# Supporting Information

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### SI Materials and Methods

**Animal Preparation and Anesthesia.** All animal experiments described here were performed following the rules of the International Council for Laboratory Animal Science, European Union regulation 86/609/EEC, and were approved by the Ethical Committee for Animal Research of Hospital Nacional de Parapléjicos. Data were collected from six male adult Wistar rats (250–350 g) anesthetized with i.p. urethane (1.5 g/kg). This anesthetic was chosen because it allows consistent single-neuron VPM recordings to be performed through long stimulation protocols (1). The state of anesthesia was monitored by checking the absence of vibrissal movements and tail pinch withdrawal reflex, to maintain a constant level of anesthesia at stage III-3/4 (2). Supplemental doses were applied throughout the course of the experiments when necessary (1/4 of the original dose). The body temperature was kept constant at 37 °C by means of an electric heating pad controlled by a rectal temperature sensor.

**Whisker-Pad Stimulators.** Before starting the surgery for the electrophysiological recordings, we implanted eight electrical whisker stimulators in the whisker pad. The rationale for using electrical stimuli instead of mechanical stimuli was to minimize any contribution of direction selectively (3–8) on the information conveyed about stimulus location. The insertion technique is similar to the one described by Moxon et al. (9). Briefly, each stimulator consisted of a Teflon insulated twisted pair of stainless steel wires (California Wire). Insulation was stripped on one end of the wires for 1 mm, and the two wires were then offset by 2–3 mm to avoid short circuit and bent to form a hook that served to anchor the stimulator at the base of the specific whisker. A small incision (1 cm) was made on the left side of the face ∼2 cm caudal to the vibrissal area. Then, a regular 30-gauge needle was used to tunnel each whisker-pad stimulator under the skin to a specific whisker. The whisker-pad stimulators were anchored to eight whiskers [A (2, 4), C (1, 3, 5), and E (1, 3, 5)], so that there was always at least one whisker that separated each stimulator in all directions. The final position of each stimulator was checked by sending short pulses of current (50 μs) through the specific whisker pad to elicit muscle activation. For each stimulator, the intensity of the pulses (0.2–1 mA) was adjusted so that the corresponding whisker moved ∼2 mm at 5 mm from the base in any direction. To ensure that no other whiskers were moving, the stimulated whisker was observed under magnification using a stereomicroscope (LEICA M300; Leica Microsystems). Finally, a suture point near the original incision on the face was used to avoid relative movement of the stimulator with respect to the face. The above procedure was repeated until all eight stimulators were implanted. The rationale of using pulses of short duration (50 μs) was to reproduce impulsive stimuli, to maximize the overall responsiveness of VPM neurons, which can be selective to different kinetic features of whisker stimuli (10).

**Electrophysiological Recordings.** After all whisker-pad stimulators were implanted and the wire ends secured in place, the animal's head was fixed in a stereotaxic frame (SR-6R; Narishige Scientific Instruments). Craniotomies were drilled over the right hemisphere above the VPM nucleus at −3 to −5 mm anteroposterior and 3–5 mm medio-lateral from bregma (11). Pairs of VPM neurons were recorded simultaneously by using two high-impedance tungsten electrodes (2–4 MOhms at 1 kHz). Electrodes were slowly and independently lowered down to the VPM (4–6 mm ventro-dorsal) at a speed of ∼100 μm/min by means of hydraulic micromanipulators (Narishige Scientific Instruments). The continuous signal recorded at the electrode was band-passed (200 Hz to 7 kHz), amplified (Neurolog; Digitimer), digitized at 20 kHz (CED), and stored for off-line analysis. The digitized signal was shown on a computer screen connected to a CED system, using Spike 2 software (v5.03; CED). Electrical activity from each of the electrodes was monitored on the screen during the descent to the VPM.

**Stimulation Protocol.** Once at least a single neuron was isolated at each electrode in the VPM, a stimulation protocol was performed, using two of the eight whisker-pad stimulators. The whisker-pad stimulators used were selected so that each stimulator was required to activate at least one of the neurons and to minimize the artifact due to electrical stimulation. The intensity for each pulse, as previously defined, ranged from 0.2 to 1 mA. A train of 1,600 electrical stimuli was applied through the whisker-pad stimulators, with an interstimulus interval of at least 2 s plus an average random interval of 200 ms, alternating between the two whisker-pad stimulators (800 stimuli to each whisker-pad stimulator). Once the protocol was completed, the electrodes were moved to identify additional pairs of neurons. All stimuli were generated using a Master-8 electrical stimulator (AMPI), with ISO-Flex stimulus isolators (AMPI).

