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NEUROSYSTEMS

Neuron synchronization in the rat gracilis nucleus facilitates sensory transmission in the somatosensory pathway

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Abstract

We have studied the role of the temporal correlation of multiple cell discharges in the facilitation of the somatosensory information transmission from the gracilis nucleus to the primary somatosensory (SI) cortex in anesthetized rats. Pairs of gracilis neurons or gracilis–SI cortical neurons were recorded during application of 20-ms tactile stimuli in control conditions and after electrical corticofugal stimulation. Cross-correlation of neural spike trains showed significant changes in synchronization of the neuron firing provoked by the corticofugal stimulation. To quantify the time–frequency alterations in the functional association within neuron pairs we used the wavelet coherence measure. We show that electrical stimulation of the SI cortex induces a short-lasting facilitation of tactile responses of projecting gracilis neurons if their receptive fields (RFs) overlap with the RF of the stimulated cortical area (matching condition). Moreover, synchronization of discharges of gracilis neurons with a common RF is increased by activation of the corticofugal projection. Synchronization is favored by a stimulus induced synchronous oscillatory activity of projecting neurons in the range 3–10 Hz. In the matching condition synchronous discharges in the gracilis increment the number of spikes elicited in the SI cortex. Thus the efficacy of the sensory transmission from the gracilis nucleus to the SI cortex is modulated by the corticofugal projection through two complementary mechanisms: (i) by changing the responsiveness (number of elicited spikes) of individual gracilis neurons; and (ii) by a dynamic consolidation of gracilis neurons with a common RF into microcircuits generating synchronous spikes.

Introduction

Sensory processing in the central nervous system involves synchronous activity of neurons with a common receptive field (RF). Experimental data suggest that neuron synchronization is caused by synaptic interaction between related cells and/or cells with a common sensory input (Eckhorn *et al.*, 1988; Nicolelis *et al.*, 1995; Eblen-Zajjur & Sandkuhler, 1997). Spatially distant neurons with overlapping RFs can exhibit a highly synchronous firing, whereas neurons with different RFs do not show similar temporal relationships (Gray *et al.*, 1989). Thus neuron aggregates assembled by common afferent inputs determine the topographic maps representing RFs at multiple levels of the sensory pathways. These maps provide a structural framework that constrains spatial distribution of the sensory inputs and can be modified by higher-level processes such as attention or learning (see for review King, 1997; Buonomano & Merzenich, 1998).

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Discovery of neuron sensory aggregates raised the question: what role does the neuron synchronization play in the sensory information coding? Indeed, there is evidence that synchronization among neurons may be crucial for perception, that oscillatory patters may be relevant for the functional interaction and generation of neuron ensembles, and that synchronous activity favors the existence of rhythmic couplings that engage functionally related neurons in oscillatory networks. Oscillatory activity could contribute to spike synchronization among spatially distributed neurons, which would enhance spatial summation of the postsynaptic potentials provoked by these neurons on their target cells (Singer, 1993; Singer & Gray, 1995).

On the other hand, experimental data show that the corticofugal system modifies ongoing subcortical sensory processing and reorganizes RFs in the visual, auditory and somatosensory systems (Sillito *et al.*, 1994; Yan & Suga, 1996; Malmierca & Nuñez, 1998, 2004; Canedo & Aguilar, 2000; Jen *et al.*, 2002; Zhang *et al.*, 2005, 2008). Cortical neurons exert: (i) highly focused facilitating feedback to subcortical neurons, whose RFs overlap with those of the cortical neurons (matching condition); and (ii) a widespread inhibition of neurons with different RFs (non-matching condition).

Although cellular mechanisms of the corticofugal modulation have been well studied in the gracilis neurons, little is known about the information processing at the neuron ensemble level. Sensory inputs from the hindlimbs go to gracilis neurons that also receive corticofugal fibers through the pyramidal tract (Weisberg & Rustioni, 1976; Martinez-Lorenzana *et al.*, 2001). The corticofugal projection modulates the processing of somatosensory information (see for review Nuñez & Malmierca, 2007).

Recently, we reported on the coherence in response firing patterns elicited in gracilis neurons by tactile stimulation and its modulation by the corticofugal feedback from the primary somatosensory (SI) cortex (Castellanos *et al.*, 2007). This analysis revealed that the functional coupling between the sensory stimulus and the neural response is enhanced by electrical stimulation of the SI cortex in the matching condition. Consequently, we hypothesized that synchronization of the spiking activity among gracilis neurons with the same RF may also be increased by the SI cortex stimulation. In this study we report this functional synchronization among adjacent gracilis neurons and with neurons in the SI cortex in control conditions and after electrical corticofugal stimulation.

Materials and methods

Data were obtained from 62 urethane-anaesthetized (1.6 g/Kg i.p.) young adult Wistar rats (Iffa-Credo, France) of either sex, weighing 200–250 g. This level of anesthesia induced a stable slow activity in the EEG for 2–3 h (Nuñez *et al.*, 2000) and it is the standard dosage for surgical procedures (Trurmon *et al.*, 1996). It is characterized by the absence of withdrawal from a pinch applied to the forelimb and the presence of corneal and eyelid reflexes. At these conditions, we recorded stable sensory responses. Animals were placed in a stereotaxic frame, lidocaine 2% was applied over body surfaces in contact with the frame and over the incisions, and the body temperature was kept constant at 37°C with a feedback-controlled heating pad. Supplemental doses of urethane (0.5 g/Kg i.p.) were given to maintain areflexia. Experiments were carried out in accordance with the European Communities Council Directive (86/609/EEC) and approved by the Ethic Board of the Universidad Autonoma de Madrid.

Extracellular recording

The cisterna magna was opened to introduce the recording microelectrodes at a 60° angle over the surface of the gracilis nucleus. Single-unit recordings were performed in the gracilis nucleus (A, −13.6 to −14.6 mm; L, 0.2–1.0 mm from bregma; H, 0.0–0.5 mm from the surface of the nucleus, according to the atlas of Paxinos & Watson (1998) by means of tungsten microelectrodes (1–2 MΩ; World Precision Instruments, WPI, Sarasota, FL, USA). The position of the recording electrode was visually controlled under a dissecting microscope. Also, stereotrodes (a pair of tungsten microelectrodes with 125 μm tip separation and 2 MΩ resistance; World Precision Instruments) were used to obtain simultaneous recordings in the gracilis nucleus. Electrical signals were hardware-filtered (0.3–3 KHz) and amplified by DAM-80 preamplifiers (World Precision Instruments). Extracellular potential was digitized at 10 KHz with an interface unit (CED 1401; Cambridge Electronic Design, Cambridge, UK) and stored on a personal computer using SPIKE 2 software (CED) for further offline analysis. Spike identification and sorting were performed offline using the SPIKE 2 software (CED). We restricted the spike sorting procedure to those recordings where a stable multiunit activity exceeded twice the background noise amplitude. The noise amplitude was defined as σ = median ($|V(t)|/0.6745$), where $V(t)$ is the bandpass-filtered extracellular potential.

