Somatosensory Cortical Neurons Decode Tactile Input Patterns and Location from Both Dominant and Non-dominant Digits

Highlights

- The location and layer of a neocortical neuron are considered predictive of function.
- Each neuron processes information from different, non-adjacent digits.
- The spike output indicates both which digit and which input pattern was delivered.
- No difference between neurons that depend on location and layer in these respects.

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In Brief

Enander and Jörntell record spike responses in neocortical neurons in primary somatosensory cortex provided with tactile inputs from non-adjacent digits. Regardless of whether the neurons were located in specific digit-dominated subregions, each neuron decoded the tactile inputs from either digit as well as the input location.
SUMMARY

For neurons of the primary somatosensory cortex, the anatomy of the thalamocortical connections supports a digit-wise specialization, whereas the intracortical connections suggest cross-digit integration. To evaluate the digit-wise specialization in individual somatosensory neurons, we explored the decoding of eight spatiotemporally complex tactile input patterns delivered to two non-adjacent digits in the anaesthetized rat. A striking finding was a good decoding performance for the eight input patterns to the non-dominant digit of the neuron, which in some cases was even better than for the same inputs to the dominant digit. Moreover, individual neurons decoded not only the pattern received but also to which digit it was delivered. These neuronal decoding properties were uniform throughout the cortical layers. Our results indicate that non-trivial tactile inputs to a single digit engage a wide processing circuitry throughout the digit region and suggest a low impact for somatotopy on the organization of the information processing.

INTRODUCTION

The mapping of receptive fields of individual neurons has been and still is a cornerstone in many neurophysiological studies of the brain (Kaas et al., 1979; Mountcastle, 1957; Penfield and Boldrey, 1937; Woolsey et al., 1942). The primary somatosensory cortex (S1) is believed to be modular on the basis that neurons receiving input from different digits, and different submodalities of tactile input, have been reported to show a digit-wise somatotopy (Mountcastle, 1997). The somatotopical organization is supported by the anatomy of the thalamocortical connections that have a point-to-point relationship between the digit-wise representations in the thalamus and in the cortex (Jones, 2000; Jones and Friedman, 1982; Liao et al., 2013).

However, the neocortex is internally characterized by a high number of horizontal connections between the neurons across different parts of the cortex. This has, for example, been shown with local tracer injections in S1 cortex that resulted in extensive labeling of axons terminating well outside the boundaries for defined digit representations (Liao et al., 2013). Furthermore, recent work suggests that the axonal ramifications of individual pyramidal neurons can extend to and distribute synaptic terminals across very large parts of the cortex (Gerfen et al., 2018). In fact, individual thalamocortical axons, even in the distinctly organized barrel cortex (Petersen, 2007), have rich branching patterns and cortical terminations well outside their primary cortical representations (Arnold et al., 2001; Oberlaender et al., 2011). These findings indicate that individual neurons in the somatosensory cortex, although provided with a dominant thalamocortical input from single digits or whiskers, are also provided with synapses representing information from non-dominant input.

Interactions at the neuronal level between inputs from different digits or whiskers have indeed been reported in both monkeys and rodents (Foffani et al., 2008; Lipton et al., 2010; Manns et al., 2004; Moore and Nelson, 1998; Moore et al., 1999; Tutunculer et al., 2006). In the monkey, using multi-electrode array recordings of neuronal responses to localized mechanical skin stimulation of a small part of a digit, all cortical neurons at least within a radius of 5 mm (the reach of the array) were found to be activated, although to various degrees (Reed et al., 2010). The functions of such interactions have only been sparsely characterized, for example spike synchronization across large cortical distances and lateral inhibition have been proposed (Qi et al., 2016), but it is clear “that traditional depictions of RFs [receptive fields] do not reflect the extent of integration that occurs” (Reed et al., 2008).

Here, we aim to perform a more detailed investigation of the nature of the interactions between digit representations. We use a recently introduced approach that is based on low-intensity electrical activation of tactile afferents in predefined complex spatiotemporal patterns, with the primary purpose of obtaining maximal reproducibility for a varied set of non-trivial tactile input patterns. This approach overcomes limitations associated with mechanical skin stimulation (see Discussion) and provides a highly sensitive tool to analyze the precision by which the spike output of a neuron contains information about the type of tactile input received (Genna et al., 2018; Oddo et al., 2017). We find that individual neurons can decode tactile input patterns to a non-adjacent, non-dominant digit at a comparable or even a higher level than for inputs to the dominant digit while also decoding which digit the input originated from. Furthermore, we
found no layer-wise specialization between neurons for either of these functions.

RESULTS

We recorded extracellular spikes from a total of 89 neurons in the paw region of the somatosensory cortex in anesthetized rats (Figure 1A) (recording depths: mean, 0.845 mm; range, 0.338–1.312 mm). The aim was to investigate the nature of their responses to spatiotemporal patterns of activation of the tactile afferents applied to the volar skin of two separate digits. These spatiotemporal patterns were delivered through two electrical interfaces each composed of a set of four stimulation sites (pairs of needle electrodes), on each digit (Figure 1B). In order to minimize the risk of stimulus spread, we used digit 2 and digit 5, which are innervated by the median and the ulnar nerve, respectively. We used single stimulation pulses as a search stimulus to target the recordings to the parts of the cortex with the largest field potentials for one of the digits. The search stimulus was presented to digit 2 in one set of experiments (7 rats; N = 53 neurons) and digit 5 in the other set (5 rats; N = 36 neurons).

