Title

Spike count, spike timing and temporal information in the cortex of awake, freely moving rats

Journal

Journal of Neural Engineering, 11(4)

ISSN

1741-2560

Authors

Scaglione, Alessandro
Foffani, Guglielmo
Moxon, Karen A

Publication Date

2014-08-01

DOI

10.1088/1741-2560/11/4/046022

Peer reviewed

Published in final edited form as:
J Neural Eng. 2014 August ; 11(4): 046022. doi:10.1088/1741-2560/11/4/046022.

Spike count, spike timing and temporal information in the cortex of awake, freely moving rats

Alessandro Scaglione[1,2], **Guglielmo Foffani**[1,3], and **Karen A. Moxon**[1]

[1]School of Biomedical Engineering, Science and Health Systems, Drexel University, 3141 Chestnut Street, PA 19104, Philadelphia, USA [2]National Institute on Aging, National Institutes of Health, Baltimore, Maryland [3]Neurosignals Group, Hospital Nacional de Parapléjicos, SESCAM, Finca la Peraleda s/n, 45071 Toledo, Spain

Abstract

Objective—Sensory processing of peripheral information is not stationary but is, in general, a dynamic process related to the behavioral state of the animal. Yet the link between the state of the behavior and the encoding properties of neurons is unclear. This report investigates the impact of the behavioral state on the encoding mechanisms used by cortical neurons for both detection and discrimination of somatosensory stimuli in awake, freely moving, rats.

Approach—Neuronal activity was recorded from the primary somatosensory cortex of five rats under two different behavioral states (quiet vs. whisking) while electrical stimulation of increasing stimulus strength was delivered to the mystacial pad. Information theoretical measures were then used to measure the contribution of different encoding mechanisms to the information carried by neurons in response to the whisker stimulation.

Main Results—We found that the behavioral state of the animal modulated the total amount of information conveyed by neurons and that the timing of individual spikes increased the information compared to the total count of spikes alone. However, the temporal information, i.e. information exclusively related to when the spikes occur, was not modulated by behavioral state.

Significance—We conclude that information about somatosensory stimuli is modulated by the behavior of the animal and this modulation is mainly expressed in the spike count while the temporal information is more robust to changes in behavioral state.

INTRODUCTION

How neurons use spikes to represent or ‘encode’ information about external stimuli is a fundamental subject in systems neuroscience. On the one hand, neurons can represent information by the total number of spikes emitted in a given time window (Adrian, 1926). With this code, generally referred to as spike count, the total information that can be carried by a neuron is primarily limited by its firing rate (Scaglione et al., 2011). On the other hand,

Corresponding author: Karen Moxon, PhD, Associate Professor, School of Biomedical Engineering, Science and Health Systems, Drexel University, 3141 Chestnut Street, PA 19104, Philadelphia, USA, karen.moxon@drexel.edu, Phone: 215.895.1959, Fax: 215.895.0570 (fax).

neurons can use the time of arrival of individual spikes to represent information (Optican and Richmond, 1987; Bialek et al., 1991; Hopfield, 1995; deCharms and Merzenich, 1996; Victor and Purpura, 1996; de Ruyter van Steveninck et al., 1997; Borst and Theunissen, 1999; Reich et al., 2001; Chase and Young, 2006; Butts et al., 2007). With this code, generally referred to as spike timing, neurons can carry additional information compared to spike count alone (MacKay and McCulloch, 1952). Spike count and spike timing are not mutually exclusive, as spike timing information generally includes spike count information (Nelken et al., 2005; Foffani et al., 2009). Therefore, only the additional information conveyed by spike timing over spike count can be genuinely considered as temporal information, i.e. information exclusively related to when the spikes occur (Foffani et al., 2009).

In the rat trigeminal system, the role of spike timing has been investigated for the encoding of both stimulus location (Arabzadeh et al., 2004; Foffani and Moxon, 2004; Panzeri et al., 2001; Petersen et al., 2002) and stimulus dynamics (Bale and Petersen, 2009; Montemurro et al., 2007). In both the whisker cortex and thalamus, spike timing conveys more information than spike count (Ghazanfar et al., 2000; Panzeri et al., 2001; Foffani and Moxon, 2004; Arabzadeh et al., 2006). Similar results have been obtained in the forepaw cortex and thalamus, suggesting that spike timing plays an important role throughout the somatosensory system (Foffani et al., 2004; Blanc and Coq, 2007; Foffani et al., 2008; Foffani et al., 2009). However, these studies were conducted in anesthetized preparations.

In general, somatosensory processing is not a static experience: rats gather tactile information about the surrounding environment by actively sweeping their whiskers (Welker, 1964; Carvell and Simons, 1990). Consequently, during whisking the somatosensory system is subject to volleys of excitatory inputs that arise at the lowest level of the trigeminal system (trigeminal ganglion, Vg), while during quiet immobility there is no output from the Vg (Leiser and Moxon, 2007). Importantly, these changes in behavioral state from quiet to whisking modulate cortical neuronal responses to unexpected stimulation of the whisker pad (Fanselow and Nicolelis, 1999). However, little is known about the relationship between the behavior of the animal and the neural encoding mechanisms used by cortical neurons.

Here we investigated the impact of the behavioral state of the animal on the encoding mechanisms used by neurons in the rat barrel cortex to represent information. We recorded the activity of cortical neurons in response to three different strengths of punctuate electrical stimuli delivered to the mystacial pad while monitoring the behavior of the animal. The encoding mechanisms of these neurons for both stimulus detection and discrimination were then investigated using Shannon mutual information (Shannon, 1948) between the neural activity and the stimulus set, separately estimating spike count information, spike timing information and temporal information (Foffani et al., 2009).

METHODS

Animal preparation and experimental setup

Data were collected from 5 adult rats (250–350 g). Each rat was implanted into the barrel field region of the rat somatosensory cortex with a rectangular, 2 by 8, electrode array of insulated stainless steel micro-wires (50 μm diameter). The micro-wires had a length of about 5 mm and a row/column spacing of 350 μm (center-to-center), so the area covered by the entire array was about 400 μm by 2500 μm (Neurolinc, NY, USA). Animals were anesthetized with Isoflurane (1.5 to 3%) and then placed in a stereotaxic frame (Cartesian Research inc., Sandy OR). The skin over the skull was retracted and the surface of the skull cleaned of blood and fascia. Bregma and lambda were then identified on the skull and their relative distance was marked and aligned with respect to the horizontal plane. A mark on the skull was made at 2.5 mm posterior to bregma and 5.5 mm lateral. A rectangular craniotomy, centered on the marked location, of 3 by 2 mm was then performed on the left side of the skull. The 16-channel array was first rotated by 30 degrees with respect to the sagittal plane, centered over bregma and then moved to the final position given above. We tried to align the center of the array on whisker C5 accordingly to the rat brain atlas (Paxinos and Watson, 1986) so that the array should span over about 10 barrels, row C-D arc 1 to 5 (Chapin and Lin, 1984). The array was then lowered to layer V–VI of the cortex (CTX) at a depth of about 1.8 mm ventral from the pial surface. The electrophysiological properties of the cells were monitored, checking that strong responses to manual deflection of the whiskers were observed when moving the whiskers corresponding to the array target area. In addition to the craniotomy for the array, burr holes were drilled for five stainless steel screws that were embedded in the skull. Four of these screws served to anchor the array and to hold the ground wires in place. The last screw was used as an EEG electrode positioned over the whisker barrel cortex contralateral to the location of the array. The array was then cemented in place, along with the ground and the EEG wire, using dental cement to anchor them to the screw.

Whisker-pad Stimulator surgery—Before the electrode array implantation surgery, a whisker-pad stimulator was implanted to be able to deliver electrical stimuli to the mystacial pad (Scaglione et al., 2011). The advantages of such stimulator are that: 1) the stimulator can be targeted to preferentially stimulate nerve fibers from a particular whisker or group of whiskers, providing a selectivity of afferent activation that was not available with the cuff electrode (Fanselow and Nicolelis, 1999) and 2) any contribution of direction selectively (Bale and Petersen, 2009; Simons and Carvell, 1989) on neuronal activity in response to the stimulation is minimized. The whisker-pad stimulator was implanted following a similar procedure as described in Moxon et al. (2007). In short, a twisted pair of Teflon coated stainless steel wires with a diameter of 0.004 in. (California Fine Wire Co., Ca) was tunneled with an 18 gauge needle from the original incision in the scalp to the whisker pad of the rat to stimulate sensory afferents. Prior to the implantation, the wires were inserted into the needle and the insulations at the end of the wires were stripped (1–2 mm on the needle tip side and 10–15 mm on the luer-lock side). The bare ends of the wires on the needle tip side were offset by 2–3 mm and bent over the needle tip in order to form a hook to anchor the both wires at the base of a single whisker follicle. The wire was then tunneled

to the whisker pad using the 18 gauge needle as a guide; when the whisker pad area was reached, the wires were anchored to the base of the “target whisker” and the guide needle was gently removed. The bare ends of the wire on the implant side were inserted in a conductive grease filled connector (MS303-120; Plastics One Inc). Finally, the whisker-pad stimulator was tested applying a biphasic pulse of approximately 0.2 mA to evoke a whisker twitch. The electrode array implantation was then performed, as described previously, and once the array was cemented in place, the whisker-pad stimulator connector was then glued, with acrylic cement, at least 5 mm posterior the electrode array connector.