Figure 1A illustrates a typical multiunit activity recording where spikes of several neurons are observable with the naked eye. Single-unit activity was discriminated by threshold spike detection and template matching controlled by clustering. We used SPIKE 2 software for the offline spike sorting. The algorithm first performs crude spike detection by capturing windows around events defined by the voltage crossing of a user-defined threshold ($> 2\sigma$). Then, spike sorting is performed with a combination of template matching and a principal component analysis-based cluster cutting. Figure 1B shows two isolated spike waveforms corresponding to two different neurons. If different cells fire simultaneously (with a delay of < 1 ms) the extracellularly recorded waveforms are distorted and can hardly be classified. Consequently, we discarded such events from further analysis. This may reduce the estimated synchronization between neurons in gracilis nuclei (see also Discussion); however, as such events were found infrequently they did not significantly affect the results.

To identify gracilis cells projecting to the thalamus, bipolar stimulating electrodes (120 μm diameter blunt-cut nichrome wire) were aimed at the medial lemniscus (A, 6.5; L, 0.5–1.5; H, 8–9 mm). Antidromic firing was evoked by means of brief rectangular pulses (0.1–0.3 ms, 50–500 μA). Spikes were identified as antidromic if they had a constant latency after the stimulus, were blocked by a spontaneous spike close to the stimulus and followed high (> 100 Hz) stimulation rates.

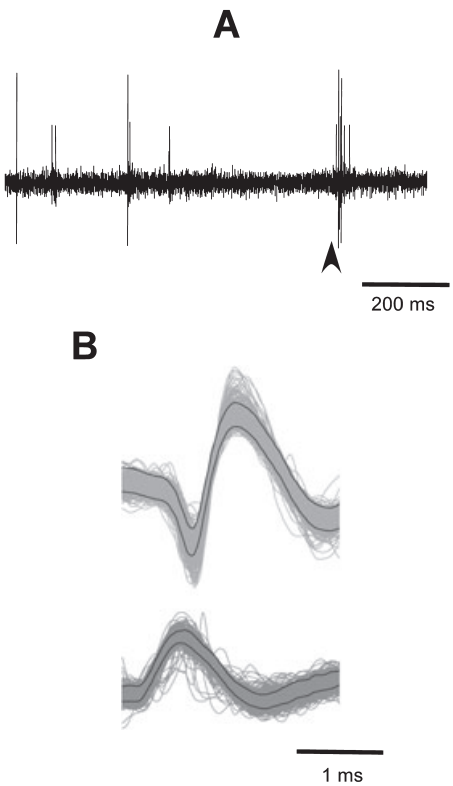

FIG. 1. Sorting of extracellularly recorded spikes. (A) An example of a 1-s epoch of high-pass-filtered (f_{cut} = 300 Hz) extracellular potential. Spikes have significantly different shape and amplitude. The arrowhead indicates a tactile stimulus application. (B) Superimposed waveforms of spikes belonging to two clusters as extracted by the spike sorter implemented in SPIKE 2 software.

Extracellular recordings were also obtained from the SI cortex. Single-unit recordings in the SI cortex (A, 1–3 mm from bregma and L, 2.5–4 mm from the midline) were made at 900–1500 μm below the surface, using tungsten microelectrodes (2–5 MΩ; World Precision Instruments). The same preprocessing as described above was applied to spikes recorded from the SI cortex.

Somatosensory and cortical stimulation

The gracilis neurons were carefully mapped by means of a loud speaker driven by the amplified neuron activity using a small hand-held brush to locate neurons responding to weak mechanical stimuli of the hindlimb. The RF was defined by the limits at which stimuli elicited changes in the unit activity. Tactile stimulation was performed by an electronically gated solenoid with a probe of 1 mm in diameter that produced < 0.5 mm skin deflections. Control tactile stimulation consisted in 0.5-Hz tactile pulses lasting 20 ms and delivered at the RF over 60 s. This stimulation frequency was selected to avoid interference between tactile stimuli.

Similar tactile stimulation was used to determine the RF of SI cortical neurons in order to place a stimulating electrode in the SI cortex and to stimulate a cortical area with an RF similar to (matching condition) or different from (non-matching condition) that of the gracilis neuron. After detecting the RF in the SI cortex, a bipolar stimulating electrode (120 μm diameter blunt-cut stainless steel wire) was aimed at the same site as the recording electrode (1.0 mm depth; cortical layer 5). Electrical stimulation of the selected cortical area was performed by a Grass S88 stimulator (Quincy, MA, USA) coupled to a photoelectric stimulus isolation unit. Trains of brief rectangular pulses (0.1–0.3 ms) at 50 Hz, lasting 500 ms, were applied. A current intensity twice as high as the threshold eliciting spike firing was selected (10–100 μA).

Data analysis

Unit recordings from pair of neurons were accepted for data analysis when the amplitude of spikes was at least twice as large as the amplitude of the background noise, and the fluctuation of the unit amplitude was < 10% throughout the recording.

Correlation analysis

Summed peristimulus time histograms (PSTHs) were calculated, using 1 ms bin width. Autocorrelation histograms (ACHs; 1 ms bin width) were generated to detect rhythmicity in the spiking activity of neurons. ACHs containing three or more peaks at equidistant time intervals were considered to be oscillating. Peaks and valleys in the correlograms were considered statistically significant when the heights of two or more contiguous bins exceeded 95% confidence limits. To quantify the cell rhythmicity an exponential fit of the ACH peaks was calculated:

$$P = P_0 \mathrm{e}^{-t/},$$

where P_0 and τ are the parameters describing the ACH amplitude at the central bin and the decay time constant of the oscillatory peaks, respectively. Higher values of τ indicate a slower decrease in the ACH peaks, i.e., their better reproducibility and, thus, a higher cell rhythmicity. Cross-correlation histograms (CCHs; 1 ms bin width) were also used to quantify relationships between pairs of two gracilis neurons recorded simultaneously, and between gracilis and SI cortical neurons. The degree of synchronization was estimated using the cross-correlation index (CCI):

$$\mathrm{CCI} = A/\alpha,$$

where A is the mean amplitude of the CCH taken in the range 25 ms before to 25 ms after the central peak and the normalization constant (α) is given by:

$$\alpha = (M_1 + M_2)/2M_1M_2,$$

and M_1 and M_2 are the total numbers of spikes fired by cells 1 and 2, respectively. All data are shown as mean value ± SEM. Statistical significance was determined using two-tailed t-tests.

Wavelet coherence

To quantify the time–frequency functional association between neural spike trains we used the wavelet coherence (Castellanos *et al.*, 2007).

Once spikes of a single cell had been identified we represented them as a series of δ-functions:

$$x(t) = \sum_i \delta(t - t_i)$$

where $\{t_i\}$ are the time instances of spike occurrences. Then, the continuous wavelet transform of a spike train $x(t)$ is given by:

$$W(p,z) = \frac{1}{\sqrt{p}} \int_{-\infty}^{+\infty} x(t) \Psi^* \left(\frac{t-z}{p} \right) dt \quad (1)$$

where Ψ is the 'mother wavelet' and parameters p and z define the wavelet time scale and temporal localization, respectively. In this study we used Morlet wavelets whose frequency content is given by $f \approx 1/p$. Then, the wavelet power spectrum can be estimated as:

$$E(p,z) = \frac{1}{\sqrt{\pi} r k_0} |W(p,z)|^2 \quad (2)$$

where r is the mean firing rate for the neuron.