Responses to Single-Pulse Stimulations Defined the Dominant Input of the Neuron

For each neuron recorded, the spike responses evoked by the isolated single-pulse stimulation were used to generate peristimulus time histograms (PSTHs). PSTHs were generated for each of the eight stimulation sites (Figure 1C). A response to the single-pulse stimulation (defined as a 0- to 30-ms post-stimulus activity equal to or exceeding 2 SDs from baseline) to all eight stimulation sites occurred only for two neurons (one example in Figure 1C). Neurons responding to all four stimulation sites from one digit but from none of the stimulation sites of the
other digit were also rare (digit 2, n = 5; digit 5, n = 0), whereas neurons responding to at least one of the stimulation sites of one of the digits but from none of the sites of the other digit were more common (digit 2, n = 23; digit 5, n = 3). The remaining 63 neurons responded to inputs from both digits in various mixtures.

The intensity of the responses evoked by the single-pulse stimulations to either digit was used to identify the dominant input digit of the neuron. To quantify the response intensity to isolated single-pulse stimulation from the PSTHs, we used the sum of the z-score during the first 100 ms after the stimulus (area under the curve [AUC]) for each of the four stimulation sites on the digit. A dominant input was defined if the mean AUC for one digit exceeded the mean AUC of the other digit by more than 10%. In cases where the differences in the AUCs did not reach the 10% threshold, the neuron was defined as being “equidominated.” Figure 1D illustrates the AUCs for inputs to the two digits for all neurons, split up on basis of the search stimulus (see Table S1 for statistical comparisons). It can be noted that, in experiments using digit 5 as the search stimuli, digit 2 still provided the most intense responses across the population of neurons.

The latency from the single-pulse stimulus to the onset of the detected response was found to be 14 ± 8 ms (mean ± SD) for digit 2 and 17 ± 10 ms for digit 5. The differences in the response latencies for inputs to the two digits were statistically significant (paired t test, p = 0.030; t = −2.20). Altogether, the population data of the response magnitude and the latency times indicate a relative dominance in the cortical neuronal population of input from digit 2 over digit 5 (Iwamura et al., 1983).

Neuronal Decoding of the Spatiotemporal Stimulation Patterns

The eight predefined spatiotemporal stimulation patterns were presented 50 times each in a pseudo-random order. As the stimulation patterns were all presented to the two digits separately, we obtained a total of 16 separate stimulation patterns repeated for a total of 800 times. The corresponding PSTHs indicated that, on average, the neurons had unique temporal response profiles to many of the spatiotemporal stimulation patterns (Figure 2).

The repeated stimulus presentations made it possible to quantify the precision with which individual neuronal responses could be used to identify the stimulus pattern (Figure 3A). The quantified value of this precision across all of the eight stimulation patterns constituted the decoding performance of the neuron. To analyze the decoding performance for each neuron in relation to our set of eight predefined spatiotemporal stimulation patterns applied to each digit (“inter-pattern decoding per digit”), we used a modified version of a previously published method based on the identification of differences in the temporal profiles of individual responses (Oddo et al., 2017) (principal-component analysis [PCA] on bootstrapped responses was combined with a k-nearest neighbor [kNN] classifier that reported the accuracy by which the responses could identify the stimulation pattern; see STAR Methods for details). For each neuron and each set of spatiotemporal stimulation patterns, the decoding performance could be visualized in a confusion matrix (Figure 3A). In previous studies where we studied the responses of S1 neurons to input from a single digit, we reported “accuracy” as the measure of decoding performance. The accuracy is the proportion of correctly classified responses (i.e., the sum of the true positives in the diagonal
for neurons subdivided by search stimulus (Figure 1D), when a t test, p < 0.0001). In contrast, the decoding performance for inputs from digit 5 was significantly different (depending on the digit for which the neuron had the best decoding of the spatiotemporal input patterns (i.e., inter-pattern, per digit decoding performance). If such a relationship existed, it would indicate that the digit dominating the input of the neuron would also define the focus of its processing. In contrast, we found that there was no strong relationship between the digit dominance and the digit with the best inter-pattern decoding performance (Figure 3B), i.e., a neuron that was dominated by digit 5 input could often decode input from digit 2 with a higher precision than it decoded input from digit 5, and vice versa. Another interesting feature revealed by this plot, is the gradual rather than binary transition between digit dominances. A linear regression model fitted to the relative difference in response intensity to single-pulse stimulation between the digits as the independent variable, and the relative difference in inter-pattern decoding per digit as the dependent variable resulted in a weak coefficient of determination (Figure 3B), i.e., the digit dominance of the neuron did not predict which digit had the best inter-pattern decoding.