Experimental setup

After the surgery, animals were allowed a recovery period of a week before starting the experimental recording sessions. In each session, animals were removed from their home cage and a headstage was attached to the electrode array connector (nano-miniature connector, Omnetics, MN) on the top of their head to record the neural activity. Electrical signals recorded at each electrode were then amplified and bandpass filtered (0.8 Hz to 8 KHz). The signal was then digitized (40 kHz sampling rate) using a multi-channel data acquisition system (Plexon Inc., Dallas TX) and sent to a computer for data storage. A second connector was then attached to the animal’s head to supply current to the whisker-pad stimulator. Animals were then placed for 2 hours on a clear 12 × 12 inches plexiglass platform (no walls to minimize contact of the whiskers) while stimuli of varying duration were pseudo-randomly delivered to the whisker pad (see *Stimulation Protocol*). The TTL pulses defining the time of the stimuli were time locked to and stored with the neural data. While on the platform, animals were free to move. The entire session was videotaped with a CCD camera (Mintron, Freemont CA) that recorded two views of the animal, a frontal and a bottom view, by means of a mirror positioned at a 45-degree angle below the platform. The video recording was then used for behavioral discrimination (see *Behavioral Analysis*). One session per animal was recorded and used for subsequent analyses.

Stimulation Protocol—Electrical stimuli were delivered to the whisker-pad stimulator using an iso-flex stimulus isolator (AMPI Jerusalem, Israel) connected to a Master-8 stimulator (AMPI Jerusalem, Israel). The Master-8 stimulator was connected to a computer and controlled using a custom built MATLAB algorithm (Mathworks Inc., Natick MA). The algorithm was designed to deliver square pulses with a duration chosen at random between 60, 120 and 240 μs. Each pulse was separated by an interval of 2 seconds plus a uniformly distributed random interval of 0–200 ms. The order of the stimuli was randomized to avoid the animal anticipating a particular stimulus during a recording session and changing its behavior accordingly. By changing the duration of the pulse, we were able to deliver stimuli of different strengths similar to increasing mechanical deflection of the targeted whisker. To this end, the amplitude of the current for the stimulus was chosen so that the medium strength stimulus (120 μs) generated neuronal responses similar to those obtained with mechanical deflection of the same whisker by moving it forward by approximately 5° from its base (Foffani and Moxon, 2004). The amplitude of the current ranged from 0.5 to 1.5 mA for all the animals included in the study. Neural data collected around each single pulse constituted a single trial used for the data analysis. A total of 2700 trials, 900 trials for each stimulus strength, were obtained in each animal. However, only in 22% of the total trials

(596 ± 94 per animal) the behavior of the animal was clearly identified (see below) and those trials were used for subsequent analyses.

Behavioral Analysis—To time lock the behavior to the data acquisition system, each recording session was videotaped and timestamps, generated by the neuron recording system (Plexon, Inc Dalas TX), were acquired on each video field. A Panasonic AG-DS-555 video recording system was used to identify the behavior of the animal during each video field (17 ms window). Similar to our previous work (Leiser and Moxon,2007) the behavior of the animal was classified accordingly to three different categories: 1) awake with no movement (*quiet*); 2) when the animal was sweeping its whiskers with minimal locomotion activity (*whisking*) and 3) all other conditions in which the animal was not either in a quiet or whisking state. Only the first two behavioral states were considered for subsequent analyses. Identification of the two behavioral states was qualitatively performed by human observers, who, in addition to the above criteria, were instructed to mark only video fields where the whiskers were clearly on focus with respect with the camera. Frequently in each recording session, animals spent most of the time sitting quietly. For this reason, whisking behavior was sometime encouraged by playing sound clicks to the animal to increase the number of recorded trials during this behavioral state. Most of the stimuli delivered under active whisking behavior were delivered while the animal was “standing still and whisking”, such that any locomotor behavior of the animal played a small role in our results. Whisker twitching behavior was not considered in this work and excluded from the whisking behavior. To this end, the collected EEG signal was used to confirm that for periods marked as whisking the animal was not twitching (EEG displays characteristic 12 Hz oscillations; Semba and Komisaruk, 1984) and for periods identified as quiet the animal was not sleeping (by checking for the absence of large low frequency amplitude oscillations characteristic of sleep while the animals had their eyes open). Overall a total of 439 ± 83 trials per animal were obtained during Quiet epochs and 157 ± 63 were obtained during whisking epochs.

Spike sorting—During the recording sessions, waveforms of the spikes were extracted from the digitized signal by setting a threshold of 4 times the standard deviation of the raw amplitude. The selected waveforms and their respective spike times were recorded for the duration of the experiment using Sort Client (Plexon, Dallas TX). Plexon offline sorter (Plexon, Dallas TX) was used to discard artifacts and define the units. Waveforms were presented in PCA space and clusters of spikes, each representing a unit, were selected by hand. Outliers, defined as waveforms beyond the 99% confidence interval of all the clusters, were discarded as artifact. We considered single units only those units that had: 1) a clear separation of the cluster in the PCA space; 2) a refractory rate error, percentage of spikes within a 1 msec from each of the spikes of the unit, of less than 5% and 3) presence of the same spike waveform in the time period before each of the stimulation intensities so that the stimulation was not eliciting overlapping spikes from different units recorded at the same electrode. Units that did not meet these criteria were treated as multi-units.

Data Analysis

A total of 99 units were discriminated after offline sorting (13.4 ± 1.9 electrodes with units per rat, with 1.24 ± 0.32 units per electrode), 22 of which were considered to be single units

(3.00 ± 2.34 electrodes with single units per rat, with 0.26 ± 0.23 single units per electrode). For each discriminated unit, to characterize the neuronal response, a peri-stimulus time histogram (PSTH) was generated for each stimulus strength (60, 120 and 240 μs) using a 1 ms bin size (Figure 1A–D) to allow extraction of 6 parameters: 1) background activity, defined as the mean firing rate in a pre-stimulus window of 100 ms; 2) response magnitude, defined as the background-subtracted integrated response (spikes/stimulus) between 2 and 41 ms after the stimulus; 3) peak latency, as the latency of the peak response between 2 and 41 ms after the stimulus; 4) latency of the first spike, defined as the mean across trials of the latency of the first spikes between 2 and 41 ms after the stimulus and 5) jitter of the first spike defined as the standard deviation across trials of the latency of the first spikes. In addition to those 5 parameters we calculated 6) the number of units that were significantly responsive. A unit was defined to be significantly responsive if at least three consecutive bins of the post-stimulus window passed a threshold value of the background firing rate plus three times its standard deviation and the response between the first and the last significant bin was significantly greater than the background activity (non-paired t-test $p<0.001$) (Tutunculer et al., 2006) for any of the three stimulus strengths. All statistical analyses were separately performed on single-units and multi-units. However, because we found a very good agreement between the results for the two groups of cells, in all subsequent analyses we report the results for all units irrespectively of whether they were classified as single- or multi-unit. Neurophysiological data for the single units are available in Table S1 and Table S2 of the supplementary materials.

Behavioral modulation of the responses elicited by the whisker-pad stimulator—To test the effect of the behavior of the animal on the responses elicited by the electrical stimulation of the whisker-pad, the response properties of the recorded units (as measured by the 6 parameters defined above) were compared between the two behavioral states. Statistically significant changes were assessed using two-way repeated measures ANOVA. The first factor was Behavior with two levels, Quiet and Whisking, and the second factor was Stimulus Strength with three levels 60, 120 and 240 μs.