A normalized measure of association between two spike trains is the wavelet coherence (Grinsted *et al.*, 2004):

$$c_{NM}(p,z) = \frac{\left| S\left(\frac{W_{NM}(p,z)}{p} \right) \right|^2}{S\left(\frac{E_N(p,z)}{p} \right) S\left(\frac{E_M(p,z)}{p} \right)} \quad (3)$$

where $W_{NM}(p,z) = W_N W_M^* / k_0 \sqrt{\pi r_N r_M}$ is the wavelet cross-spectrum of two simultaneously recorded spike trains and S is a smoothing operator (for details see Torrence & Webster, 1998; Grinsted *et al.*, 2004). Two linearly independent spike trains have statistically nonsignificant coherence, whereas $C(f^{-1}, z) = 1$ indicates a perfect linear relationship between the spike trains at the frequency f and localization z. We restricted the upper frequency range to 64 Hz and used 8 ms time bins to plot the coherence spectra. Further, we divided the total frequency range into six bands: stimulus (0.46–0.7 Hz), δ(0.7–4 Hz), θ(4–8 Hz), α(8–13 Hz), β (13–30 Hz) and γ (30–64 Hz).

Surrogate test of statistical significance

Although a large coherence amplitude usually indicates the presence of a consistent phase relationship (functional coupling) between two spike trains in a given time interval, it is also possible that it may be due to a random variation in the spike trains. Thus the statistical significance of the observed coherence should be cross-checked. To evaluate the significance level for the wavelet coherence we used the surrogate data test with the Monte Carlo simulation to establish a 95% confidence interval (for more details see Castellanos *et al.*, 2007).

Efficacy of the sensory transmission

To measure the efficacy of the sensory transmission between the gracilis nucleus and the SI cortex we evaluated the CCI between gracilis and SI cortical neurons. We hypothesized that if the corticofugal stimulation in the matching condition increased only the number of spikes evoked by a tactile stimulus in the gracilis cell, the proportion of spikes fired in the cortical cell to spikes in the gracilis cell should not change. Hence CCI must be the same. However, if the corticofugal stimulation also increased firing synchronization of gracilis neurons with overlapping RFs, the proportion of spikes fired in the cortical cell to spikes in the gracilis cell should increase. In this case CCI must increase. Similar results were expected from gracilis and cortical simultaneous recordings.

Results

We studied the effect of the SI cortical stimulation on synchronous activity of gracilis neurons in two experimental conditions: (i) with the RF of the electrically stimulated cortical area overlapping with the RF of the gracilis cells (matching condition); and (ii) with the RFs not overlapping (non-matching condition).

Cell classification

We analyzed the firing activity of 150 gracilis neurons, which were silent or showed a low discharge rate in spontaneous conditions (on average 2.1 ± 0.33 spikes/s; range 0–10 spikes/s). Neurons with such firing patterns are likely to be projecting to the thalamus according to previous results (Panetsos *et al.*, 1997). Indeed, selected neurons were identified as projecting cells by antidromic activation using electrical stimulation of the medial lemniscus (17 out of 34 tested cells). Neurons with discharge rates > 10 spikes/s in spontaneous conditions formed a heterogeneous population of nonprojecting neurons to the thalamus and they were thus not considered in this study.

Correlation analysis of gracilis neurons

In order to study the neuronal synchronization, 65 pairs of gracilis neurons were recorded by a single microelectrode or by a stereotrode. As results were similar for neuron pairs recorded by a single microelectrode (50 neuron pairs) or by the two tips of a stereotrode (15 neuron pairs), we pooled the data. Tactile stimuli applied to the neuron RFs provoked a mean response of 2.1 ± 0.3 spikes per stimulus (ranging from 0.5 to 3 spikes per stimulus; Fig. 2A, control). Pairs of neurons (n = 42) with overlapping RFs showed CCHs with a peak at bin zero in all cases, indicating that the two neurons tended to fire synchronously during tactile stimulation (Fig. 2A, control). During spontaneous firing these neuron pairs showed a flat CCH (data not shown), which indicates that the synchronization of responses was due to the tactile stimulus. As the peak of the CCH is centered on the zero bin, the observed synchronization is likely to be due to a common synaptic input.

In agreement with previous results (Malmierca & Nuñez, 1998, 2004; Canedo & Aguilar, 2000), cortical stimulation induced a short-lasting facilitation of tactile responses in most gracilis neurons (69%) with overlapping RFs [matching condition; n = 29 neuron pairs; Fig. 2A, after electrical stimulation of the SI cortex (AESC)]. The central peak in the CCH for two gracilis neurons increased after the SI cortical stimulation (50 Hz, 500 ms) in the matching condition, suggesting that the synaptic interaction between the gracilis neurons has been strengthened (Fig. 2A, AESC). In contrast, cortical stimulation in the non-matching condition decreased the CCH central

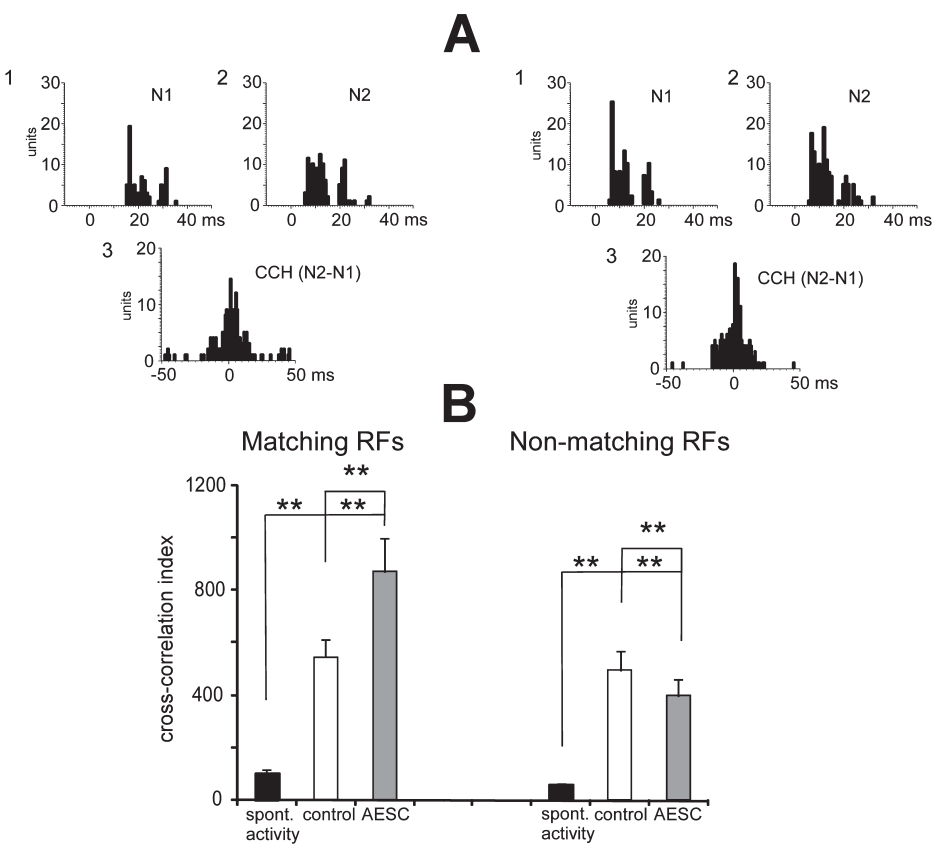

FIG. 2. Corticofugal stimulation increased neuron synchronization in the matching condition. (A1 and 2) PSTHs for two gracilis neurons recorded simultaneously in control and AESC. Both neurons respond to tactile stimuli delivered at the second toe. (A3) CCH between the two neurons. The central peak is due to synchronous firing. The sensory responses and the synchronous activity became more prominent. (B) Bars correspond to the mean CCI in pairs of gracilis neurons (n = 23 neuron pairs in the matching condition and n = 23 in the non-matching condition) calculated for 60 s in spontaneous conditions, during 60 s of tactile stimulation of the RF in control conditions, and during 60 s of tactile stimulation after AESC. $^{**}P < 0.01$.

peak in 16 out of 23 neuron pairs and did not modify it in the remaining seven neuron pairs (data not shown).