**Single-Neuron Discrimination.** Neurons were discriminated offline using Plexon offline software. Spikes were extracted from the raw signal by setting a threshold of at least 6× the SD of the amplitude of the analog signal. Discrimination between single neurons was meticulously achieved with offline analysis, on the basis of the shape of the action potentials, using voltage threshold methods, spike sorting protocols, and template matching algorithms in a complementary way (12, 13).

**Dataset.** A total of 48 neurons were discriminated from the six animals during 10 stimulation protocols. For each neuron we computed a peri-stimulus time histogram (PSTH) (14) around the time of stimulus for each stimulator (location). We first determined whether neurons were responsive to the stimuli, by measuring the background activity, defined as the average firing rate (Hz) in a 400-ms period before the stimulus, and the response magnitude, defined as the average number of spikes (spikes per stimulus) emitted in a 20-ms poststimulus window. A neuron was considered responsive if the response magnitude was greater than three times the background firing rate in a window equivalent to the poststimulus window (both expressed in spikes per stimulus). Only responsive neurons ($n = 40$) were considered for further analyses. Of these neurons, 11 contributed to only one pair, 18 contributed to two pairs, and 11 to three pairs. In all pairs the 2 neurons were always recorded from different electrodes. The overall dataset consisted of 40 single neurons, combined in 40 pairs.

**Data Analysis.** ***Assessing trial-to-trial spike-count variability.*** To quantify the trial-to-trial spike-count variability in the response of VPM neurons to the stimuli, we used the Fano factor, which was measured as the ratio between the variance and the mean (across trials) of the number of spikes emitted in a poststimulus window of 20 ms. For a Poisson process the theoretical Fano factor = 1. However, when only a finite number of Poisson trials are experimentally available, the measured Fano factor is not exactly 1 and asymptotically follows a gamma distribution with dependence on the number of trials (15). In our experimental conditions (800

trials), the 95% confidence interval for the Fano factor of a Poisson process is between 0.9 and 1.1. Therefore, we considered our neurons to display sub-Poisson firing only if the Fano factor was <0.9. On the one hand, Poisson firing represents a response variability characterized by absence of autocorrelation in the spike count: If a spike occurs, the probability of observing a second spike is the same as for the first spike, because all spikes are completely independent. On the other hand, sub-Poisson firing represents a response variability characterized by autocorrelation in the spike count (16): If a spike occurs, the probability of observing a second spike decreases (or increases). In the following sections we equivalently refer to information due to trial-to-trial spike-count variability or due to count autocorrelations.

***Information conveyed by trial-to-trial spike-count variability in single neurons.*** To rigorously assess the contribution of trial-to-trial spike-count variability (i.e., count autocorrelations) to the information conveyed about stimulus location by single-neuron responses, we used our general Poisson exact breakdown of the mutual information (17). In brief, we first estimated the information ($\hat{I}_{\text{lin}}$) obtained by substituting the probability distributions of the responses with equivalent Poisson distributions, with mean ($\mu$) equal to the average number of spikes emitted by the neuron in the poststimulus window. For example, the equivalent Poisson probability that the response ($r$) of a neuron is equal to $k$ spikes is given by $\hat{P}(r=k)=\mu^k e^{-\mu}/k!$. We then calculated the information ($I$) considering the probability distributions of the recorded responses ($P(r = k)$). By taking the difference between these two measures of information, we obtained an estimate of the amount of information ($\hat{I}_{\text{cor-auto}}$) conveyed by trial-to-trial spike-count variability (i.e., count autocorrelations). For single neurons, this information can be expressed by the formula

$$\hat{I}_{\text{cor-auto}} = I - \hat{I}_{\text{lin}} = H(R) - H(R|S) - (H(\hat{R}) - H(\hat{R}|S)),$$

where $H(R)$ and $H(\hat{R})$ are the entropies of the single-neuron responses $R$ and of the equivalent Poisson responses $\hat{R}$, and $H(R|S)$ and $H(\hat{R}|S)$ are the corresponding entropies conditional to the stimuli $S$. Note that throughout the paper the "hat" ($\hat{}$) always indicates the use, at least in part, of Poisson equivalent distributions.