Spike count information, spike timing information and temporal information—To quantify the information conveyed in the neuronal responses about the stimuli by each of the recorded units, Shannon mutual information was estimated (Shannon, 1948). The mutual information that a set R of neuronal responses conveys about a set S of stimuli is estimated as:

$$I(\mathbf{R},\mathbf{S})=\sum_{s\in\mathbf{S}}\sum_{r\in\mathbf{R}}P(s)P(\boldsymbol{r}|s)\cdot\log_2\frac{P(\boldsymbol{r}|s)}{P(\boldsymbol{r})} \quad 1$$

where $\boldsymbol{r}$ represents the neuronal response emitted by a unit, P(s) denotes the probability with which the stimulus s is presented, and P($\boldsymbol{r}$|s) denotes the probability with which the response $\boldsymbol{r}$ occurs in response to the stimulus s. For spike count information, $\boldsymbol{r}$ is an integer number representing the number of spikes emitted on a single trial in the overall response time window (r_{count}). For spike timing information $\boldsymbol{r}$ is a vector of integer numbers whose length is determined by the bin size chosen to represents the responses ($\boldsymbol{r}_{\text{timing}}$). For sufficiently

small bin sizes, $\boldsymbol{r}$ is a binary number (or a vector of Booleans), i.e. a sequence of zeros and ones that represent the absence (0) or presence (1) of spikes in specific bins within the overall response time window. Importantly, r_{count} and $\boldsymbol{r}_{timing}$ are not independent. In fact r_{count} = sum($\boldsymbol{r}_{timing}$). This implies that spike timing information includes both spike count information (how many spikes occurred) and temporal information (when they occurred) (Nelken et al., 2005; Foffani et al., 2009):

$$I_{spike-timing} = I_{spike-count} + I_{temporal} + \Delta I \quad 2$$

where ΔI represents the synergy/redundancy between temporal information, $I_{temporal}$, and spike count information, $I_{spike\text{-}count}$. In the limit case in which temporal spike patterns change between stimuli with no differences in spike count, $I_{spike\text{-}count}$=0, ΔI=0 and all information is purely temporal information. If both spike counts and temporal spike patterns change between stimuli (and therefore can be used to discriminate between stimuli), then $I_{spike\text{-}count}$>0, $I_{temporal}$>0 and ΔI is negative, taking into account the redundancy between spike count information and temporal information. Note that with the assumption of negligible synergy between $I_{spike\text{-}count}$ and $I_{temporal}$, the additional information of spike timing over spike count (i.e. $I_{spike\text{-}timing} - I_{spike\text{-}count}$) represents a lower bound of temporal information. It is important to remark that temporal information, as defined here, refers to the information exclusively related to when the spikes occur, independently of the spike count information (Foffani et al., 2009). This definition should not be confused with other rigorous definitions related to 'temporal coding' or 'temporal encoding' (e.g. Theunissen & Miller, 1995).

Stimulus detection and stimulus discrimination—We investigated both the problem of discriminating the stimulus response from background, i.e. *stimulus detection*, and the problem of discriminating the responses between the three stimulus strengths, i.e. *stimulus discrimination*. For this reason, Shannon mutual information was estimated in three different ways based on the definition of the stimulus set **S**. To analyze *stimulus detection*, mutual information was estimated either to: 1) detect any of the stimulus strengths vs. background or 2) detect each single stimulus strength vs. background. In the first case, the stimulus set consisted of two elements S_B and S_A that were obtained grouping neuronal responses for all stimulus strengths (S_A) and their respective pre-stimulus background activity (S_B). Instead, in the second case, for each of the three stimulus strengths (60, 120 and 240 μs) the stimulus set consisted of two elements S_i and S_{0i} (where i=1,2 and 3) that represent, respectively, one of the three stimulus strengths and the respective background activity preceding the stimulus. Second, to analyze *stimulus discrimination*, the stimulus set consisted of three elements S_i (where i=1,2 and 3) that represent the three stimulus strengths (60,120 and 240 μs). All responses were obtained considering a 40 ms long post-stimulus window.

Behavioral modulation of spike count and spike timing information for stimulus detection—To extract the amount of spike timing information, Shannon mutual information was estimated between the responses and the stimulus set for *stimulus detection* using a post-stimulus window size of 40 ms and a binsize of 8 ms. This binsize was chosen as a tradeoff between maximization of spike-timing information based on previous studies (Ghazanfar et al., 2000; Panzeri et al., 2001; Foffani et al., 2004) and minimization of the

upward bias of mutual information estimates (see *Bias correction of the mutual information*, below). This information was compared to the one obtained from the spike count using the same post-stimulus window but a binsize of 40 ms to remove any timing component from the neural activity. It is important to note that because the spike timing information is estimated using a binsize manipulation method, any additional information obtained using this 8 ms binsize compared to spike count constitutes a lower bound of the total temporal information carried by spike timing considering smaller binsizes (Panzeri and Schultz, 2001). Statistically significant differences in the information encoded by the units were assessed using a two or three-way repeated measure ANOVA testing the effect of three factors: 1) Behavior with levels Quiet and Whisking, 2) Encoding with levels Count and Timing and, when comparing each single stimulus vs. background, 3) Stimulus strength with levels 60, 120 and 240 μs.

Behavioral modulation of spike count and spike timing information for stimulus discrimination—To test the effect of spike timing on *stimulus discrimination* we repeated the previous analysis estimating Shannon mutual information between the responses and the stimulus set for *stimulus discrimination* using a window size of 40 ms and a binsize of 40 ms for spike count and 8 ms for spike timing. Statistically significant differences in the information encoded by the units were assessed using a two-way repeated measure ANOVA testing the effect of two factors: 1) Behavior with levels Quiet and Whisking and 2) Encoding with levels Count and Timing.

Nature of the spike timing code—To further investigate the nature of the spike timing code, the information carried by the neurons about *stimulus discrimination* was further analyzed 1) to study any relationship between temporal information and the magnitude of the neuronal responses across stimuli, 2) to quantify the information contained in the 1st spike and 3) to determine if temporal information alone is greater than spike count information and whether they both depend on behavioral state.

To study the relationship between temporal information and the magnitude of the neuronal responses across stimuli, a *Response Magnitude Difference Index* was defined as the sum over the three stimuli of the absolute differences between the response magnitude of the unit to each individual stimulus and the mean response magnitude of the unit to all three stimuli (see first paragraph of the data analysis section for the definition of response magnitude). This index is zero if the response magnitude of the three stimuli is the same and increases as a function of the differences between response magnitudes of the individual stimuli. We said above that the additional information conveyed by spike timing over spike count ($I_{spike\text{-}timing} - I_{spike\text{-}count}$) represents a lower bound of the temporal information in the code. We therefore estimated the *Fraction of Temporal Information* in the code as:

$$\frac{I_{timing} - I_{count}}{I_{timing}} \quad 3$$

We tested the correlation between the *Fraction of Temporal Information and the Response Magnitude Difference Index*.

To assess the informational contribution of the first spike, neuronal responses were generated discarding all the subsequent spikes in any given trial, and the information was estimated considering an increasing time window from 2 to 41 ms post-stimulus – with a binsize of 8 ms – and compared to the information obtained from the spike timing considering the same post-stimulus window using all spikes.

To determine if temporal information alone is greater than spike count information, we considered only the first spike in each single-trial response and only responsive trials (i.e., trials with spikes) (Foffani et al., 2009). In this condition, spike count information is identically zero, so all spike timing information estimated is indeed temporal information. Because the mean latency of the first spike was 15 ms, a window of 20 ms was considered for the analysis using a binsize of 2 ms. Note that for this analysis we could use a smaller binsize because the upward bias of the mutual information is less problematic with only one spike per trial. The information extracted with this method was then compared with the information obtained considering the spike count of the responsive trials considering all spikes and compared across behavioral states. Statistically significant differences in the information encoded by the units were assessed using a two-way repeated measure ANOVA testing the effect of two factors: 1) Behavior with levels Quiet and Whisking and 2) Encoding with levels Count and Temporal.

Bias correction of the mutual information—Because the estimates of the neuronal response probabilities are subject to statistical fluctuations, due to the finite number of trials, an upward bias is observed in the estimate of the mutual information when equation 1 is used (Treves and Panzeri, 1995; Panzeri and Treves, 1996; Victor, 2002; Golomb et al., 1997; Kraskov et al., 2004; Panzeri et al., 2007; Ince et al., 2010). In particular, it can be shown that this bias is a function of the ratio between the number of trials and the number of possible responses, and is negligible when this ratio is greater than or equals to approximately 32. Alternatively an unbiased estimator needs to be employed to work in regime where this ratio is smaller than 32. To reduce the bias in the estimates of the mutual information in our data, we applied the Panzeri-Treves correction (Panzeri and Treves, 1996) to the shuffled-unconditional shuffled estimator, $I_{sh\text{-}ush}$, as proposed by Ince and colleagues (Ince et al., 2010). This approach allows unbiased estimates of the mutual information to be obtained even when the ratio of the number of trials to the number of possible responses is equal to or greater than 8 (Ince et al., 2010). In our dataset this condition was respected for 22 units (9 of which were single units) out of the 99 total for all information analyses using all spikes (40 ms window, 8 ms binsize). For the last analysis, in which we estimated temporal information with only one spike per trial, the number of possible responses is greatly reduced and unbiased estimates could be obtained from a total of 39 units, considering a binsize of 2 ms and a 20 ms time window.

Statistical Analyses—Throughout the text values are reported as mean plus or minus the 95% confidence interval. Statistical analyses were performed either as paired t-test or repeated measures ANOVA (as described in the corresponding sections above). For all ANOVAs we report only those interactions between the main factors that were statistically significant (for a complete list of factors and their p-values please refer to table S3 in the

supplementary materials). Post-hoc analyses on significant interactions were performed adjusting the p-value using the Bonferroni correction method. Mean differences between levels of all of the single factors and those interaction factors that were statistically significant are reported in Table S4 of the supplementary materials.