**Figure 3. Inter-pattern, per Digit Decoding Performance Had a Weak Relationship to Input Dominance**

(A) Example data from the same neuron as in Figure 2. Confusion matrix showing inter-pattern, per digit decoding of responses to stimuli for digit 2 and digit 5, respectively. The stimulation pattern label predicted by the classifier is shown along the y axis and the actual label along the x axis. The numbers in the diagonal indicate the decoding level for each specific stimulation pattern, where the chance level is 12.5% (given by eight classes of input). Please refer to kNN and Confusion Matrices in STAR Methods for further details.

(B) Scatterplot of the inter-digit difference in F-score decoding performance against the inter-digit difference in response magnitude (used to define the dominant digit) across all neurons (N = 89). The gray area indicates the ±10% limits within which the difference in response intensity was considered too low to identify a dominant digit input (“equidominant” inputs). Neurons above this area are defined as digit 2 (“D2”) dominated, and those below were digit 5 (“D5”) dominated. Also indicated are a linear regression model (red line) and its 95% confidence interval (CI; blue dashed lines) (R² = 29.2%; slope, 1.07 [95% CI, 0.77–1.36]; intercept, 0.07; p < 0.0001).

The Dominant Input Did Not Provide the Best Decoding Performance

A main hypothesis we tested was whether the digit with the dominant response intensity to single-pulse stimulations was also the digit for which the neuron had the best decoding of the spatiotemporal input patterns (i.e., inter-pattern, per digit decoding performance). If such a relationship existed, it would indicate that the digit dominating the input of the neuron would also define the focus of its processing. In contrast, we found that there was no strong relationship between the digit dominance and the digit with the best inter-pattern decoding performance (Figure 3B), i.e., a neuron that was dominated by digit 5 input could often decode input from digit 2 with a higher precision than it decoded input from digit 5, and vice versa. Another interesting feature revealed by this plot, is the gradual rather than binary transition between digit dominances. A linear regression model fitted to the relative difference in response intensity to single-pulse stimulation between the digits as the independent variable, and the relative difference in inter-pattern decoding per digit as the dependent variable resulted in a weak coefficient of determination (Figure 3B), i.e., the digit dominance of the neuron did not predict which digit had the best inter-pattern decoding.

Weak Relationship between the Decoding of the Same Patterns to the Two Digits

The analysis of Figure 3A also allowed a quantification of the F-score decoding of each individual stimulation pattern. Figure 4 plots the decoding of each pattern for one digit against the decoding of the same pattern to the other digit for each neuron (inter-pattern, inter-digit decoding performance for individual patterns). The relationship between the decoding of the same input patterns between digits was weak. Figure 4 also illustrates that the decoding of the individual input patterns fell down to chance when the stimulation pattern labels were shuffled between the responses.

Individual Neurons Could Encode Both Input Pattern and Input Location

The next question we asked was whether the neurons in addition to separating the spatiotemporal stimulation patterns when
rons report which of the patterns were delivered (Figure 3), they 
that, in most cases, not only did the spike responses of the neu-
to digit 2 and to digit 5 shown separately. This analysis implies 
decoding is shown in Figure 5B, for neurons with dominant input 
to the input was provided to, the full set of responses (i.e., to the to-
tal of 16 stimulation patterns) were pooled and analyzed together 
neuronal responses, rather than the spatiotemporal structure 
tensity was a function of the intensity of the stimulation patterns 
i.e., the number of stimulation pulses) rather than their spatio-
temporal structures. A related issue was previously investigated 
by Oddo et al. (2017), where scrambling of the stimulation pat-
terns in time or in space was found to disrupt the decoding per-
formance. However, here we investigated whether there was a 
simple relationship between the number of pulses in the spatio-
temporal stimulation patterns and the number of action poten-
tials of the neuronal responses, expressed as the mean number 
of SDs from the baseline for each stimulation pattern (Figure 
S1A). The decoding analysis was repeated with the number of 
pulses as the “labels” (corresponding to the stimulation pat-
terns in the previous analysis above) and the number of action 
potentials as the response dimension (i.e., the rate code). For 
the kNN analysis of the rate codes, the mean decoding perfor-
mance was 6.7% (SD, 0.85%; chance level at 6.25%) as shown 
by the example confusion matrix (Figure S1B). These results indi-
cate the hypothesis that it is the intensity of the stimuli (i.e., the 
number of pulses) that determines the differences between the 
n neuronal responses, rather than the spatiotemporal structure 
of the stimulation patterns, is implausible.

Finally, we tested whether there was a relationship between 
recording depth, sorted by putative cortical layer and the decod-
ing performance, net inter-pattern decoding performance be-
tween digits or net response magnitude (Figures 7 A–7F). None 
of the parameters correlated with depth. However, the response 
magnitude tended to be higher between 0.8 and 1.1 mm, which 
is in agreement with previous observations on the tendency of 
average response magnitudes to tactile afferent stimulation to 
be higher in thick tufted layer 5 pyramids (de Kock et al., 
2007), which are most often found at 1.1 ± 0.1 mm (Oberlaender et al., 2011).

