quantifying symmetry of the distribution of sample differences about its median. For samples failing this assumption (absolute value skewness > 0.6), we instead used the sign test (MATLAB ‘signtest’).

A Supplementary Methods Checklist is available.
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