Results were considered statistically significant for $p<0.05$. In addition to the p-value and the value of the F-statistic, for repeated measures ANOVA the partial eta squared (η_p^2) was reported when comparing the effect of the behavior on the different coding mechanisms analyzed (spike count, spike timing and temporal information). This value represents the ratio of total variance attributable to a single factor of the repeated measure ANOVA model, i.e. a measure of the effect of one factor on the variance observed in the data. Using this measure it was possible to compare the effect of different factors from different analyses.

RESULTS

Behavioral modulation of the responses elicited by the whisker-pad stimulator

Neuronal activity was recorded from five rats allowed to move freely on a plexiglass platform while three different electrical stimulations of increasing stimulus strength (i.e. duration of the stimulus, see Methods) were randomly delivered to the mystacial pad via a whisker-pad stimulator (Moxon et al., 2007; Scaglione et al., 2011). Ninety-nine units were discriminated and the neuronal responses to the stimuli were separated into two groups based on whether the stimulus had been presented when the animal was sitting quietly or whisking. To quantify the impact of the behavior on the neuronal responses, their magnitude and latency were quantified and compared across behaviors (quiet or whisking) and stimulus strengths.

The behavior of the animal modulated the background activity of the units. First, as might be expected from studies of activity in the trigeminal ganglion during different behavioral states (Leiser and Moxon, 2007), there was a significant increase in background firing rate (Figure 1E) of about 35% during whisking (31±27 Hz) compared to quiet (23±17 Hz; paired t-test, mean difference 7.6 ± 2.6 Hz, $t(98)=5.84$, $p<0.001$), when considering the whole dataset of 99 units. For this reason the background activity was subtracted when considering the magnitude and peak of the responses to the different stimuli.

When considering the responsiveness of the units, both the number of responding units and the response magnitude were modulated by the animal's behavior (Figure 1G–H). Significantly more units responded to the whisker-pad stimulator during quiet (11.47 ± 3.05 units/animal) than during whisking (4.73 ± 3.08 units/animal; ANOVA Factor Behavior $F(1,4)=134.22$, $p<0.001$). Moreover, the magnitude of the response of those units was significantly greater during quiet (0.60 ± 0.13 spikes/stimulus) compared to whisking (0.42 ± 0.11 spikes/stimulus; ANOVA Factor Behavior $F(1,98)=34.35$, $p<0.001$). Post-hoc analysis on the statistically significant interaction (ANOVA Factor Behavior*Stimulus $F(2,196)=15.26$, $p<0.001$) revealed that this difference was significant when comparing Quiet vs Whisking for all but the smallest stimulus strength (60 μs, $p=0.608$; 120 $p<0.001$ and 240 μs $p<0.001$). This extends earlier work on the impact of behavioral state on the magnitude of the response (Fanselow and Nicolelis, 1999) to show that during active

behavioral states (i.e. whisking), the recorded units are less responsive to unexpected stimuli, similarly to what has been shown for the forepaw somatosensory system (Chapin and Woodward, 1981, 1982a-b).

As expected, response magnitude and number of responding units were modulated by the strength of the stimulus. For example, the unit in Figure 1A–D had a significant response to the stronger stimuli (120 and 240 μs) but not to the weakest (60 μs). Because our whisker-pad stimulator activates a smaller and more consistent region of the whisker pad compared to a nerve cuff electrode (Fanselow and Nicolelis, 1999), it was possible to observe the effect of increasing stimulus strength on the spread of the response in the barrel cortex (Figure 1F). As the strength of the stimulus increased, a greater number of significantly responsive units were recorded per electrode site (ANOVA Factor Stimulus $F(2,8)=9.63$, $p=0.007$; Figure 1F–G). The same trend was observed when considering the response magnitude: as the strength of the stimulus increased, the response magnitude increased during both quiet and whisking (ANOVA Factor Stimulus $F(2,196)=77.65$, $p<0.001$; Figure 1H).

When considering the temporal features of the responses (Figure 1I–K), whisking compared to quiet seemed not to affect the peak latency (ANOVA Factor Behavior $F(1,98)=0.04$, $p=0.842$; quiet 13.7 ± 1.1 ms, whisking 13.8 ± 1.3 ms) while it slightly but significantly increased both the latency (ANOVA Factor Behavior $F(1,98)=25.85$, $p<0.001$; quiet 6.06 ± 0.09 ms, whisking 6.16 ± 0.11 ms) and the jitter of the 1st spike (ANOVA Factor Behavior $F(1,98)=26.30$, $p<0.001$; quiet 1.05 ± 0.05 ms, whisking 1.13 ± 0.04 ms). Post-hoc analysis on the statistically significant interaction (1st spike latency: ANOVA Factor Behavior*Stimulus $F(2,196)=4.21$, $p=0.016$; jitter of the 1st spike: ANOVA Factor Behavior*Stimulus $F(2,196)=8.53$ $p<0.001$) revealed that this difference was significant when comparing Quiet vs Whisking only for the strongest stimulus strength for the latency of the 1st spike (60 μs, $p=0.098$; 120 μs, $p=0.079$; and 240 μs, $p<0.001$) and all but the smallest stimulus strength for the jitter of the 1st spike (60 μs, $p=0.533$; 120 μs, $p=0.002$ and 240 μs, $p<0.001$). Moreover, as the strength of the stimulus increased, the peak latency (ANOVA Factor Stimulus $F(2,196)=26.16$, $p<0.001$; Figure 1H), the latency of the 1st spike (ANOVA Factor Stimulus $F(2,196)=128.55$, $p<0.001$; Figure 1I) and the jitter of the first spike (ANOVA Factor Stimulus $F(2,196)=108.31$, $p<0.001$; Figure 1J) significantly decreased for both behavioral conditions.

Therefore, the behavioral state of the animal modulates the activity of the recorded units in response to peripheral stimuli affecting the magnitude and, to lesser extent, the latency of their response. These results suggest that the information about the peripheral stimuli might be processed differently depending on the behavioral state of the animal.

Behavioral modulation of the information about stimulus detection

Since the behavior of the animal affects the activity of the units in response to the electrical stimulation of the whisker pad, we first tested if the changes observed in the neuronal responses imply changes in the coding mechanisms of the units when discrimination between background and any of the stimuli, i.e. *stimulus detection*. To this end, Shannon mutual information between the neuronal responses and the stimuli was estimated

considering two different coding mechanisms: spike count (one single 40 ms bin) and spike timing (five 8 ms bins). The analysis was performed on a subset of 22 units that respected the conditions required to ensure proper bias correction for the estimate of the information (see *bias correction of the mutual information*). To ensure that the subset of units represented a good sample of the entire set of recorded units, we verified that the mean response of the subset showed the same modulation to the behavior and the stimulus strength (data not shown) as the entire set of 99 units.

In accordance with the results obtained above, the behavior of the animal also modulated the information carried by the neuronal responses. In fact, significantly more information could be extracted during quiet epochs compared to whisking epochs (ANOVA Factor Behavior $F(1,21)=24.91$, $p<0.001$; Figure 2A). Moreover this increase was observed for both spike count information and spike timing information (Figure 2A). When considering the ability to discriminate separately each of the three different stimuli from background, as the intensity of the stimulus increases more information could be extracted (ANOVA Factor Stimulus $F(1,21)=14.20$, $p<0.001$; Figure 2B–C). Post-hoc analysis on the significant interaction (ANOVA Factor Behavior*Stimulus $F(2,42)=5.56$, $p=0.007$) revealed that this difference was significant when comparing, during Quiet or Whisking behavior, 240 vs 60 μs and 240 vs 120 μs (Quiet: $p<0.001$ and $p<0.001$ Whisking: $p=0.010$ and $p=0.021$) but not 120 vs 60 μs (Quiet: $p=0.05$; Whisking: $p=0.98$).

When considering the different encoding mechanisms across behavioral states, spike timing carried more information than spike count (ANOVA factor Encoding $F(1,21)=26.50$, $p<0.001$ Figure 2B). Post-hoc analysis on the significant interaction (ANOVA Factor Encoding*Stimulus $F(2,42)=7.05$, $p=0.002$) revealed that this difference was significant when comparing Timing vs Count for all stimuli (60 μs, $p=0.016$; 120 μs, $p=0.049$ and 240 μs $p<0.001$). Note that the significant interaction is due to the fact that more information could be extracted when comparing Timing vs Count as the intensity of the stimulus increases (see Table S3) which correlates well with the changes induced by the stimulus on both the jitter and the latency of the 1st spike. Importantly, the additional information carried by spike timing over spike count was similar across the two behaviors (0.049 ± 0.020 bits during quiet; 0.045 ± 0.031 bits during whisking, paired t-test $t(21)=0.24$, $p=0.613$). Therefore, for stimulus detection, the precise time of arrival of the spikes carried additional information over the total number of spikes emitted in the same post-stimulus window regardless of the animal's behavior.

Similarly to what we obtained with the neurophysiological properties of the responses, the behavior of the animal modulated the information about stimulus detection by increasing the information extracted during quiet compared to whisking, suggesting that during the quiet state an unexpected transient stimulus is more perceptible than during whisking in accordance with the model proposed by Nicolelis and colleagues (Nicolelis and Fanselow, 2002).