To quantify the level of synchronization among gracilis neurons, we evaluated the CCI in (i) spontaneous conditions, (ii) during 60 s of the tactile stimulation at the corresponding RF and (iii) the same as in (ii) but after the SI cortical stimulation (Fig. 2B). In the matching condition, the mean CCI increased from 102 ± 14.5 to 546 ± 62.8 during the control tactile stimulation of RFs ($P < 0.001$, $n = 42$ neuron pairs; Fig. 2B, spont. activity vs. control). SI cortical stimulation led to a further CCI increase during 2–5 min (872 ± 130; $P = 0.005$, $n = 23$ neuron pairs; Fig. 2B, AESC).

In the non-matching condition, the CCI of gracilis neurons also increased during tactile stimulation of their RFs in respect to the values at spontaneous conditions (from 59 ± 4.7 in control to 495 ± 73 during tactile stimulation; $n = 12$, $P < 0.001$). However, the CCI was reduced to 398 ± 63 after the SI cortical stimulation ($n = 23$ neuron pairs, $P = 0.007$; Fig. 2B, AESC).

Rhythmic activity of gracilis neurons

In spontaneous conditions all gracilis neurons showed nonrhythmic discharge patterns. During tactile stimulation 48% of the gracilis neurons (30 out of 62 cells) exhibited a rhythmic activity in the range 3–15 Hz (8.6 ± 1.9 Hz). No differences were observed between rhythmic and nonrhythmic cells, as has been indicated previously (Nuñez *et al.*, 2000). Figure 3A shows the ACHs of two representative neurons recorded during application of tactile stimuli at their RFs. The ACHs have periodic peaks, indicating the tendency to fire rhythmically at ∼10 Hz. CCH for this neuron pair also shows periodic peaks at 0.1-, 0.2- and 0.3-s time intervals on both sides, suggesting the presence of synaptic coupling between the neurons triggered by the tactile stimulus, and that the coupling was rhythmic. Corticofugal stimulation (matching condition) increased the response to the tactile stimulus of the gracilis neurons, as described above, and also the rhythmic pattern evoked during tactile stimulation became more pronounced (Fig. 3B, ACH). An exponential curve was fitted to the ACH peaks to quantify the cell rhythmicity in control and after corticofugal stimulation. At control the mean τ was 24.4 ± 3.4 ms and it was increased to 40.8 ± 5.9 ms after corticofugal stimulation in the matching condition ($n = 30$, $P < 0.001$). Thus the rhythmic ACH peaks decayed more slowly after the corticofugal stimulation than in the control condition, indicating an increase in the neuron rhythmicity provoked by the stimulation. Besides, the rhythmic activity frequency was not modified (8.5 ± 1.3 Hz). The CCH also has periodic peaks at the same time intervals as in the control conditions. In some cases ($n = 11$) neuron rhythmicity only appeared after SI stimulation with a latency of ∼30 s (data not shown). A facilitation effect was not observed when the SI stimulation was performed in the non-matching condition.

Coherence analysis of gracilis neuron firing

We evaluated wavelet coherence between firing responses and tactile stimulus events for 16 gracilis neurons. Then, we grouped the obtained coherence plots (three-dimensional functions of time and frequency)

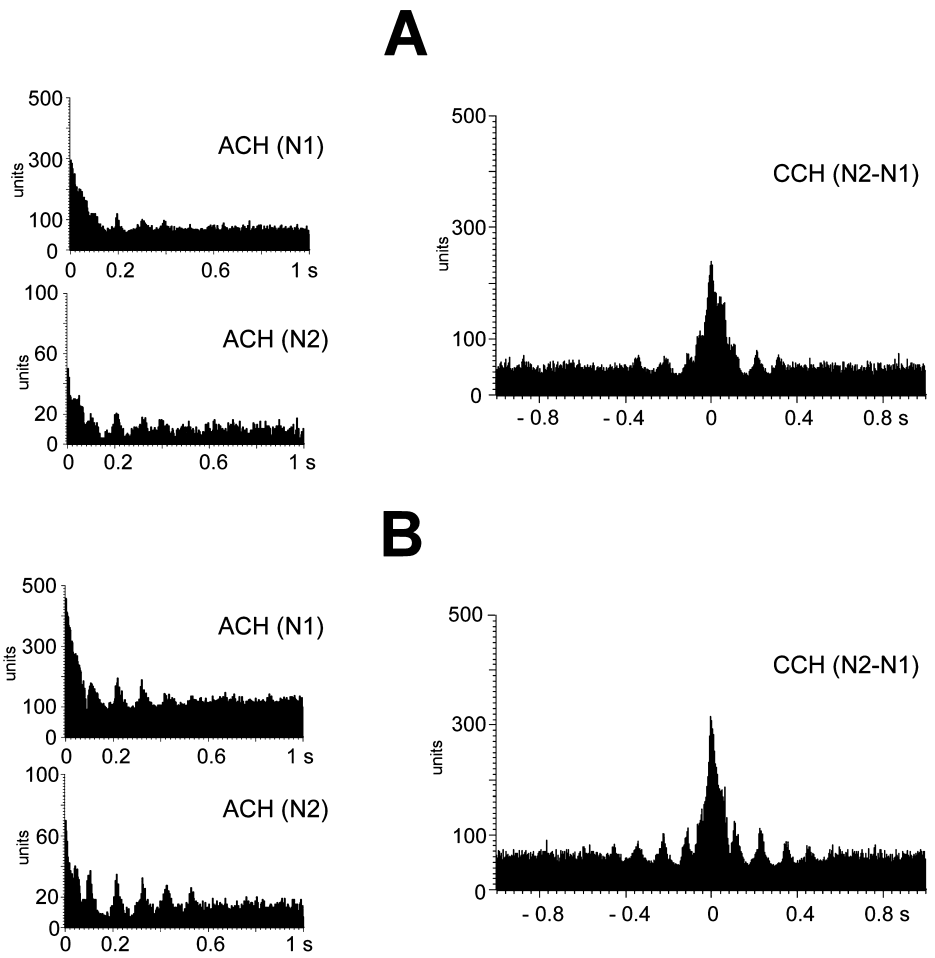

FIG. 3. Corticofugal stimulation facilitated common rhythmic activity in gracilis neurons. ACHs and CCHs of two representative neurons, (A) during tactile stimulation of their RFs and (B) during tactile stimulation of their RFs but after the SI cortical stimulation in the matching condition. Correlated rhythmic activity was increased by the corticofugal stimulation.

into three major groups according to the effects of the electrical SI cortex stimulation on the neurons' responses to tactile stimuli: 'I'-effect or increase in the response coherence, 'D'-effect or decrease, and 'No'-effect.