**DISCUSSION**

The present study shows that the temporal patterns in the spike 
output of individual neurons of the somatosensory cortex can 
signal tactile quality, as encoded in the spatiotemporal tactile 
input patterns, from non-adjacent digits. The decoding of tactile 
input patterns could even be better for inputs from the non-domi-
nant digit than from the dominant digit, suggesting a low impact
of somatotopy on the organization of the information processing (Figure 3). At the same time, individual neurons could also signal which of the digits the input originated from (Figures 5 and 6). Hence, the output of each neuron, regardless of layer location (Figure 7), potentially contains information of both the “what” and the “where” components of tactile inputs for two non-adjacent digits. This principle is unlikely to be confined to the two digits we explored, or even to digits. Therefore, our findings have major implications for current models of cortical processing of tactile inputs that focus on local, functional specialization.

The Approach to Stimulus Delivery Was Required for a High-Resolution Analysis

Skin-object interactions generate spatiotemporal patterns of tactile afferent inputs and the rationale of our approach is that it can provide a varied set of such patterns with very high pattern reproducibility (Oddo et al., 2017). In this context, the spatiotemporal patterns of electrical tactile afferent stimulation offer an advantage that even refined mechanical stimulation cannot match. For example, for repeated activations, even higher energy mechanical stimulation in identified receptive fields of single tactile primary afferents will generate variable spike output responses in the afferent (Johansson et al., 1982). In addition, as a skin-object interaction potentially results in the activation of thousands of skin sensors (Shao et al., 2016), even shifts on the micrometer scale in the location of a mechanical skin stimulation from one trial to another can result in quite different patterns of skin sensor activation. For more natural forms of stimulation, which are lower-energy spatiotemporal patterns of skin strain (Hayward et al., 2014; Jörntell et al., 2014), the mechanical time constants of the skin makes it difficult to achieve reproducible skin stimulation, and for the rodent there is not yet a solution overcoming these problems. The electrical spatiotemporal tactile activation patterns eliminate all of these potential sources of variability and focuses the analysis to the cortical network. This approach makes it possible to deliver a high number of repetitions of a relatively large set of complex spatiotemporal tactile input patterns in a random order within a short span of time. A major advantage compared to other studies of somatosensory processing is that individual responses have an impact in the analysis (the decoding performance indicates the accuracy by which the responses to repeated inputs identify the input) rather than relying on averaged responses. The advantage is demonstrated in a comparison with a previous analysis of neuronal responses to identify the direction of whisker bending of two adjacent whiskers (Kida et al., 2005). Kida et al. used the average number of spikes across multiple trials and plotted them against the approximate direction at which a whisker was bent to evoke a response. In this type of analysis, the same responses were

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**Figure 5. Inter-pattern, Inter-digit Decoding Performance Indicated That Neurons Decoded Both Input Pattern and Digit**

(A) Confusion matrix for inter-pattern, inter-digit decoding performance, i.e., the decoding of all stimulation patterns from both digits, for the same neuron as in Figure 3A. Note that the chance level is 6.25% (given by 16 classes of input).

(B) Distributions of the neuronal inter-pattern, inter-digit decoding performance (i.e., full-set decoding performance). The bin width is 2.5%. The gray dashed vertical line indicates the chance level, 6.25%. The red histogram is for digit 2-dominated neurons, the blue histogram is for digit 5-dominated neurons, and the black is for equidominated neurons.

(C) Scatterplot of the mean of the inter-pattern, per digit decoding performance (i.e., the mean performance for digit 2 and digit 5 inputs) on the y axis (chance level, 12.5%; gray dashed vertical line), and the full-set decoding performance on the x axis (chance level, 6.25%; gray dashed horizontal line). Each neuron contributes with one data point (N = 89; red/D2-dominated = 61; blue/D5-dominated = 20; black/equidominated = 8). A fitted linear model is drawn as dark gray line with the CIs indicated by dashed light gray lines (R² = 97.4%; slope, 0.90 [95% CI, 0.88–0.93]; intercept, 0.07; p < 0.0001).
Moreover, under ketamine predictive signal reflecting an attempt to interpret the sensory by the sensory stimulus itself rather than an internally generated to unequivocally identify a recorded cortical response as evoked for multiple directions, so the neuronal responses could not be used to unambiguously tell in which direction the whisker was bent nor which of the whiskers that were bent.

**Dual-Digit Inputs versus Somatotopy**

Our neuron search was guided by stimuli applied to one of the digits, but we often found that neurons responded to both digits even though the differences in response intensities could be large. A relevant question is whether the neurons we collected had unorthodox whole-paw receptive fields? We do not believe this to be the case, because our approach, to record in areas that exhibited the largest local field potentials from input to either digit 2 or digit 5 is in line with the standardized techniques to define the respective subareas of S1. The notion of a pure single digit peripheral input in S1 likely stems from studies using low-intensity, near-threshold stimuli to define receptive fields, which results in a “tip of the iceberg” effect that is not representative of the actual integration of the neuron (Reed et al., 2008). In our case, we did not use bare minimum stimuli to define the inputs to the individual neurons. Instead, we used the same stimulus intensity consistently, and hence it was not surprising that we often detected responses to both digits 2 and 5 in individual neurons. Notably, the existence of such double-digit receptive fields has previously been reported for the forepaw region of S1 in the rat (Tutunculer et al., 2006) using a mechanical indentation with a similarly predefined stimulation intensity.