Behavioral modulation of the information about stimulus discrimination

Since the behavior of the animal modulated the amount of information about stimulus detection, we investigated the effect of the behavior on the information extracted when

discriminating between the three different stimulus strengths, i.e. *stimulus discrimination* (Figure 3A–B). As done previously, Shannon mutual information between the neuronal responses and the stimuli for the subset of 22 units was estimated considering two different coding mechanisms: spike count and spike timing.

As for the case of stimulus detection, there was significantly more information about stimulus discrimination during quiet than whisking (ANOVA factor Behavior $F(1,21)=6.19$, $p=0.021$) and for both behaviors spike timing carried additional information compared to spike count (Figure 3C) (ANOVA factor encoding $F(1,21)=77.76$, $p<0.001$). Therefore, in addition to the possibility that an unexpected stimulus is more perceptible during quiet than during whisking, features of the stimulus may be more discriminable. Furthermore, when considering the two behaviors separately, the additional information carried by spike timing over spike count was not statistically different across the two behaviors (0.073 ± 0.028 bits during quiet; 0.057 ± 0.023 bits during whisking, paired t-test $t(21)=0.853$, $p=0.403$). Therefore, the result that the additional information conveyed by spike timing over spike count is independent of the animal's behavior is extended to stimulus discrimination.

These results suggest that, in the rat barrel cortex of awake, freely moving animals, the behavior of the animal modulates the information about stimulus discrimination. Moreover, there is an important role for the timing of neuronal spikes in response to the different types of stimuli regardless of the animal's behavioral state.

Nature of the spike timing code

Spike timing carried additional information compared to spike count regardless of the behavioral condition, but the additional information conveyed by spike timing over spike count represents only a lower bound of the temporal information in the code. We therefore further investigated the nature of the spike timing code, and specifically the contribution of temporal information, in the stimulus discrimination paradigm. First we tested the idea that temporal information becomes particularly critical when the response magnitudes of the stimuli are similar. To this end, the fraction of temporal information in the code was correlated with a response magnitude difference index that provides a measure of how much different are the response magnitudes across the three stimulus strengths (see methods for more details). The fraction of temporal information negatively correlated with the response magnitude difference for both quiet (Figure 4A, $r=-0.645$, $p<0.001$) and whisking (Figure 4B, $r=-0.577$, $p<0.001$; Figure 4A–B).

Second, the information contained in the first spike of the responses was estimated for both quiet and whisking. Most of the information was carried by the first spike for both quiet (Figure 4C) and whisking (Figure 4D), 84.8% and 82.7% of the total information respectively, suggesting that the latency of the 1st spike in response to the three different stimuli carries the vast majority of the information. In these 22 units (9 of which were single units), first spikes represented 65% and 70% of spikes when considering the neuronal responses observed under quiet or whisking behavior respectively, which means that the remaining 35% and 30% of spikes provided little information that was not already conveyed by first spikes.

Third, to determine if temporal information alone is greater than spike count information and depends on the animal's behavior, the temporal information was directly estimated on 39 units for stimulus discrimination (twice the number of units used above and with a smaller binsize of 2 ms). Importantly, the fact that spike timing extracted more information than spike count does not necessarily imply that the temporal information is greater than spike count information because spike timing theoretically contains both temporal information and spike count information (Nelken et al., 2005; Foffani et al., 2009; see Methods). To be able to directly estimate the amount of temporal information in our dataset, the previous spike timing analysis was repeated using only the 1st spikes of the responses and selecting only responsive trials. In this way the amount of information contained in the spike count is zero, as the number of spikes is the same for each stimulus, and all the information is due to temporal components of the responses. This temporal information was compared to the spike count information for the same responsive trials (Figure 4E) and the two information estimates were compared between the two behavioral states. Results show that the temporal information was greater than the spike count information (ANOVA factor encoding $F(1,38)=40.48$, $p<0.001$) while the amount of information extracted was similar across behavioral states (ANOVA factor behavior $F(1,38)=1.15$, $p=0.29$). Note that the spike count information looses its behavioral dependence in this analysis because we had to consider only responsive trials, suggesting that the absence of responses was critical for the behavioral modulation of information. We conservatively verified that temporal information did not change between quiet and whisking (paired t-test $t(28)=1.255$, $p=0.217$). The influence of the animal's behavior on temporal information, as measured by the effect size of the factor behavior, was almost negligible ($\eta_p^2=0.029$, weak effect) compared to the effect measured for spike count and spike timing information considering stimulus detection ($\eta_p^2=0.556$, strong effect) or stimulus discrimination ($\eta_p^2=0.269$, moderate effect).

Therefore, when disambiguating the contribution of the temporal information from the spike timing, the behavioral state of the animal does not have any effect on the temporal component of the information. In other words, temporal information is robust with respect to changes of the animal's behavior.

DISCUSSION

We examined the role of spike timing on the encoding of somatosensory information across different behavioral states in awake, freely moving animals. For both spike timing and spike count, the information conveyed about stimulus detection and stimulus discrimination was modulated by the animal's behavior. Moreover, regardless of the animal's behavior, spike timing carried additional information over spike count alone in awake, freely moving animals. Temporal information, i.e. the information conveyed by the timing of individual spikes, was particularly critical when the magnitudes of the responses to the different stimuli were similar. In addition, the great majority information carried by the units about stimulus discrimination was readily available in the timing of the first spike. Finally, temporal information alone was greater than spike count information and the behavioral state of the animal did not influence the amount of temporal information extracted. Therefore, while there are important differences in the way information is processed across behavioral states,

temporal information, which is greater than spike count information, is more robust across behaviors.

Behavioral modulation of the neural activity

Our results support earlier work demonstrating that the behavior of the animal modulates the magnitude of the neuronal response to a stimulus and the receptive field size of neurons, and extend our understanding to the effect on the latency of the response. It is known that the behavioral state of the animal modulates the responsiveness of neurons (Chapin and Woodward, 1981, 1982a-b) including those in the rat whisker system (Fanselow and Nicolelis, 1999; Moxon et al., 2007) such that the magnitude of the response to an unexpected stimulus is greater during a behaviorally quiet state compared to an active, exploratory state.

Furthermore, several studies have shown that the size of the receptive fields is modulated by the state of the animal. For example, the receptive field size increases with progressively lighter states of anesthesia (Nicolelis et al., 1993; Nicolelis and Chapin, 1994; Friedberg et al., 1999; Erchova et al., 2002; Aguilar and Castro-Alamancos, 2005) and is even larger in awake animals during quiet immobility (Ferezou et al., 2006; Krupa et al., 2004). This would allow for better integration of the information about peripheral stimuli. Although in our study we cannot directly estimate the receptive field size of each unit, since our stimulus was delivered to one location on the whisker pad, our result about the spread of the response across behavioral states supports the idea that the receptive field size of an unexpected stimulus would be reduced during whisking compared to quiet. These results are similar to those obtained in the supragranular layer of awake, freely moving mice by Ferezou et al. (2006), who suggested that during whisking the decrease of the spread of the response might be generated by the greater variability of the whisker position before the stimulus. Indeed this result is similar to what was previously observed in the forepaw somatosensory cortex - the phase of the step cycle during treadmill locomotion can affect the response of units to an electrical stimulation of the paw (Chapin and Woodward, 1981, 1982a-b). In this context, our results would suggest that during quiet all of the information about an unexpected stimulus is allowed to pass to the cortex while during whisking some of the information is gated-out or attenuated and this gating may depend on the phase of the whisk.

Finally, our results show that the latency of the responses is also weakly modulated by the behavior. Although the behavior did not modulate the latency of the peak of the response, it slightly increased both the latency and the jitter of the first spike during whisking compared to quiet. These results provide insights into the effect of the behavior on the information represented by the neurons.

Behavioral modulation of the information extracted from the neuronal responses

In addition to the modulation of the neuronal activity, we estimated the information that can be extracted from the responses to an unexpected stimulus. Our results showed that information extracted considering both the total number of spikes emitted in a given time window (spike count information) and the timing of individual spikes (spike timing information) are modulated by the behavior of the animal and is higher during quiet

compared to whisking. Moreover, this result holds whether one is considering stimulus detection or stimulus discrimination.

Taken together, our results show that during whisking compared to quiet fewer cells respond to the stimulus and the information about the stimulus decreases for both stimulus detection and stimulus discrimination. Although the result about stimulus discrimination seems to be in contradiction with the observation that rats use their whiskers in discrimination tasks (Welker, 1964; Carvell and Simons, 1990), our results could be due to the relative saliency of the stimulus itself such that an unexpected stimulus is more salient during quiet than during whisking. Another possibility is that, during whisking, information about unexpected stimuli is gated by the animal in order to separate information coming from active discrimination (i.e. whisking). This regulation could be mediated by the action of the basal forebrain, which has been previously shown to respond to motivational stimuli (Wilson and Rolls, 1990; Richardson and DeLong, 1991; Whalen et al., 1994; Lin and Nicolelis, 2008), and its activation has been shown to improve the encoding properties of neurons in primary sensory cortex (Goard and Dan, 2009). Therefore, it is possible that in the presence of stimuli relevant to the active whisking, more information about stimulus discrimination would be conveyed during whisking behavior compared to quiet.