In agreement with our previous results (Castellanos *et al.*, 2007) the wavelet coherence confirmed the presence of a strong but time-varying functional coupling between the neural response and stimulus events in the stimulus frequency band. After the electrical stimulation of the SI cortex in the matching condition we obtained the I-effect in 72%, the D-effect in 16% and No-effect in 12% of the cases.

In addition to the stimulus coherence we also quantified the mean wavelet coherence among spike trains for 12 pairs of gracilis neurons. Figure 4 shows the mean firing coherence between gracilis neurons for the control tactile stimulation and after the electrical stimulation of the SI cortex in the matching condition. The coherence progressively decreased with frequency, which means that the gracilis neurons were mostly functionally coupled at relatively low frequencies. The corticofugal stimulation led to a statistically significant coherence increase in the stimulus, δ (0.7–4 Hz) and θ (4–8 Hz) frequency bands, whereas at higher frequencies differences did not reach statistical significance (Fig. 4A and B). It is noteworthy that the frequency bands in which we found a change in the coherence of firings correspond to the frequency range of the oscillation observed in gracilis neurons provoked by tactile stimulation (Fig. 3). The later indirectly confirms the hypothesis of the oscillatory integration of neurons into microcircuits under tactile stimulation of their RFs favored by SI corticofugal stimulation.

If the stimulus–response coherence of both neurons in a pair were facilitated by the SI cortical stimulation, we called such pair II, i.e., I-effect for both neurons (matching condition). If the cortical stimulation facilitated only one of the neurons and inhibited the other, we called such a pair ID (I effect for one neuron and D for the other). In this case for one neuron we have matching, but for the other non-matching, conditions. Finally, DD neuron pair means that the SI cortical stimulation did not facilitate the response of either neuron. The latter case corresponds to the non-matching condition. Among the neuron pairs we found: II, 67%; ID, 25%; and DD, 8%.

Figure 4A shows the mean firing coherence for the II neuron pairs. Such pairs exhibited an increase in coherence after the cortical stimulation in the stimulus, δ and θ frequency bands. For the ID neuron pairs the effect of the electrical cortex stimulation did not modify the coherence (Fig. 4D), and the same was true for the DD neuron pairs (data not shown).

The wavelet transform enables study of the dynamic properties of the firing coherence in the frequency and time domains simultaneously. Previously we have shown (Castellanos *et al.*, 2007) that the strength of the functional coupling between the stimulus and neuron response varies in time. Here we obtained similar result for the neuron pairs. Figure 4B and E show 2-D plots of coherences for II and ID neuron pairs. Interneuron coherence was higher and less intermittent for the II pairs. In both cases the electrical cortical stimulation increased the coherence, although time intervals with no significant coherence remained. Figure 4C and F show the mean (over the whole time interval) wavelet coherences between the neurons. SI cortical

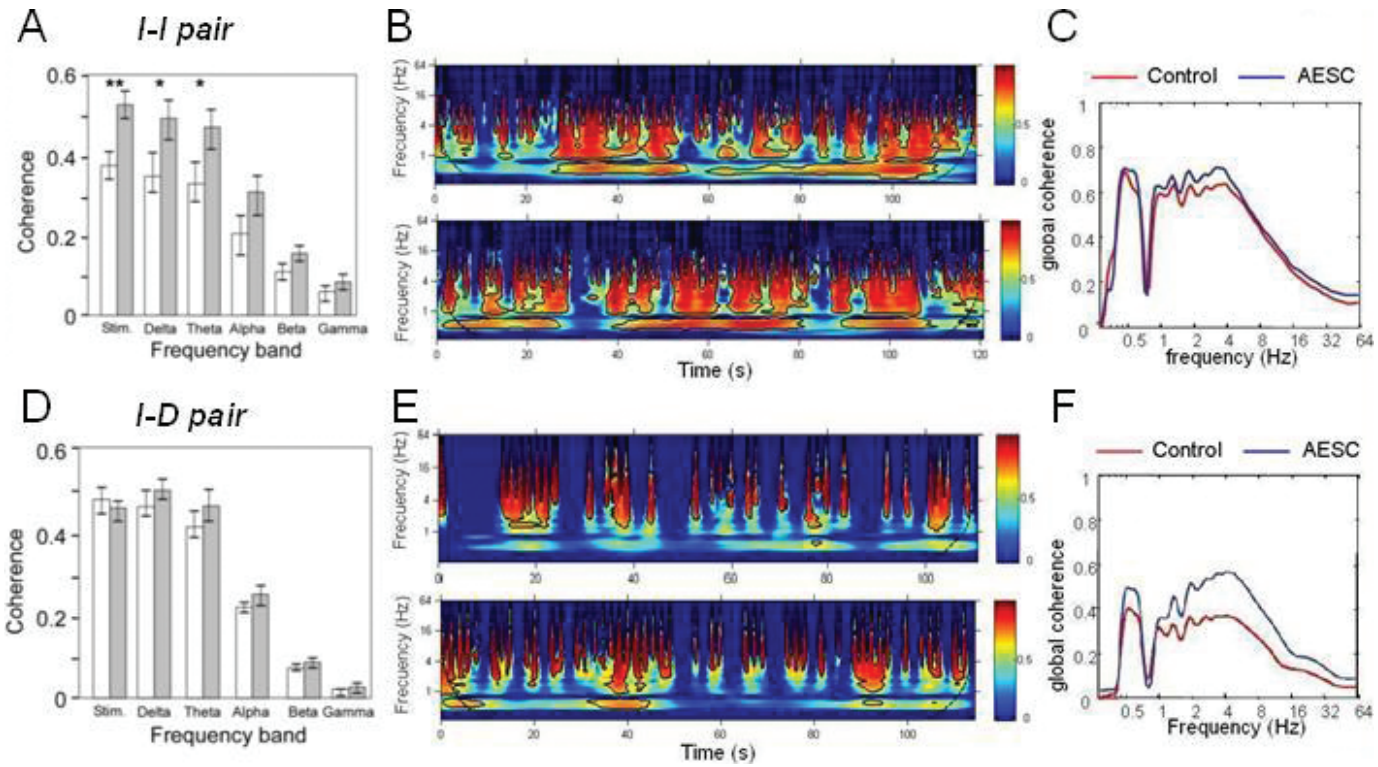

FIG. 4. Time-spectral analysis of the influence of electrical stimulation of the SI cortex on the coherence of firing of gracilis neuron pairs. (A and D) Mean wavelet coherence for II and ID neuron pairs (I and D denote increase and decrease, respectively, in the single-neuron stimulus–response coherence provoked by electrical stimulation of the SI cortex). II neuron pairs increased their interneuron coherence after the cortical stimulation (grey bars; white bars are control) in the stimulus, δ and θ frequency bands, whereas there were no statistically significant coherence changes for ID neuron pairs (*$P < 0.05$, **$P < 0.01$). (B and E) 2-D time–frequency plots of the interneuron coherence for ID and II pairs, respectively. Upper and lower subplots correspond to control and AESC conditions, respectively. Color intensity from blue to red codifies the strength of the functional coupling. (C and F) Coherence plots of the time-averaged interneuron coherences corresponding to those shown in B and E.

stimulation increased the interneuron coherence over a wide frequency range. The differentiation was stronger for the ID pairs, which suggests a complex effect of the corticofugal fibers on the neural gracilis circuitry.