Our results suggest that the dominant input to a neuron, which defines the somatotopical organization of the cortex, likely has a low predictive value for the functions of the neuron in tactile processing. In the apparent dichotomy between the somatotopically organized thalamocortical projections and the widespread horizontal branching of intracortical connections, our study would suggest that the latter may have a much larger defining role for understanding these functions.

**Implications for Cortical Models of Tactile Processing**

At the general level, functional localization would make sense if the cortex would benefit from parceling its processing into distinct groups of neurons. However, functional localization in principle is a sparsification of the representation, as opposed to a representational densification where a large number of neurons are engaged in the processing of any conceivable (tactile) event. There are clear theoretical arguments against the idea of exaggerated sparsification as this leads to the drawback of poor generalization capability, i.e., network structures acquired from learning one type of condition are applicable to exactly the same type of event but will perform poorly in novel, but related, contexts (Spanne and Jörntell, 2015).
As even single neurons can be used to identify the location of the stimulus on the skin (Figure 6; see also Oddo et al., 2017, where it was shown that the spike output of cortical neurons could also identify which of the four stimulation sites on digit 2 that was activated), the identification of the location, or the “where,” of the input becomes a trivial task. Identifying the “what” of the input, or the quality of the tactile input, may instead be a more resource-consuming task that requires a much larger

Figure 7. Recording Depth Was Not Predictive of Decoding or Digit Decoding Specialization
(A) Scatterplot of inter-pattern, per digit decoding performance for digit 2 against neuron depth. Differences between layer-segmented distributions was not statistically significant (Kruskal-Wallis test; p = 0.79, H = 0.5). Within each putative cortical layer, a box plot is overlaid that summarizes the data points within that layer. The distance from the cortical surface is indicated along the y axis. Layer delimitations are indicated with dashed horizontal lines, with depth indicated to the left and putative layer name to the right. The y axes are the same for all panels.
(B) Inter-pattern, per digit decoding performance for digit 5 plotted against depth. Differences between layer-segmented distributions was not statistically significant (Kruskal-Wallis test; p = 0.13, H = 4.1).
(C) Inter-pattern, inter-digit decoding performance (full-set decoding) versus depth. Differences between layer-segmented distributions were not statistically significant (Kruskal-Wallis test; p = 0.83, H = 0.4).
(D) Net difference between digit 2 and digit 5 decoding (inter-pattern, per digit decoding) versus depth. Differences between layer-segmented distributions were not statistically significant (Kruskal-Wallis test; p = 0.43, H = 1.7).
(E) Mean inter-digit, per pattern decoding performance for each neuron versus its recording depth. Differences between layer-segmented distributions were not statistically significant (Kruskal-Wallis test; p = 0.83, H = 0.4).
(F) Mean firing frequency (in hertz) during evoked responses versus depth. Differences between layer-segmented distributions were not statistically significant (Kruskal-Wallis test; p = 0.38, H = 1.9).
processing capacity and therefore could form a reasonable explanation for the widespread integration of inputs in the somatosensory cortex (neurons with poorer decoding might, for example, be more engaged in other types of input patterns). An important related question is the format of the information that underlies the cortical identification of the “what” of tactile inputs.

We have previously shown that, at the level of the cuneate nucleus, which is the first processing stage for tactile afferent input before it reaches the thalamus and the cortex, the individual neurons can already extract different high-level features from haptic interactions, the so-called fundamental haptic input features (Jörntell et al., 2014). These high-level features are not arbitrary but correspond to the types of fundamental interactions, as defined by the laws of contact mechanics, that can arise in the interactions between any two objects (Hayward, 2011). It is likely that a particular haptic feature corresponds to a set, or a family, of spatiotemporal patterns of skin sensor activation. The identification of such patterns, regardless of the location of their origin, may be a prioritized function of the neurons in the somatosensory cortex. In addition, these features would need to be matched against internal expectations about the properties of the tactile input (Bar, 2007; Friston, 2010), which is likely another explanation for the widespread integration of inputs in the so-matosensory cortex. J. Physiol. 229, 152–168.

STAR METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found with this article online at https://doi.org/10.1016/j.celrep.2019.02.099.
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STAR METHODS

KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Deposited Data      | This paper | [https://figshare.com/articles/Spike_time_files/7687604/1](https://figshare.com/articles/Spike_time_files/7687604/1) |
| Experimental Models: Organisms/Strains | Sprague-Dawley WT | Taconic | N/A |
| Software and Algorithms | Spike2 | CED | ced.co.uk |
|                      | MATLAB | MathWorks | [https://www.mathworks.com/](https://www.mathworks.com/) |
|                      | Python | Python | [https://www.python.org/](https://www.python.org/) |

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Henrik Jörntell, BMC F10 Tornävägen 10, Lund University, SE-221 84 Lund, Sweden, Tel: +46 46 222 77 64, e-mail: henrik.jorntell@med.lu.se.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Rats
Adult Sprague-Dawley rats of male sex (N = 12, weight mean = 357.5 g, range = 324-418 g) were used in the acute experiments. All animal experiment procedures in the present study were in accordance with institutional guidelines and were approved in advance by the Local Ethics Committee of Lund, Sweden (permit IDs M118-13 and 13193-2017).