Spike timing and spike count code—The aim of this study was to investigate the role of spike timing on the information carried by neurons in the rat somatosensory system in awake, freely moving animals. Previous studies, conducted in anesthetized preparations, showed that spike timing carries more information than spike count in both the barrel cortex (Ghazanfar et al., 2000; Panzeri et al., 2001; Petersen et al., 2002) and in the somatosensory representation of the forepaw (Foffani et al., 2004; Foffani et al., 2008) when considering stimulus location. These results have been extended to both the ventro-postero medial thalamus (VPM) (Ghazanfar et al., 2000; Montemurro et al., 2007) and the ventro-postero-lateral thalamus (VPL) (Foffani et al., 2009) considering stimulus location or stimulus dynamics. Here we extend these results to the somatosensory cortex of awake, freely moving animals, drawing a parallel with other studies that obtained similar results in the auditory system (Huetz et al., 2009; Mickey and Middlebrooks, 2003). Therefore, spike timing could serve as a general coding substrate for sensory information in behaving animals.

In addition we further extended this result by examining differences across the behavioral state of the animal. The fact that in our study we found that spike timing carried additional information over spike count regardless of the behavior of the animal and the discrimination paradigm (*stimulus detection* vs *stimulus discrimination*) suggests that spike timing is a general feature of how neurons encode information that is independent of the state of the animal (anesthetized, quiet, whisking). If spike timing is such a general feature of encoding then it is likely to play a pivotal role in how neurons encode information about features of more natural peripheral stimuli (Bialek et al., 1991; Hopfield, 1995; Victor and Purpura, 1996; Panzeri et al., 2001; Chase and Young, 2006).

Nature of the spike timing code: information in the first spike and temporal Information—When considering spike timing, under all conditions studied to date,

including awake, freely moving animals studied here, the very first spike emitted in response to a stimulus conveys most of the information about that stimulus (Furukawa et al., 2000; Reich et al., 2001; Van Rullen and Thorpe, 2001; Panzeri et al., 2001). Moreover, spike timing is particularly more informative than spike count when the response magnitude is similar across different features of the stimulus (Arabzadeh et al., 2006; Foffani et al., 2008; Montemurro et al., 2007). This phenomenon is related to the contribution of the temporal information or information conveyed solely by the latency of the first spike (Foffani et al., 2009). Here we show that this role for temporal information holds true for awake, freely moving animals as well. In this way, information about the stimulus could be rapidly processed using only the first spike (Thorpe et al., 2001; VanRullen et al., 2005) across different awake behavioral states.

Despite the fact that both spike timing and spike count information were greater during quiet than during whisking, there was no significant difference in the temporal information across behaviors. In addition, the influence of the animal's behavior on temporal information was almost negligible compared to the effect on spike count information or spike timing information. Therefore, temporal information is weakly affected by the behavior of the animal considering a 2ms bin size. Of course, the fact that there was a small difference in the mean jitter of the first spike across behaviors (~1ms) leaves open the possibility that there are differences in temporal information at smaller bin sizes. However, our results show that the behavior of the animal produced a consistent positive increase in the latency of the first spike for each of the stimulus strengths tested preserving the relative differences in latencies between stimulus strengths. Therefore, spike timing likely represents a robust coding mechanism (Panzeri and Diamond, 2010) employed by the brain to consistently encode the same information across different behavioral states.

In conclusion information about peripheral stimuli is modulated by the behavior of the animal and this modulation is mainly expressed in the spike count while the temporal information is more robust to changes in the behavioral state of the animal.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by grant R01 NS05741 from the National Institutes of Health, grant #89500 from Shriners Hospital for Children and grant #P113 from Internationale Stiftung fur Forschung in Paraplegie.

References

Adrian ED. The impulses produced by sensory nerve endings: Part I. J Physiol. 1926; 61:49–72. [PubMed: 16993776]

Arabzadeh E, Panzeri S, Diamond ME. Whisker vibration information carried by rat barrel cortex neurons. J Neurosci. 2004; 24:6011–6020. [PubMed: 15229248]

Arabzadeh E, Panzeri S, Diamond ME. Deciphering the spike train of a sensory neuron: counts and temporal patterns in the rat whisker pathway. J Neurosci. 2006; 26:9216–9226. [PubMed: 16957078]

Bale MR, Petersen RS. Transformation in the neural code for whisker deflection direction along the lemniscal pathway. J Neurophysiol. 2009; 102:2771–2780. [PubMed: 19741100]

Bialek W, Rieke F, de Ruyter van Steveninck RR, Warland D. Reading a neural code. Science. 1991; 252:1854–1857. [PubMed: 2063199]

Blanc JL, Coq JO. Coding processes involved in the cortical representation of complex tactile stimuli. J Physiol Paris. 2007; 101:22–31. [PubMed: 18042355]

Borst A, Theunissen FE. Information theory and neural coding. Nat Neurosci. 1999; 2:947–957. [PubMed: 10526332]

Butts DA, Weng C, Jin J, Yeh CI, Lesica NA, Alonso JM, Stanley GB. Temporal precision in the neural code and the timescales of natural vision. Nature. 2007; 449:92–95. [PubMed: 17805296]

Carvell GE, Simons DJ. Biometric analyses of vibrissal tactile discrimination in the rat. J Neurosci. 1990; 10:2638–2648. [PubMed: 2388081]

Chapin JK, Lin CS. Mapping the body representation in the SI cortex of anesthetized and awake rats. J Comp Neurol. 1984; 229(2):199–213. [PubMed: 6438190]

Chapin JK, Woodward DJ. Modulation of sensory responsiveness of single somatosensory cortical cells during movement and arousal behaviors. Exp Neurol. 1981; 72:164–178. [PubMed: 7202621]

Chapin JK, Woodward DJ. Somatic sensory transmission to the cortex during movement: gating of single cell responses to touch. Exp Neurol. 1982a; 78:654–669. [PubMed: 6293865]

Chapin JK, Woodward DJ. Somatic sensory transmission to the cortex during movement: phasic modulation over the locomotor step cycle. Exp Neurol. 1982b; 78:670–684. [PubMed: 6293866]

Chase SM, Young ED. Spike-timing codes enhance the representation of multiple simultaneous sound-localization cues in the inferior colliculus. J Neurosci. 2006; 26:3889–3898. [PubMed: 16611804]

de Ruyter van Steveninck RR, Lewen GD, Strong SP, Koberle R, Bialek W. Reproducibility and variability in neural spike trains. Science. 1997; 275:1805–1808. [PubMed: 9065407]

deCharms RC, Merzenich MM. Primary cortical representation of sounds by the coordination of action-potential timing. Nature. 1996; 381:610–613. [PubMed: 8637597]

Erchova IA, Lebedev MA, Diamond ME. Somatosensory cortical neuronal population activity across states of anaesthesia. Eur J Neurosci. 2002; 15:744–752. [PubMed: 11886439]

Fanselow EE, Nicolelis MA. Behavioral modulation of tactile responses in the rat somatosensory system. J Neurosci. 1999; 19:7603–7616. [PubMed: 10460266]

Ferezou I, Bolea S, Petersen CC. Visualizing the cortical representation of whisker touch: voltage-sensitive dye imaging in freely moving mice. Neuron. 2006; 50:617–629. [PubMed: 16701211]

Foffani G, Chapin JK, Moxon KA. Computational role of large receptive fields in the primary somatosensory cortex. J Neurophysiol. 2008; 100:268–280. [PubMed: 18400959]

Foffani G, Morales-Botello ML, Aguilar J. Spike timing, spike count, and temporal information for the discrimination of tactile stimuli in the rat ventrobasal complex. J Neurosci. 2009; 29:5964–5973. [PubMed: 19420262]

Foffani G, Moxon KA. PSTH-based classification of sensory stimuli using ensembles of single neurons. J Neurosci Methods. 2004; 135:107–120. [PubMed: 15020095]

Foffani G, Tutunculer B, Moxon KA. Role of spike timing in the forelimb somatosensory cortex of the rat. J Neurosci. 2004; 24:7266–7271. [PubMed: 15317852]

Furukawa S, Xu L, Middlebrooks JC. Coding of sound-source location by ensembles of cortical neurons. J Neurosci. 2000; 20:1216–1228. [PubMed: 10648726]

Ghazanfar AA, Stambaugh CR, Nicolelis MA. Encoding of tactile stimulus location by somatosensory thalamocortical ensembles. J Neurosci. 2000; 20:3761–3775. [PubMed: 10804217]

Goard M, Dan Y. Basal forebrain activation enhances cortical coding of natural scenes. Nature Neuroscience. 2009; 12:1444–1449.