Correlation between gracilis and SI cortical neurons

We performed simultaneous extracellular recordings in the gracilis nucleus and in the SI cortex. Nineteen pairs of gracilis–SI cortical neurons were selected for the analysis. All pairs exhibited a peak on the right side of CCH during tactile stimulation, indicating that the SI cortical neuron firing was preceded by a firing of the gracilis neuron, i.e., there was a causal effect (Fig. 5A, left). The mean delay between gracilis and cortical firing was 8.4 ± 0.2 ms.

In spontaneous conditions, most of the gracilis and cortical cells were not correlated, with mean CCI = 137 ± 16.8 (Fig. 5B, left, spont. activity). During tactile stimulation in the matching condition the CCI rose to 554 ± 58.3, indicating functional coupling between gracilis and SI cortical neurons. The CCI further increased to 738 ± 66.7 after 2 min of the corticofugal stimulation ($P < 0.001$, $n = 12$) and remained at that level for 5–10 min without significant fluctuations in its value (Fig. 5B, left). The better synchronization between the representative pair of gracilis and cortical neurons can be also perceived in the CCH (Fig. 5A, right). In the non-matching conditions during the control tactile stimulation the CCI was lower (368 ± 46.2; Fig. 5B, right, control). This may be due to the absence of a direct projection from the gracilis to SI cortical neurons with different RFs. As we expected, the corticofugal stimulation inhibited or did not alter the CCI (265 ± 32.4, $n = 7$; Fig. 5B, right, AESC).

In the matching condition the wavelet coherence between gracilis and SI cortical neurons also significantly increased over the value at spontaneous conditions during tactile stimulation (Fig. 5C). The

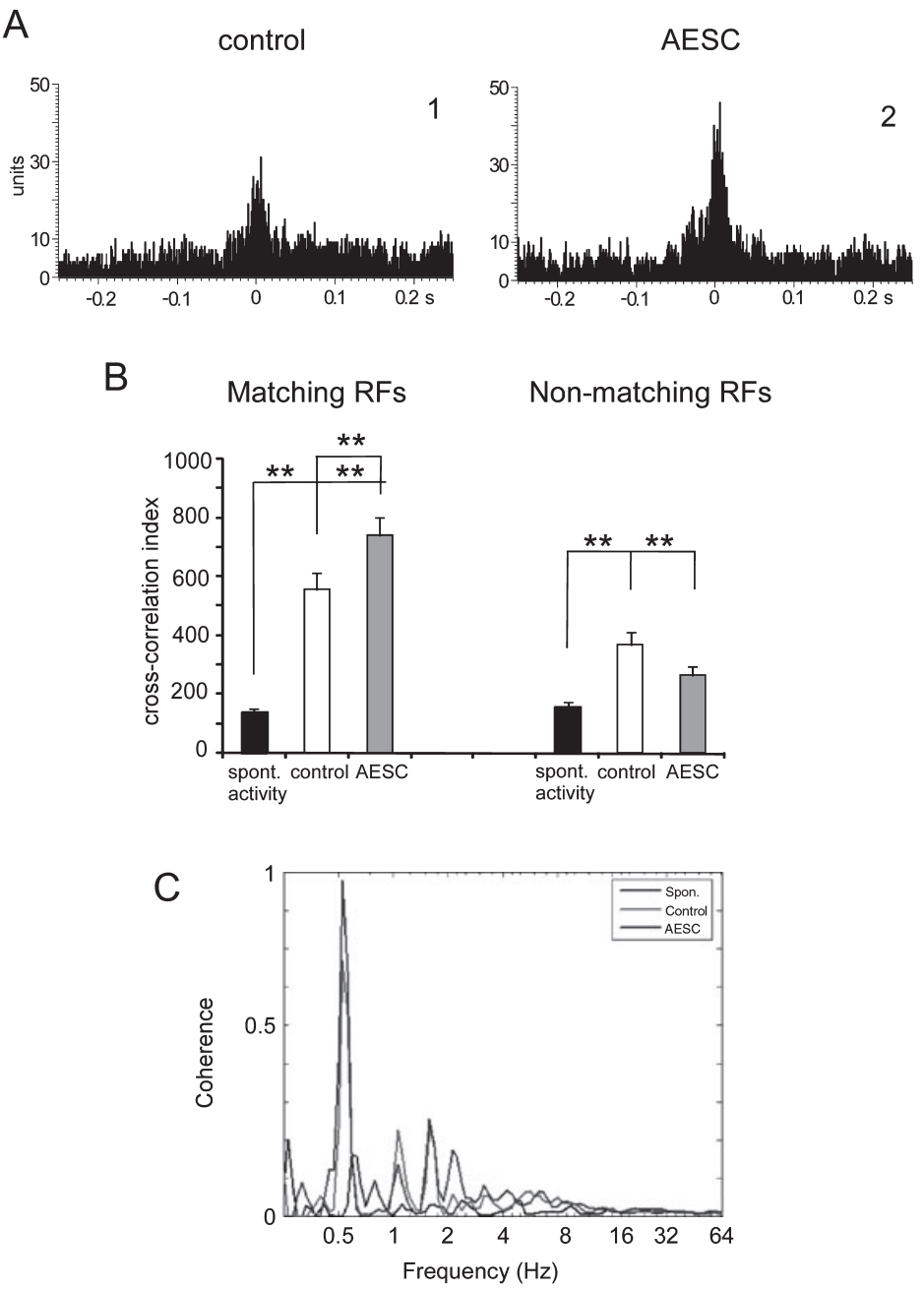

FIG. 5. Synchronous activity between gracilis and SI cortical neurons. (A) CCHs of a representative pair of a gracilis and SI cortical neurons in the matching condition. 1, control tactile stimulation; 2, the same as in (1) but after electrical stimulation of the adjacent SI cortical area (AESC). The peak corresponding to correlated firings was larger after the cortical stimulation, which indicates an increase in the synchronous responses of the neurons. (B) CCI for the gracilis and cortical neuron pair. Mean CCI increased in the matching condition and stayed unchanged (slightly decreased) in the non-matching condition ($n = 12$ neuron pairs in the matching condition and $n = 7$ in the non-matching condition; $^{**}P < 0.01$). (C) Coherence plot of the mean wavelet coherence for a gracilis–cortex neuron pair in spontaneous, control and AESC conditions.

coherence increased further after SI cortical stimulation. Again, as was observed above, the most prominent coherence changes were in the δ and θ frequency bands.

This supports the hypothesis that the firing synchronization of gracilis neurons, engaged into a microcircuit by the corticofugal projection, increases the efficacy of the sensory information transmission to the cortex.