METHOD DETAILS

Surgical procedures
Adult Sprague-Dawley rats were prepared and maintained under anesthesia with ketamine (100 mg/ml) and xylazine (20 mg/ml) mixture (concentration ratio of 20:1). Prior to the induction of anesthesia the animal was sedated with isoflurane (3% mixed with air for 30-60 s). Anesthesia was induced with an intraperitoneal injection (ketamine (100 mg/ml):xylazine (20 mg/ml) concentration ratio of 15:1, 1.5 ml/kg), and further maintained with a continuous infusion through an intravenous catheter inserted into the right femoral vein (approximately 5 mg/kg ketamine per hour). The absence of withdrawal reflexes to noxious pinch to the hindpaw was used to characterize adequate anesthesia until the brain was exposed.

The decision to run the neuronal recording experiments under anesthesia was motivated by that we needed to make sure that the mechanical stability of the brain was consistently high throughout the experiments. This was important in order to be able to run the long-term in vivo patch clamp recordings necessary to expose the neurons to a sufficient number of stimulus presentations. Further theoretical considerations relating to the interpretation of our results can be found in the Discussion.

A craniectomy with an approximate extent of 3.5 mm caudally and 2 mm laterally relative to a reference point located 1.5 mm rostral and 3.0 mm lateral to the bregma was made in the right hemicranium. The craniectomy exposed the primary somatosensory cortex for the forelimb, with the paw and digit areas located in the most rostral part of the exposure (Chapin and Lin, 1984) (Figure 1A). An ECoG electrode was placed in the rostral end of the craniectomy. For recording stability, a cap of agarose (0.03 g/ml dissolved in physiological saline) was made to cover the exposed part of the brain.

The exposed brain was inspected with a microscope during both insertion and extraction of the recording electrodes. The state of the rat was continuously evaluated based on skin tone, respiration rate and ECoG signal. The ECoG signal was monitored for irregular occurrences of sleep spindles, indicating deep sleep. The duration of the anesthesia did not exceed 12 hours.

For the present set of experiments, an effort was made to try to minimize the possible bias for finding neurons primarily activated by either digit. Thus, two types of experiments were performed. For one set of rats (N = 7) the search stimuli used was presented to digit 2 and electrode tracks were made within the estimated area of maximal field potentials in layer III for that input, which also approximately overlapped the anatomical location of the digit 2 area estimated by Chapin and Lin (1984). For the other set (N = 5) the search stimuli was presented to digit 5 and electrode tracks were made within the estimated area of maximal field potentials in layer III for that input, which also approximately overlapped the anatomical location of the digit 5 area estimated by Chapin and Lin (1984).
each experiment, we made in the order of ten tracks, where the majority were made in the field potential focus for the respective search stimulus.

**Recordings**

All recordings were made in vivo in the right hemisphere, in the region of the primary somatosensory cortex of the forepaw (Figure 1A) at the stereotaxic coordinates reported under Surgical procedures. This was further established by presence of local field potentials evoked by electrical stimulation of either digit 2 or digit 5.

The recorded signal was continuously monitored on two displays and via loudspeakers. During slow advancement of the recording electrode (approximately 0.002 mm per second) with an electrical stepping motor the skin stimulation sites for either digit 2 or 5 were activated with one pulse per second as search stimuli. Individual neurons were recorded with patch clamp pipette extracellularly in loose patch current clamp recording mode. Patch clamp pipettes were pulled from borosilicate glass capillaries to 10-30 MOhm, using a Sutter Instruments (Novato, CA) P-97 horizontal puller. The pipettes were filled with an electrolyte solution with the same composition as previously described (Oddo et al., 2017). According to spike duration, as defined by (Bartho et al., 2004), none of our recorded neurons were interneurons (tough to peak time: 87ms ± 42, half-amplitude duration: 31ms ± 15).

All data was digitized at 100 KHz using CED 1401 mk2 hardware and Spike2 software (Cambridge Electronic Design, CED, Cambridge, UK). Spikes were identified using in-house software, where spike identification was based on matching to manually constructed templates (spike times data available at: https://figshare.com/articles/Spike_time_files/7687604/1). All spike detection was carefully controlled by visual inspection of raw data traces throughout the recordings. A recording was deemed as of sufficient quality if at least one complete protocol was recorded and all needle electrodes were functional. The recording depth from the surface of the brain was saved. After the recording session the animal was sacrificed.

**Stimulation**

Two sets of four pairs of intracutaneous needle electrodes were inserted, with an interneedle distance of 2-3 mm, into predetermined sites of the skin on the volar side of digit 2 and 5 of the left forepaw (Figure 1B). These two sets constituted the electrical interfaces to the tactile afferents, through which the eight predefined spatiotemporal patterns and single pulse stimulation of skin afferents were delivered (Figure 2) (the stimulation patterns are indicated as F5, S5, F10, S10, F20, S20, F∞ and S∞; they were exactly the same patterns as in the paper by Oddo et al. (2017)). These stimulus patterns had variable duration but lasted less than 350 ms. Consecutive onsets of stimulus pattern delivery were separated by 1.8 s. Additionally, for each of the two sets stimulation sites, isolated single pulses to each of the four stimulation sites were delivered. These isolated single pulses were delivered in trains of five consecutive pulses at 3 Hz as 20 separate trains for each individual stimulation site. The eight spatiotemporal patterns and four single pulse stimulation trains were presented up to 50 times for each digit in a pseudo-random order (for a total of 880 stimulus presentations per neuronal recording). Thus, each finger was stimulated with the same collection of spatiotemporal patterns and single pulse trains.