Golomb D, Hertz J, Panzeri S, Treves A, Richmond B. How well can we estimate the information carried in neuronal responses from limited samples? Neural Comput. 1997; 9:649–665. [PubMed: 9097477]

Hopfield JJ. Pattern recognition computation using action potential timing for stimulus representation. Nature. 1995; 376:33–36. [PubMed: 7596429]

Huetz C, Philibert B, Edeline JM. A spike-timing code for discriminating conspecific vocalizations in the thalamocortical system of anesthetized and awake guinea pigs. J Neurosci. 2009; 29:334–350. [PubMed: 19144834]

Ince RA, Senatore R, Arabzadeh E, Montani F, Diamond ME, Panzeri S. Information-theoretic methods for studying population codes. Neural Netw. 2010; 23:713–727. [PubMed: 20542408]

Kraskov A, Stogbauer H, Grassberger P. Estimating mutual information. Phys Rev E. 2004; 69:066138.

Krupa DJ, Wiest MC, Shuler MG, Laubach M, Nicolelis MA. Layer-specific somatosensory cortical activation during active tactile discrimination. Science. 2004; 304:1989–1992. [PubMed: 15218154]

Leiser SC, Moxon KA. Responses of Trigeminal Ganglion Neurons during Natural Whisking Behaviors in the Awake Rat. Neuron. 2007; 53:117–133. [PubMed: 17196535]

Lin SC, Nicolelis MA. Neuronal ensemble bursting in the basal forebrain encodes salience irrespective of valence. Neuron. 2008; 59:138–149. [PubMed: 18614035]

MacKay DM, McCulloch WS. The limiting information capacity of a neuronal link. B Math Biol. 1952; V14:127–135.

Mickey BJ, Middlebrooks JC. Representation of auditory space by cortical neurons in awake cats. J Neurosci. 2003; 23:8649–8663. [PubMed: 14507964]

Montemurro MA, Panzeri S, Maravall M, Alenda A, Bale MR, Brambilla M, Petersen RS. Role of precise spike timing in coding of dynamic vibrissa stimuli in somatosensory thalamus. J Neurophysiol. 2007; 98:1871–1882. [PubMed: 17671103]

Moxon KA, Devilbiss DM, Chapin JK, Waterhouse BD. 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. 2007; 1147:105–123. [PubMed: 17368434]

Nelken I, Chechik G, Mrsic-Flogel TD, King AJ, Schnupp JW. Encoding stimulus information by spike numbers and mean response time in primary auditory cortex. J Comput Neurosci. 2005; 19:199–221. [PubMed: 16133819]

Nicolelis MA, Fanselow EE. Thalamocortical optimization of tactile processing according to behavioral state. Nat Neurosci. 2002; 5:517–523. [PubMed: 12037519]

Optican LM, Richmond BJ. Temporal encoding of two-dimensional patterns by single units in primate inferior temporal cortex. III. Information theoretic analysis. J Neurophysiol. 1987; 57:162–178. [PubMed: 3559670]

Panzeri S, Diamond ME. Information Carried by Population Spike Times in the Whisker Sensory Cortex can be Decoded Without Knowledge of Stimulus Time. Front Synaptic Neurosci. 2010; 2:17. [PubMed: 21423503]

Panzeri S, Petersen RS, Schultz SR, Lebedev M, Diamond ME. The role of spike timing in the coding of stimulus location in rat somatosensory cortex. Neuron. 2001; 29:769–777. [PubMed: 11301035]

Panzeri S, Senatore R, Montemurro MA, Petersen RS. Correcting for the sampling bias problem in spike train information measures. J Neurophysiol. 2007; 98:1064–1072. [PubMed: 17615128]

Panzeri S, Schultz SR. A unified approach to the study of temporal, correlational, and rate coding. Neural Comput. 2001; 13:1311–1349. [PubMed: 11387048]

Panzeri S, Treves A. Analytical estimates of limited sampling biases in different information measures. Network-Comp Neural. 1996; 7:87–107.

Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. 2. Sydney; Orlando: Academic Press; 1986. p. xxvip. 237

Petersen RS, Panzeri S, Diamond ME. The role of individual spikes and spike patterns in population coding of stimulus location in rat somatosensory cortex. Biosystems. 2002; 67:187–193. [PubMed: 12459298]

Reich DS, Mechler F, Victor JD. Temporal coding of contrast in primary visual cortex: when, what, and why. J Neurophysiol. 2001; 85:1039–1050. [PubMed: 11247974]

Richardson RT, DeLong MR. Electrophysiological studies of the functions of the nucleus basalis in primates. Advances in experimental medicine and biology. 1991; 295:233–252. [PubMed: 1776570]

Scaglione A, Moxon KA, Aguilar J, Foffani G. Trial-to-trial variability in the responses of neurons carries information about stimulus location in the rat whisker thalamus. Proc Natl Acad Sci U S A. 2011; 108:14956–14961. [PubMed: 21873241]

Shannon CE. A mathematical theory of communication. Bell Syst Tech J. 1948; 27:379–423.

Theunissen F, Miller JP. Temporal encoding in nervous systems: a rigorous definition. J Comp Neur. 1995; 2(2):149–62.

Thorpe S, Delorme A, Van Rullen R. Spike-based strategies for rapid processing. Neural Networks. 2001; 14:715–725. [PubMed: 11665765]

Treves A, Panzeri S. The Upward Bias in Measures of Information Derived from Limited Data Samples. Neural Comput. 1995; 7:399–407.

Tutunculer B, Foffani G, Himes BT, Moxon KA. Structure of the excitatory receptive fields of infragranular forelimb neurons in the rat primary somatosensory cortex responding to touch. Cereb Cortex. 2006; 16:791–810. [PubMed: 16120794]

Van Rullen R, Thorpe SJ. Rate coding versus temporal order coding: what the retinal ganglion cells tell the visual cortex. Neural Comput. 2001; 13:1255–1283. [PubMed: 11387046]

VanRullen R, Guyonneau R, Thorpe SJ. Spike times make sense. Trends Neurosci. 2005; 28:1–4. [PubMed: 15626490]

Victor JD. Binless strategies for estimation of information from neural data. Phys Rev E. 2002; 66

Victor JD, Purpura KP. Nature and precision of temporal coding in visual cortex: a metric-space analysis. J Neurophysiol. 1996; 76:1310–1326. [PubMed: 8871238]

Welker WI. Analysis of Sniffing of the Albino Rat. Behaviour. 1964; 22:223–244.

Whalen PJ, Kapp BS, Pascoe JP. Neuronal activity within the nucleus basalis and conditioned neocortical electroencephalographic activation. J Neurosci. 1994; 14:1623–1633. [PubMed: 8126559]

Wilson FA, Rolls ET. Learning and memory is reflected in the responses of reinforcement-related neurons in the primate basal forebrain. J Neurosci. 1990; 10:1254–1267. [PubMed: 2329375]

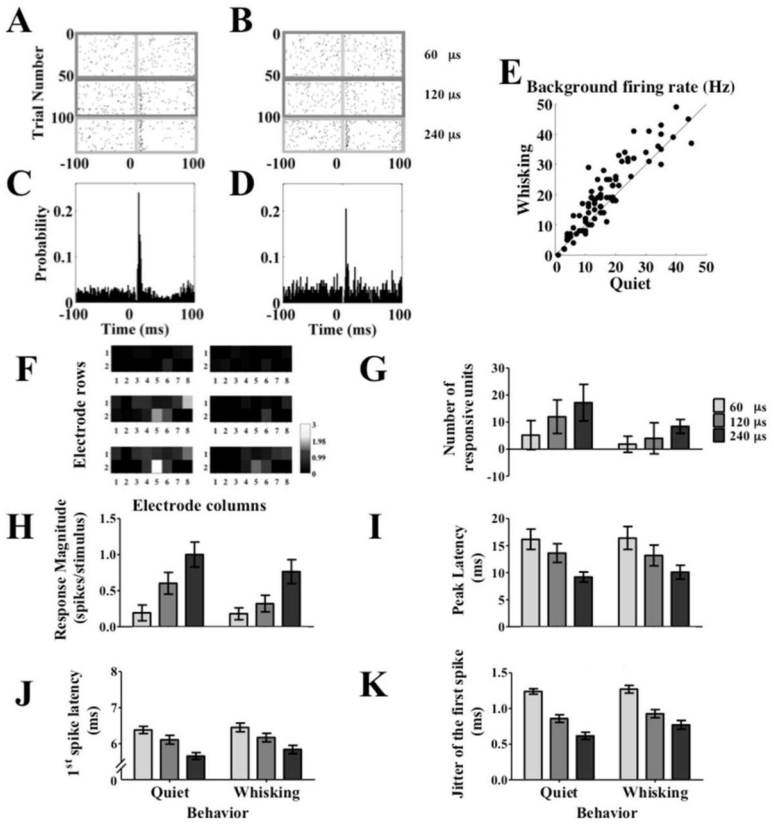

Figure 1. Behavioral modulation of neurophysiological parameters

A–D. Raster plots and peri-stimulus time histograms (PSTHs) of an example unit in response to the electrical stimulation of the whisker-pad for quiet (A,C) or whisking behavior (B,D). For the raster plots, boxes highlight trials with the same stimulus strengths (60, 120 and 240 µs from the top to the bottom). The PSTHs, instead, are obtained from combining all trials across the different stimulus strengths. **E.** Background firing rate is greater during whisking than during quiet. **F**. Spread of the response from a rectangular multi-electrode array for the three different stimulus strengths (rows, 60, 120 and 240 µs from the top to the bottom) across the two different behavioral states (columns, left quiet and right whisking). Each square represents the position of an electrode in the array; the color represents the average response magnitude (spikes per stimulus, see colormap) of each unit recorded at a particular location. **G–K.** Average of the number of responsive units (G), response magnitude (H), peak latency (I), 1st spike latency (J) and jitter of the 1st spike (K) for each stimulus strength in the two behavioral states. Each one of the three colored bars represents one stimulus strength (see legend figure 1G) grouped on the x-axis across behaviors (upper index). For all graphs error bars denote 95% confidence intervals around the mean.