***Contribution of the variability within neurons to the redundancy between neurons.*** To quantify the contribution of each neuron to the information conveyed by the respective pair, we calculated the total amount of information carried by the pair ($I$) and the amount of information carried by each neuron of the pair as if they were independent ($I_{\text{lin}}$),

$$I = H(\mathbf{R}) - H(\mathbf{R}|S)$$

$$I_{\text{lin}} = \sum_{i=1,2} \left[ H(R_{N_i}) - H(R_{N_i}|S) \right],$$

where $H(\mathbf{R})$ and $H(\mathbf{R}|S)$ are the entropy and the conditional entropy of the observed responses $\mathbf{R}$ of the neuron pair, and $H(R_N)$ and $H(R_N|S)$ are the entropy and the conditional entropy of the observed responses of one of the neurons of the pair ($N_i$). The difference between these two quantities ($I - I_{\text{lin}}$) is usually referred to as the synergy/redundancy term $\Delta_{\text{syn}}$ (18, 19) and we used it to determine whether the neurons are *synergic* (positive difference) or *redundant* (negative difference) with respect to the pair. For each pair, we estimated both the $I_{\text{lin}}$ and the $\Delta_{\text{syn}}$ terms, considering an increasing poststimulus time window ranging from 0 to 20 ms.

The role of synergy and redundancy can be assessed by measuring the similarity ($I_{\text{sig-sim}}$) in the conditional probabilities of the responses across stimuli (i.e., signal similarity) or by measuring the cross-correlations ($I_{\text{cor}}$) between the conditional responses of the neurons across stimuli trials (i.e., noise correlations) (18–21). These terms were quantified by entropy differences as

$$I_{\text{sig-sim}} = H_{\text{ind}}(\mathbf{R}) - \sum_{i=1,2} H(R_{N_i})$$

$$I_{\text{cor}} = -H_{\text{ind}}(\mathbf{R}) + H(\mathbf{R}) - H(\mathbf{R}|S) + \sum_{i=1,2} H(R_{N_i}|S),$$

where $H_{\text{ind}}(\mathbf{R})$ is the entropy that the observed responses would have if neurons were independent. For each pair, we then compared the information extracted by the two terms, considering increasing poststimulus time windows ranging from 0 to 20 ms.

When considering more than one neuron, autocorrelations from different neurons can interact, contributing to the signal similarity between neurons (16). To estimate this contribution of variability within neurons to the redundancy between neurons ($\hat{I}_{\text{sig-sim-auto}}$), we calculated the redundancy due to the observed responses ($I_{\text{sig-sim}}$) and subtracted from it the amount of redundancy that would be obtained if the neurons where free of autocorrelation ($\hat{I}_{\text{sig-sim}}$). This result can be written as

$$\begin{aligned}\hat{I}_{\text{sig-sim-auto}} &= I_{\text{sig-sim}} - \hat{I}_{\text{sig-sim}} \\ &= H_{\text{ind}}(\mathbf{R}) - \sum_{i=1,2} [H(R_{N_i})] - \left( H_{\text{ind}}(\hat{\mathbf{R}}) - \sum_{i=1,2} [H(\hat{R}_{N_i})] \right),\end{aligned}$$

where $H_{\text{ind}}(\mathbf{R})$ and $H_{\text{ind}}(\hat{\mathbf{R}})$ are the entropies the responses would have if neurons were independent, considering the observed or the equivalent Poisson responses, respectively. The sum of the two autocorrelation terms ($\hat{I}_{\text{cor-auto}} = \hat{I}_{\text{lin-auto}} + \hat{I}_{\text{sig-sim-auto}}$) of the general Poisson exact breakdown (16, 17) was then used to quantify the net effect of count autocorrelations (i.e., trial-to-trial spike-count variability) on the information conveyed by the pairs of neurons.

All information analyses were performed on poststimulus time windows of increasing size, from 1 ms to 20 ms poststimulus.

***Bias correction.*** Information theory measures are affected by the well-known bias introduced by limited sampling of the observed neural responses (22). In our dataset, the maximum number of responses to a stimulus was 7 for single neurons and 49 for pairs of neurons. Because the number of trials was 800, the ratio of the responses to trials $\bar{R}/N$ was 0.009 for single neurons and 0.061 for pairs of neurons. We therefore could use the quadratic extrapolation correction for the bias, which was proved to provide good performance for $\bar{R}/N < 0.25$ (22). The bias correction procedure was implemented using the *Information Breakdown Toolbox* (ibTB) (23). We did not correct for the bias those terms in which the observed probabilities of the responses were substituted with equivalent Poisson probabilities because these probabilities depend only on one parameter (average number of spikes emitted), which is very well sampled using 800 trials per stimulus.

***Statistical analyses.*** Values of information are given as mean ± SD. Comparisons between information values were performed with paired $t$ tests after logarithmic transformation. Pearson's correlation coefficient was used for correlations between measures. Results were considered statistically significant at $P < 0.05$.