For each skin site, the stimulation pulse was set to an intensity of 0.5 mA with a duration of 0.14 ms (DS3 Isolated Stimulator, Digitimer, UK), which is 2.5 times greater than the threshold for activating tactile afferents (Bengtsson et al., 2013; Rasmusson and Northgrave, 1997), but well below the threshold intensity for A-delta (peak activation requires 6–10 times threshold intensity) and C-fibers (Ekerot et al., 1987).

**Generation of the spatiotemporal stimulation patterns**

The generation of the spatiotemporal stimulation patterns has been described in detail previously (Oddo et al., 2017). Briefly, we used an artificial fingertip equipped with a set of neuromorphic sensors to transduce a set of tactile events by indenting the sensorized skin of the fingertip against a set of predefined shapes using a cyclic motion (Figure S2A). The core element of the sensorized fingertip was a Micro Electro Mechanical System (MEMS) sensor with 4 transducing piezoresistors implanted at the base of a cross-shaped structure. The MEMS was packaged with polymeric compliant material (Dragon Skin, Smooth-On, USA). MEMS data were sampled at 380 Hz per sensor output by a 24-bit Analog to Digital Converter (ADS1258, Texas Instruments, USA) integrated in the fingertip, and acquired via a serial peripheral interface by a Field Programmable Gate Array (Cyclone II FPGA, Altera, USA). The FPGA acquired the information, which in principle corresponds to the receptor potentials of skin sensors (Woo et al., 2015). These ‘receptor potentials’ were converted to spike trains by the neuromorphic artificial touch system which uses a customized implementations of Izhikevich spiking neuron model. The four needle electrode pairs, of the interfaces (one interface for each digit) were 1-to-1 connected to the four neuromorphic sensors of the artificial fingertip (see Figure S2B for needle electrode pair locations on digit 2 and 5).

The artificial fingertip allowed us to synthesize spatiotemporal patterns of skin sensor activation at quasi-natural rates that follow a natural overall temporal modulation, or ‘envelope’ (Middleton et al., 2006), that the biological skin sensors display under dynamic indentation. The eight spatiotemporal patterns were derived from interacting with four different objects using two different adaptation rates on the neuromorphic sensors, slow (S) and fast (F). However, as shown previously (Oddo et al., 2017), under the type of dynamic indentation movement used, available evidence indicates that there is in principle little difference in the spike activation between slowly and rapidly adapting tactile mechanoreceptors (Jenmalm et al., 2003; Johansson et al., 1982). Hence, the eight spatiotemporal patterns should be regarded as eight different types of skin-object interactions. Just like natural tactile inputs, the input we provided...
can be expected to be distributed and processed through the neuronal networks in the cuneate nucleus, thalamus and neocortical circuitry before it reached the neurons we recorded from. Hence, the measured decoding is bound to reflect at least in part the inherent processing mechanisms of the brain.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

**Single pulse trains PSTH and Z-score**

For data display, we made persistimulus time histograms (PSTH) with a bin width of 5 ms. For the isolated single pulse stimulations, each PSTH included 100 ms pre-stimulus and 100 ms post-stimulus time. Subsequently, we calculated the z-score (i.e., the deviation from the mean expressed as the number of standard deviations (s.d.s.)) for each bin of the PSTHs.

**Definition of dominant digit input based on response intensity to single pulse stimulation**

To quantify the response intensity to isolated single pulse stimulation from the PSTHs, we used the sum of the z-score during the first 100 ms after the stimulus (area under the curve, AUC) for each of the four stimulation sites on the digit investigated. The means of the AUCs evoked from the two digits was used as the basis to define the dominant digit input. If the mean AUC of the responses evoked from one digit exceeded the mean AUC of the responses evoked by the other digit by more than 10%, it was defined as being dominated by input from that digit. If the difference did not exceed 10%, the neuron was defined as being ‘equidominated’ (see Figure 3B).

**Latencies**

The analysis of the response latency times was also based on the PSTHs of the isolated single pulse stimulations. Pre- and post-stimulus durations was 100 ms as before. The PSTH-values were again converted to z-scores and the onset of a response was defined as the time of the first bin with a value equal to or greater than 2.0 within the first 50 ms post-stimulus. Because of the ambiguity in that each bin represented the range of 5 ms the value chosen to represent a bin, and thus response onset, was the mean of the edges (i.e., for the bin with the edges 5-10 ms the representing value became 7.5 ms). The exception being the first bin which would have been represented with 2.5 ms, which is less than the theoretically fastest conduction time. Thus, the assigned value to the first post-stimulus time bin was 4 ms.