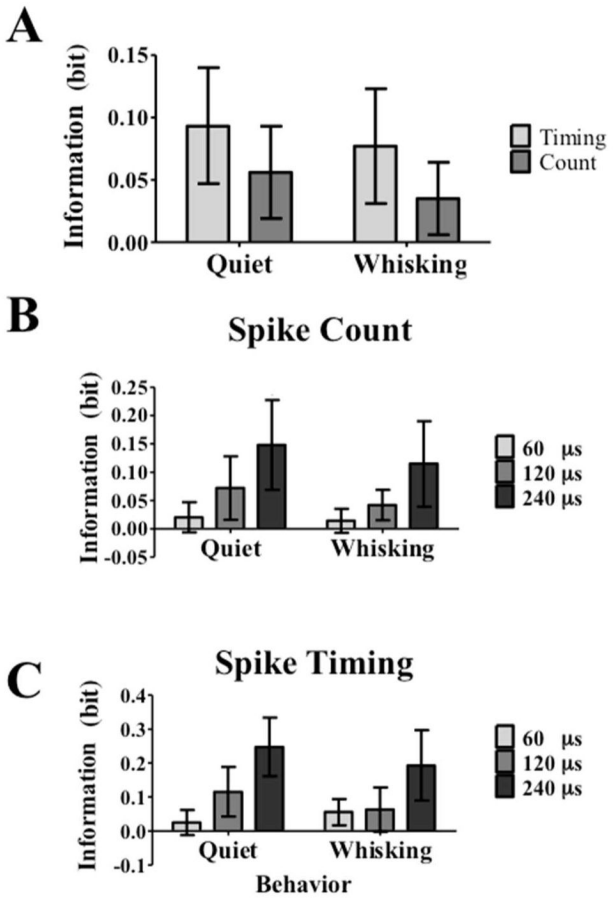

Figure 2. Behavioral modulation of information about stimulus detection

A. Averages of the *stimulus detection* information for spike count (light gray bar) and spike timing (dark gray bar) information considering all stimulus strengths for the two behavioral conditions. **B–C**. Averages of the *stimulus detection* information for each of the stimulus strengths (60 µs, light gray bar; 120 µs, dark gray bar; 240 µs, black bar) considering the spike count (B) and spike timing (C). For each graph, bars are grouped upon the behavioral state (x-axis upper index) and the y-axis represents averages across units (n=22) of the extracted information (bits). Error bars denote 95% confidence intervals around the average.

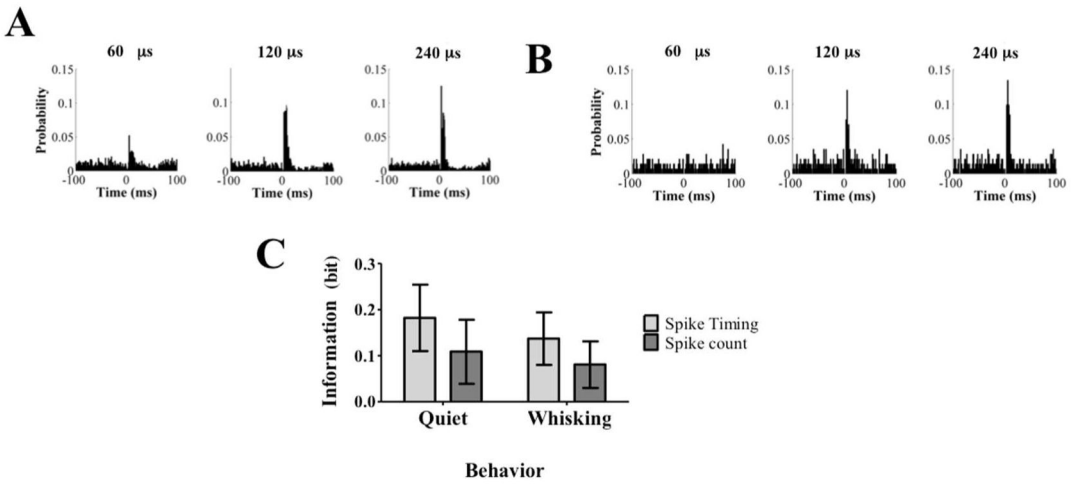

Figure 3. Behavioral modulation of information about stimlus discrimination

A–B. Peri-stimulus time histograms (PSTHs) of a unit in response to the three different stimulus intensities during quiet (A) or during whisking (B). For each PSTH the x-axis represent time (sec) referenced to the stimulus onset (time t=0) and y-axis represents the unit activity in probability. **C**. Bar plot representing the effect of the behavior on the average *stimulus discrimination* information for spike count (light gray bars) and spike timing (dark gray bars). Bars are grouped upon the behavioral state (x-axis upper index) and the y-axis represents averages across units (n=22) of the extracted information (bits). Error bars denote 95% confidence intervals around the average.

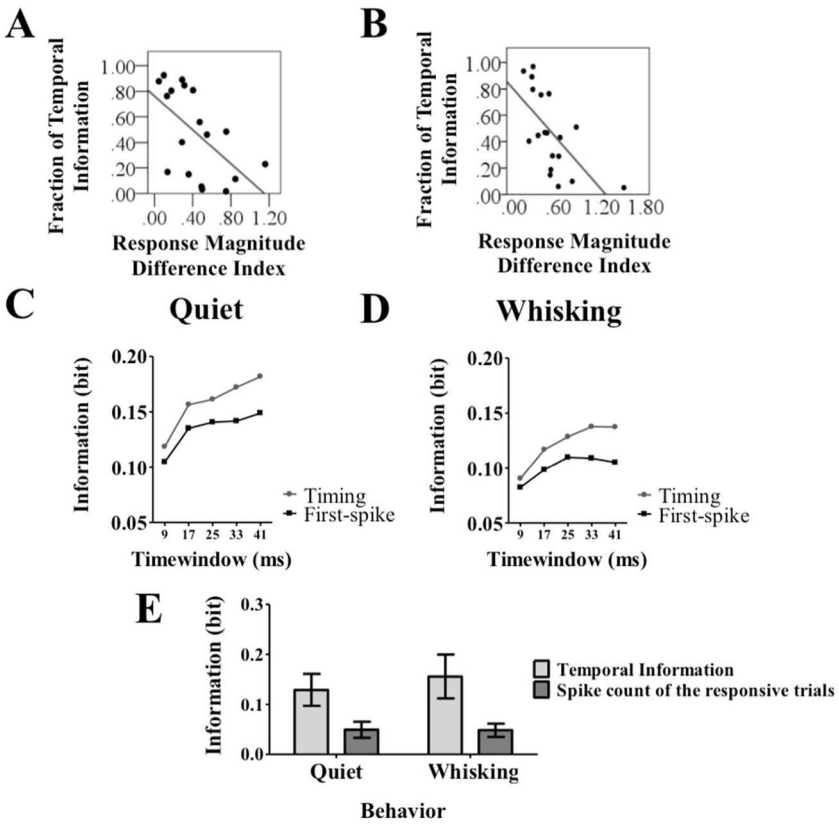

Figure 4. Nature of the spike timing

A–B. Scatter plots of the *Fraction of Temporal Information* in the code (y-axis) versus the difference in response magnitude between stimuli as measured by the *Response Magnitude Difference Index* (x-axis) for quiet (A) and whisking (B). **C–D**. Average information in bits (y-axis) extracted when considering the spike timing information (black line) or the information carried by the first spike (gray line) under quiet (A) and whisking (B) behavioral conditions for different time windows (x-axis). **E**. Averages of the temporal information (light gray bars) and the spike count information of the responsive trials (dark gray bars) across the two different behaviors. Bars are grouped upon the behavioral state (x-axis upper index) and the y-axis represents averages across cells (n=39) of the extracted information (bits). Error bars denote 95% confidence intervals around the average.