Research Report

Cholinergic modulation of sensory interference in rat primary somatosensory cortical neurons

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ABSTRACT

Sensory interaction was studied using extracellular recordings from 275 neurons in the primary somatosensory (SI) cortex of pentobarbital-anesthetized rats. Tactile stimulation was applied to the receptive field using a 1 mm diameter probe that indented the skin for 20 ms, at 0.5 Hz, (test stimulus). Tactile test responses of SI neurons decreased during simultaneous application of a gentle tickling (distracter stimuli) continuously for 60 s on a separate receptive field located in the same or the contralateral hindlimb (ipsi- or contralateral distraction). This decrease in neural response produced by distracter stimuli was interpreted as "sensory interference". Sensory interference was observed in 66% and 61% of recorded SI neurons when ipsi- or contralateral distracters were applied, respectively and was blocked by a novel stimulus obtained by increasing the stimulation frequency of the test tactile stimuli from 0.5 to 2 Hz. The number of neurons showing sensory interference in response to a contralateral distracter was not modified after corpus callosum transection, suggesting that interhemispheric connections are not crucial for sensory interference. In contrast, the number of neurons showing sensory interference decreased in animals with 192 IgG-saporin basal forebrain lesions that decreased the number of cortical cholinergic fibers. This finding indicates that cholinergic affrents from the basal forebrain are fundamental to sensory interference and suggests that the associative cortices–basal forebrain–primary somatosensory cortex network may be implicated in sensory interference.

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1. Introduction

When animals explore their environment, somatosensory stimuli are not experienced as isolated stimuli but instead they are sampled along with multiple contextual factors including other stimuli. The ability to focus on selected sensory inputs while ignoring irrelevant inputs is a critical feature of cognition. It must be remembered that sensory response properties are dynamic and depend on interaction between different stimuli (Fanselow and Nicolelis, 1999). Moreover, processing of multisensory stimuli is strongly modulated by attention and by sensory environment (Reynolds and Desimone, 2003; Petkov et al., 2004; Sussman and Steinschneider, 2006).

Recently we reported that the response to tactile stimulation in the primary somatosensory (SI) cortical neurons of...
Anesthetized rats decreased when other somatosensory stimuli were simultaneously applied outside of the receptive field (RF; Alenda and Nuñez, 2004). This decrease in the somatosensory response was interpreted as “sensory interference” and the stimulus that elicited sensory interference was considered to be a “distractor”. Those data support the idea that cortical sensory responses are not immutable. On the contrary, responses are modulated by many factors, such as the simultaneous presence of other sensory stimuli.

It has also been suggested that the basal forebrain (BF) could participate in sensory interference effects since the muscarinic receptor antagonist atropine blocked sensory interference between tactile stimuli (Alenda and Nuñez, 2004). The BF is the major source of cholinergic afferents to the neocortex (Mesulam et al., 1983; Semba and Fibiger, 1989; Semba, 2000) and cortical responses to sensory stimuli are facilitated by increase in cholinergic transmission (Sillito and Kemp, 1983; Metherate et al., 1990; Himmelheber et al., 2001). Thus, enhanced acetylcholine (ACh) release in these structures may be a neurochemical substrate for cortical arousal or selective attention (Everitt and Robbins, 1997; Sarter and Bruno, 1997; Rasmusson, 2000).

There is compelling evidence for a significant role by the BF cholinergic system in attention (Pang et al., 1993; Muir et al., 1994; Voytko et al., 1994). In vivo microdialysis studies have shown large and sustained elevations in cortical ACh release during established attentional performance (Himmelheber et al., 2000; Dalley et al., 2001). Recent in vitro studies have demonstrated that ACh reduces the efficacy of feedback and intracortical connections via the activation of muscarinic receptors, and increases the efficacy of feed-forward connections via the activation of nicotinic receptors (Gil et al., 1997; Kimura et al., 1999; Hsieh et al., 2000; Metherate and Hsieh, 2004). Thus, ACh may have multiple effects on cortical activity through activation of pre- and postsynaptic cholinergic receptors.

The current study was designed to investigate sensory interference from tactile stimuli in SI neurons of rat and to...
reveal the contribution of cholinergic projections from the BF to this sensory interference.

2. Results

In order to study sensory interference, this study made recordings of 275 neurons from the SI cortex. The selected neurons had RFs on the glabrous part of the hindlimb and were silent or displayed a low firing rate (0.1–2 spikes/s) under spontaneous conditions. Recorded neurons were located at depths of 900 to 1500 μm (corresponding to cortical layers V–VI) and all neurons were grouped together for analysis.

Tactile stimuli applied to the RF of SI cortical neurons (n=71; test tactile stimuli) evoked spike firing, which was reduced by simultaneous application of a gentle tickling with a paint-brush for 60 s on the same limb but outside the RF of the recorded neuron (ipsilateral distracter stimulus) or on the contralateral limb (contralateral distracter stimulus; Fig. 1A). In the control condition, test tactile stimuli induced a mean tactile response of 2.4±0.2 spikes/stimulus (ranging from 0.5 to 3.2 spikes/stimulus). Application of an ipsilateral distracter stimulus reduced tactile test responses to 1.5±0.2 spikes/stimulus (p<0.001, n=71, Wilcoxon matched-pairs test; Fig. 1B). We interpreted this decrease in tactile response caused by the application of distracter stimuli as “sensory interference”.