As there were four stimulation sites per digit (and as such eight stimulation sites in total per neuron) the shortest latency per digit was calculated. This yielded two latency times per neuron, one for each digit (digit 2 and digit 5). The mean and the standard deviation were calculated for the population of latencies for digit 2 and digit 5. The two populations were compared using paired t test, where the two latencies from each neuron were considered dependent.

**Decoding of neuronal responses using Principal Component Analysis**

To answer the question of whether the spike output of the neurons could be used to identify the input pattern applied to the skin of the second or fifth digit we used a slightly modified version of a previously published method (Oddo et al., 2017; Zuo et al., 2015) based on the common statistical methods Principal Component Analysis (PCA) and kNN classification, with the addition of bootstrapping. In short, bootstrapped responses were decomposed to their scalar products of the corresponding Principal Components, and thus positioned in a high-dimensional space upon which the kNN-classification was applied. In principle, this analysis allows a quantification of the precision by which the spike response of each single neuron can be used to tell which of the eight spatiotemporal tactile afferent inputs was applied for each trial.

The choice of method was due to that biological noise arising from uncontrollable variations in intrinsic brain network activation that coincides in time with the provided input will inevitably cause the neurons to respond somewhat differently even if it is exactly the same input is provided. As the responses of the neuron to repetitions of the same input were expected to vary within a certain range, we needed to quantify if the response variability still permitted the response evoked by the same input to be separated from the responses to the other spatiotemporal input patterns. The answer to that question is hence not binary, it is a probability. As we used eight different input patterns, we wanted to know with what probability each input pattern could be separated from the other seven input patterns. The combined PCA and kNN classification allowed us to do just that, as described below.

I. We converted the spike trains evoked by each stimulus presentation into continuous functions by convolving them with an exponential kernel with a characteristic time of 5 ms.

II. We randomly assigned half of the convolved responses to a training set and the remaining into a test set. Each set was handled separately for the remainder of the analysis.

III. We used bootstrapping to resample the data 500 times (see below for further details) and from the training set we extracted the N Principal Components (PCs) needed to explain 95% of the variance.

IV. Then, we computed the scalar product between each bootstrapped response and each of the N PC-vectors using the least square method. Thus, positioning each bootstrapped response in an N-dimensional space.
V. Finally, we used the k-Nearest Neighbor (kNN) classification procedure to decode the stimuli from each bootstrapped response. For each bootstrapped response in the test set the closest 9 responses in the training set were identified by calculating the Euclidean distances in the N-dimensional space. The response was classified as elicited by the same stimulus that elicited the relative majority of these neighbors.

VI. We performed 50 iterations of this decoding procedure, each with a different training and test set, and calculated the confusion matrix and all subsequent metrics from the pooled results.

The analysis of the spike responses always included the first 1000 ms of the evoked responses. As an internal control we performed each analysis a second time, but with a shuffling of the label of the stimulation pattern between the responses.

**Shuffled control decoding**

The theoretical chance level of the decoding performance when performing a kNN-classification analysis depends on the number of classes. The chance level for correct classification when there are two classes is 50%, i.e., 100% divided by the number of classes. In our setting when we had eight classes, or spatiotemporal tactile stimulation patterns, the theoretical chance level was hence 12.5% (100%/8, or 6.25% for 16 classes). For each neuron, we also tested the effect of shuffling the responses with respect to the stimulation pattern. This control decoding analysis (referred to as ‘shuffled control decoding’ in the Results) worked exactly as the decoding analysis described previous, but prior to each data split into a test and training set the labels for each stimulation pattern were shuffled.

**Bootstrapping of time-continuous signals**

The convolved, continuous responses from a neural recording were bootstrapped by first grouping them by stimulation pattern. Subsequently, a new sample of N responses were taken from this population, where N was equal to the number of available responses, using sampling with replacement. The sum of these samples was stored as a bootstrapped response. It is important to note that by sampling with replacement each bootstrapped response is not equal to the overall mean of the available responses. This separates this approach from PSTH-based methods where only the overall mean is analyzed and smaller in-group variations are lost.

**Decoding using rate code**

Decoding analysis using rate code included only one dimension, i.e., the number of pulses of the response (the rate). Since the duration of the patterns were somewhat different (as seen in Figure 2), the duration of the time window within which the number of evoked spikes was counted was determined by the duration of the stimuli, with 20 ms added at the end to allow for the response evoked by final pulse of the sequence to be included.

**kNN and confusion matrices**

k-Nearest Neighbor classification analysis is a stochastic analysis in the sense that for each iteration a new random set of data points are selected to act as the training set, and as a consequence the remaining acts as the test set. Hence, for each data point in the test set a predicted label is assigned based on the k nearest neighbors labels of the training set (as described under Decoding of neuronal responses using Principal Component Analysis point v). This predicted label is then evaluated against the true label. This means that the confusion matrix is not symmetric, i.e., the combination S10(predicted) → S5(actual) is different from S5(predicted) → S10(actual) (see Figures 3A and 3B). The process of classification is repeated and the results are pooled.

**DATA AND SOFTWARE AVAILABILITY**

Spike time data can be accessed https://figshare.com/articles/Spike_time_files/7687604/1.