1. Aguilar JR, Castro-Alamancos MA (2005) Spatiotemporal gating of sensory inputs in thalamus during quiescent and activated states. *J Neurosci* 25:10990–11002.
2. Friedberg MH, Lee SM, Ebner FF (1999) Modulation of receptive field properties of thalamic somatosensory neurons by the depth of anesthesia. *J Neurophysiol* 81: 2243–2252.
3. Bale MR, Petersen RS (2009) Transformation in the neural code for whisker deflection direction along the lemniscal pathway. *J Neurophysiol* 102:2771–2780.
4. Hemelt ME, Kwegyir-Afful EE, Bruno RM, Simons DJ, Keller A (2010) Consistency of angular tuning in the rat vibrissa system. *J Neurophysiol* 104:3105–3112.
5. Ito M (1988) Response properties and topography of vibrissa-sensitive VPM neurons in the rat. *J Neurophysiol* 60:1181–1197.
6. Li L, Ebner FF (2007) Cortical modulation of spatial and angular tuning maps in the rat thalamus. *J Neurosci* 27:167–179.
7. Minnery BS, Bruno RM, Simons DJ (2003) Response transformation and receptive-field synthesis in the lemniscal trigeminothalamic circuit. *J Neurophysiol* 90:1556–1570.
8. Simons DJ, Carvell GE (1989) Thalamocortical response transformation in the rat vibrissa/barrel system. *J Neurophysiol* 61:311–330.
9. Moxon KA, Devilbiss DM, Chapin JK, Waterhouse BD (2007) Influence of norepinephrine on somatosensory neuronal responses in the rat thalamus: A combined modeling and in vivo multi-channel, multi-neuron recording study. *Brain Res* 1147:105–123.
10. Petersen RS, et al. (2008) Diverse and temporally precise kinetic feature selectivity in the VPm thalamic nucleus. *Neuron* 60:890–903.
11. Paxinos G, Watson C (1986) *The Rat Brain in Stereotaxic Coordinates* (Academic, San Diego), 2nd Ed.
12. Aguilar J, Morales-Botello ML, Foffani G (2008) Tactile responses of hindpaw, forepaw and whisker neurons in the thalamic ventrobasal complex of anesthetized rats. *Eur J Neurosci* 27:378–387.
13. Leiser SC, Moxon KA (2007) Responses of trigeminal ganglion neurons during natural whisking behaviors in the awake rat. *Neuron* 53:117–133.
14. Gerstein GL, Kiang NY (1960) An approach to the quantitative analysis of electrophysiological data from single neurons. *Biophys J* 1:15–28.
15. Eden UT, Kramer MA (2010) Drawing inferences from Fano factor calculations. *J Neurosci Methods* 190:149–152.
16. Scaglione A, Foffani G, Scannella G, Cerutti S, Moxon KA (2008) Mutual information expansion for studying the role of correlations in population codes: How important are autocorrelations? *Neural Comput* 20:2662–2695.
17. Scaglione A, Moxon KA, Foffani G (2010) General Poisson exact breakdown of the mutual information to study the role of correlations in populations of neurons. *Neural Comput* 22:1445–1467.
18. Panzeri S, Schultz SR, Treves A, Rolls ET (1999) Correlations and the encoding of information in the nervous system. *Proc Biol Sci* 266:1001–1012.
19. Schneidman E, Bialek W, Berry MJ 2nd (2003) Synergy, redundancy, and independence in population codes. *J Neurosci* 23:11539–11553.
20. Nirenberg S, Latham PE (2003) Decoding neuronal spike trains: How important are correlations? *Proc Natl Acad Sci USA* 100:7348–7353.
21. Pola G, Thiele A, Hoffmann KP, Panzeri S (2003) An exact method to quantify the information transmitted by different mechanisms of correlational coding. *Network* 14:35–60.
22. Panzeri S, Senatore R, Montemurro MA, Petersen RS (2007) Correcting for the sampling bias problem in spike train information measures. *J Neurophysiol* 98: 1064–1072.
23. Magri C, Whittingstall K, Singh V, Logothetis NK, Panzeri S (2009) A toolbox for the fast information analysis of multiple-site LFP, EEG and spike train recordings. *BMC Neurosci* 10:81.

**Table S1. Basic neurophysiological properties of the recorded responses of single VPM neurons ($n$ = 40)**

| | Stimulus 1 | Stimulus 2 |
|---|---|---|
| Background (Hz) | 0.35 ± 0.64 | 0.37 ± 0.69 |
| Response magnitude (spikes per stimulus) | 0.56 ± 0.33 | 0.15 ± 0.22 |
| Fano factor | 0.68 ± 0.31 | 1.04 ± 0.43 |
| First-spike latency (ms) | 6.1 ± 4.5 | 10.2 ± 8.5 |
| First-spike jitter (ms) | 2.4 ± 2.5 | 4.7 ± 4.1 |

For each neuron, stimulus 1 represents the location that elicited the greatest response magnitude.