Discussion

Current knowledge of sensory information processing is mainly based on single-cell recordings, which provide limited information on the circuitry and functionality of neuron networks involved in the sensory encoding and processing. In this study we used multielectrode recordings in gracilis nucleus and primary somatosensory cortex and employed multivariate data analysis. Our major finding is that corticofugal projections do not just facilitate or inhibit sensory responses of gracilis neurons, as has been widely discussed (Sillito *et al.*, 1994; Yan & Suga, 1996; Malmierca & Nuñez, 1998, 2004; Canedo & Aguilar, 2000; Jen *et al.*, 2002), but they also make the response of those neurons with overlapping RFs significantly more coherent. The latter means that the efficacy of the sensory transmission from the gracilis nucleus to the SI cortex is modulated by the cortex through two complementary mechanisms: (i) by changing the responsiveness (number of elicited spikes) of individual gracilis neurons, and (ii) by dynamic engagement of gracilis neurons with a common RF into microcircuits generating synchronous spike firing. We have also shown that at least a proportion of the gracilis neurons sharing common RFs can be integrated by sensory afferent inputs into oscillatory networks, whose consolidation is controlled by the corticofugal projections.

Methodological considerations

To determine the level of synchronization between discharges of different neurons, the CCI was calculated using a time range of 25 ms before to 25 ms after the central peak. We chose this range because it fits the typical period of response to tactile stimuli. A similar time range has been used to analyze somatosensory neuronal responses elsewhere (see e.g. Alloway *et al.*, 1994). The CCI was normalized to the number of spikes fired by both neurons to avoid the effect of an overall increase in the firing rate. We also note that synchronous firing (within a 1–2 ms time window) of two nearby neurons is hardly separable under extracellular recordings. We excluded such events and, consequently, the obtained CCI for gracilis neurons (Fig. 2B) could be underestimated, i.e., the CCI may be slightly higher in the control and after cortex stimulation conditions, especially in the case of matching RFs.

Recently, we have described a wavelet approach which can quantify functional stimulus–neural response coupling (Castellanos *et al.*, 2007). In the present study we further improved the mathematical tool and applied it for quantification of the firing coherence of pairs of gracilis neurons. This analysis allowed us to study the functional neural coupling and the influence of the electrical stimulation of the SI cortex in different frequency bands.

Corticofugal projections dynamically consolidate microcircuits of gracilis neurons

In conditions of spontaneous firing, projecting gracilis neurons produce erratic uncorrelated spikes. However, synchronization of the firing is observed in the vast majority of gracilis neurons recorded with the same electrode under tactile stimulation of their RFs. Our results support the hypothesis that these neurons are dynamically organized in functional assemblies. Synchronous activation of primary afferent fibers and synaptic interaction between gracilis neurons can be postulated as the source of this synchronization. Moreover, the major synchronizing factor is the afferent input from common RFs. However, synaptic inputs from nearby projecting neurons in the gracilis nucleus and the activity of inhibitory interneurons also contribute to the synchronization (Soto *et al.*, 2004).

Discharges of gracilis neurons with overlapping RFs become more synchronous after electrical stimulation of the SI cortex in the matching condition. The strength of functional connections, as measured by the CCI and the wavelet coherence, significantly increases in the low-frequency range (through delta to alpha). It is unlikely that corticofugal projections alter primary afferent inputs to gracilis neurons. Therefore, we conclude that the SI corticofugal projections in the matching condition may facilitate synaptic interactions between gracilis neurons with common RFs. Moreover, firing of the neurons in the non-matching condition becomes less synchronous. Consequently, corticofugal projections dynamically consolidate functionally related gracilis cells and 'break' connections between neurons with different RFs. A similar effect of the corticofugal projection has been observed in the thalamus (Sillito *et al.*, 1994; for review, see Nicolelis, 2005).

Oscillatory activity integrates gracilis neurons with common RFs

Earlier it has been suggested that oscillations in the somatosensory cortex may facilitate association between functionally related cells in the somatosensory cortex and also between somatosensory and motor cortex (Murthy & Fetz, 1992; Roy & Alloway, 1999; Brovelli *et al.*, 2004; Sun & Luhmann, 2007; Witham & Baker, 2007). Moreover, somatosensory cortex has strong beta band oscillations which are synchronized with those in motor cortex, allowing oscillatory sensory reafference to be interpreted in the context of the oscillatory motor command which produced it (Baker, 2007). This 'association hypothesis' can also be applied to the gracilis neurons. Indeed, our previous results show that ~48% of the projecting gracilis neurons exhibit an oscillatory activity during tactile stimulation of their RFs in the 3–10 Hz frequency range (Panetsos *et al.*, 1998; Nuñez *et al.*, 2000). Our present data indicate that corticofugal stimulation in the matching condition increases the coupling (coherence) between gracilis neurons in delta and theta frequency bands if the neurons have overlapping RFs (II pairs); and there is no effect for gracilis neurons with different RFs (ID or DD pairs).

Thus, gracilis neurons which are spatially distant but share common RFs may be integrated by sensory afferent inputs into an oscillatory neural network which is improved by the corticofugal projections.

Correlated activity of gracilis and cortical neurons supports the association hypothesis

The above-mentioned results postulate that corticofugal projections elicit selective synchronization of gracilis neurons. In agreement with this prediction, we have shown that the temporal synchronization between gracilis and SI cortical neurons increases after corticofugal stimulation in the matching condition. The CCI indicated that the number of cortical spikes per spike generated in the gracilis neuron by

a tactile stimulus increases after the electrical stimulation of the cortex. This increment can be explained by a better firing synchronization of gracilis neurons, which facilitates more effective transmission of spikes to the cortex.

Functional role of the corticofugal projection

We have shown that corticofugal stimulation in the matching condition improves the transfer of the peripheral sensory stimulus from the gracilis nucleus to the SI cortex and inhibits the others. Thus, the SI cortex may exert discrimination of relevant from irrelevant stimuli even in the first relay station of the somatosensory pathway (egocentric selection; Jen *et al.*, 2002; Nuñez & Malmierca, 2007). Consequently, the corticofugal system may mediate attention in the sensory information processing to the subcortical relay stations. It contributes to the fine focusing of sensory responses in the somatosensory system, enhancing the activity of functionally related neurons and filtering out the irrelevant sensory inputs from the periphery or outside the RF. Moreover, variation of the cortical activity during the waking–sleep cycle may condition the sensory processing in the subcortical relay stations, dynamically consolidating neuron networks involved in analysis of sensory inputs. Thus the gracilis nucleus should now be seen as a dynamic relay controlled by the SI cortex according to the behavioral state.

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Abbreviations

ACH, autocorrelation histogram; AESC, after electrical stimulation of the SI cortex; CCH, cross-correlation histogram; CCI, cross-correlation index; PSTH, peristimulus time histogram; RF, receptive field; SI, primary somatosensory (cortex); τ, decay time constant of the oscillatory peaks.

References

Alloway, K.D., Wallace, M.B. & Johnson, M.J. (1994) Cross-correlation analysis of cuneothalamic interactions in the rat somatosensory system: influence of receptive field topography and comparisons with thalamocortical interactions. *J. Neurophysiol.*, **72**, 1949–1972.

Baker, S.N. (2007) Oscillatory interactions between sensorimotor cortex and the periphery. *Curr. Opin. Neurobiol.*, **17**, 649–655.

Brovelli, A., Ding, M., Ledberg, A., Chen, Y., Nakamura, R. & Bressler, S.L. (2004) Beta oscillations in a large-scale sensorimotor cortical network: directional influences revealed by Granger causality. *Proc. Natl. Acad. Sci. USA*, **101**, 9849–9854.

Buonomano, D.V. & Merzenich, M.M. (1998) Cortical plasticity: from synapses to maps. *Annu. Rev. Neurosci.*, **21**, 149–186.

Canedo, A. & Aguilar, J. (2000) Spatial and cortical influences exerted on cuneothalamic and thalamocortical neurons of the cat. *Eur. J. Neurosci.*, **12**, 2515–2533.

Castellanos, N.P., Malmierca, E., Nuñez, A. & Makarov, V.A. (2007) Corticofugal modulation of the tactile response coherence of projecting neurons in the gracilis nucleus. *J. Neurophysiol.*, **98**, 2537–2549.

Eblen-Zajjur, A.A. & Sandkuhler, J. (1997) Synchronicity of nociceptive and nonnociceptive adjacent neurons in the spinal dorsal horn of the rat: stimulus-induced plasticity. *Neuroscience*, **76**, 39–54.

Eckhorn, R., Bauer, R., Jordan, W., Brosch, M., Kruse, W., Munk, M. & Reitboeck, H.J. (1988) Coherent oscillations: a mechanism of feature linking in the visual cortex? Multiple electrode and correlation analyses in the cat *Biol. Cybern.*, **60**, 121–130.

Gray, C.M., König, P., Engel, A.K. & Singer, W. (1989) Oscillatory responses in cat visual cortex exhibit inter-columnar synchronization which reflects global stimulus properties. *Nature*, **338**, 334–337.

Grinsted, A., Moore, J. & Jevrejeva, S. (2004) Application of the cross wavelet transform and wavelet coherence to geophysical time series. *Nonlinear Processes Geophys*, **11**, 561–566.

Jen, P.H., Zhou, X., Zhang, J., Chen, Q.C. & Sun, X. (2002) Brief and short-term corticofugal modulation of acoustic signal processing in the bat midbrain. *Hear. Res.*, **168**, 196–207.

King, A.J. (1997) Sensory processing: signal selection by cortical feedback. *Curr. Biol.*, **7**, R85–R88.

Malmierca, E. & Nuñez, A. (1998) Corticofugal action on somatosensory response properties of rat nucleus gracilis cells. *Brain Res.*, **810**, 172–180.

Malmierca, E. & Nuñez, A. (2004) Primary somatosensory cortex modulation of tactile responses in nucleus gracilis cells of rats. *Eur. J. Neurosci.*, **19**, 1572–1580.

Martinez-Lorenzana, G., Machin, R. & Avendaño, C. (2001) Definite segregation of cortical neurons projecting to the dorsal column nuclei in the rat. *Neuroreport*, **12**, 413–416.

Murthy, V.N. & Fetz, E.E. (1992) Coherent 25- to 35-Hz oscillations in the sensorimotor cortex of awake behaving monkeys. *Proc. Natl Acad. Sci. USA*, **89**, 5670–5674.

Nicolelis, M.A. (2005) Computing with thalamocortical ensembles during different behavioural states. *J. Physiol. (Lond.)*, **566**, 37–47.

Nicolelis, M.A.L., Baccala, L.A., Lin, R.C.S. & Chapin, J.K. (1995) Sensorimotor encoding by synchronous neural ensemble activity at multiple levels of the somatosensory system. *Science*, **268**, 1353–1358.

Nuñez, A. & Malmierca, E. (2007) Corticofugal modulation of sensory information. *Adv. Anat. Embryol. Cell Biol.*, **187**, 1–74.

Nuñez, A., Panetsos, F. & Avendaño, C. (2000) Rhythmic neuronal interactions and synchronization in the rat dorsal column nuclei. *Neuroscience*, **100**, 599–609.

Panetsos, F., Nuñez, A. & Avendaño, C. (1997) Electrophysiological effects of temporary deafferentation on two characterized cell types in the nucleus gracilis of the rat. *Eur. J. Neurosci.*, **9**, 563–572.

Panetsos, F., Nuñez, A. & Avendaño, C. (1998) Sensory information processing in the dorsal column nuclei by neuronal oscillators. *Neuroscience*, **84**, 635–639.

Paxinos, G. & Watson, C. (1998) *The Rat Brain in Stereotaxic Coordinates*. Academic, San Diego.

Roy, S. & Alloway, K.D. (1999) Synchronization of local neural networks in the somatosensory cortex: a comparison of stationary and moving stimuli. *J. Neurophysiol.*, **81**, 999–1013.

Sillito, A.M., Jones, H.E., Gerstein, G.L. & West, D.C. (1994) Feature-linked synchronization of thalamic relay cell firing induced by feedback from the visual cortex. *Nature*, **369**, 479–482.

Singer, W. (1993) Synchronization of cortical activity and its putative role in information processing and learning. *Annu. Rev. Physiol.*, **55**, 349–374.

Singer, W. & Gray, C.M. (1995) Visual feature integration and the temporal correlation hypothesis. *Ann. Rev. Neurosci.*, **18**, 555–586.

Soto, C., Aguilar, J., Martin-Cora, F., Rivadulla, C. & Canedo, A. (2004) Intracuneate mechanisms underlying primary afferent cutaneous processing in anaesthetized cats. *Eur. J. Neurosci.*, **19**, 3006–3016.

Sun, J.J. & Luhmann, H.J. (2007) Spatio-temporal dynamics of oscillatory network activity in the neonatal mouse cerebral cortex. *Eur. J. Neurosci.*, **26**, 1995–2004.

Torrence, C. & Webster, P. (1998) The annual cycle of persistence in the El niño – Southern oscillation. *Quart. J. Ray. Meteor. Soc.*, **124**, 1985–2004.

Trurmon, J.C., Tranquilli, W.J. & Benson, G.J. (1996). *Lumb and Jones' Veterinary Anesthesia*. Williams & Wilkins, Philadelphia.

Weisberg, J.A. & Rustioni, A. (1976) Cortical cells projecting to the dorsal column nuclei of cats. An anatomical study with the horseradish peroxidase technique. *J. Comp. Neurol.*, **168**, 425–438.

Witham, C.L. & Baker, S.N. (2007) Network oscillations and intrinsic spiking rhythmicity do not covary in monkey sensorimotor areas. *J. Physiol.*, **580**, 801–814.

Yan, J. & Suga, N. (1996) Corticofugal modulation of time-domain processing of biosonar information in bats. *Science*, **273**, 1100–1103.

Zhang, Y., Hakes, J.J., Bonfield, S.P. & Yan, J. (2005) Corticofugal feedback for auditory midbrain plasticity elicited by tones and electrical stimulation of basal forebrain in mice. *Eur. J. Neurosci.*, **22**, 871–879.

Zhang, Z., Liu, C.H., Yu, Y.Q., Fujimoto, K., Chan, Y.S. & He, J. (2008) Corticofugal projection inhibits the auditory thalamus through the thalamic reticular nucleus. *J. Neurophysiol.*, **99**, 2938–2945.