To determine the number of neurons showing sensory interference, we considered SI neurons to be affected by the distracter stimuli when the tactile responses decreased by more than 10% (the mean standard error percentage for control responses). Accordingly, sensory interference was observed in 47 out of 71 neurons (66% of neurons). One minute after the application of the ipsilateral distracter stimulus, control tactile responses had partially recovered (2.1±0.2 spikes/stimulus), and it took a mean of 2.3±0.6 min for total recovery. Test tactile responses were also reduced (1.7±0.3 spikes/stimulus; p=0.02, n=71, Wilcoxon matched-pairs test) when the distracter stimulus was applied to the corresponding RF in the contralateral hindlimb (Fig. 1B). In these cases, sensory interference was observed in 43 out of 71 SI neurons (61%). No statistically significant differences were observed in the proportion of neurons affected by ipsi- and contralateral distracters (p>0.05, using the chi-square test). Moreover, most of SI neurons affected by an ipsilateral distracter stimulus were also affected by a contralateral distracter stimulus (38 out of 47 neurons; 81%).

Sensory interference was maintained in SI cortical cells during the continuous application of a distracter stimulus. Tactile stimuli applied at 0.5 Hz on the RF elicited a mean response of 2.7±0.19 spikes/stimulus in SI cortical neurons (n=17; 100%); application of a contralateral distracter in the contralateral RF decreased test tactile responses to 1.6±0.1 spikes/stimulus (p=0.001, Wilcoxon matched-pairs test), as indicated above (Fig. 2; closed circles). The effect of sensory interference lasted during the entire 2 min in which the distracter stimulus was applied. Increasing the frequency of test tactile stimuli from 0.5 Hz to 2 Hz during the application of the distracter stimuli elicited a recovery of test tactile responses to the same level as control values (n=11), abolishing sensory interference (Fig. 2; open squares). No statistically significant differences were observed between test tactile responses at 0.5 or 5 Hz in the absence of distracter stimuli (2.7±0.2 spikes/stimulus and 2.5±0.3 spikes/stimulus, respectively). Thus, sensory interference was abolished by the emergence of a novel characteristic in the test tactile stimuli.

2.1. Contribution of interhemispheric connections

It is possible that activation of other SI cortical neurons by the distracter stimulus may explain sensory interference because cortico-cortical connections contact inhibitory neurons, limiting neuronal responses in cortical neurons (Markram et al., 2004). To determine whether activation of the contralateral cortex would induce tactile response inhibition, electrical stimulation was applied in the SI cortical region that was symmetrical to the unit recording site.

Electrical stimulation of the contralateral SI cortex induced a response of 2.5±0.7 spikes/stimulus at 6±0.2 ms latency in SI cortical neurons (n=17) that was followed by a decrease in the spike firing for 118±8.3 ms. The neuronal group (n=17) recorded in this experimental paradigm had a mean tactile response of 2.5±0.2 spikes/stimulus that was considered to be 100% of the response (Figs. 3A–B, control response). The percentage of response evoked by test tactile stimuli was plotted at different intervals after the electrical stimulus of the contralateral SI cortex. Tactile responses were significantly inhibited by a previous electrical stimulus of the corresponding SI cortex at intervals <500 ms (p<0.001, n=17, Wilcoxon matched-pairs test) but were not affected with a delay of 500–1000 ms (Figs. 3A, B, cortical–tactile stimuli).

Sensory interference was studied in 39 control neurons recorded before and in 45 neurons recorded after transection of the corpus callosum in the same animals (9 animals) to test if cortico-cortical connections are involved in sensory interference. In control conditions, 69% (27 out of 39 neurons) of the neurons displayed sensory interference when the distracter stimuli was applied to the same limb and 59% (23 out of 39 neurons)
neurons) of the neurons displayed interference when the distracter stimuli was applied to the contralateral limb (Fig. 3C). After corpus callosum transection, the number of SI neurons showing sensory interference when the distracter was applied to the same hindlimb were 75% (34 out of 45 neurons) and 49% (22 out of 45 neurons) when the distracter was applied to the contralateral hindlimb. Differences in the proportion of neurons displaying sensory interference before and after corpus callosum transection were not statistically significant ($p > 0.05$, using the chi-square test), indicating that interhemispheric connections through the corpus callosum were not decisive in the generation of sensory interference.

After corpus callosum transection, the number of SI neurons showing sensory interference when the distracter was applied to the same hindlimb were 75% (34 out of 45 neurons) and 49% (22 out of 45 neurons) when the distracter was applied to the contralateral hindlimb. Differences in the proportion of neurons displaying sensory interference before and after corpus callosum transection were not statistically significant ($p > 0.05$, using the chi-square test), indicating that interhemispheric connections through the corpus callosum were not decisive in the generation of sensory interference.

2.2. Effect of lesion of cholinergic basal forebrain neurons on sensory interference

Since lesion of the corpus callosum did not decrease sensory interference and a previous study has shown that sensory interference in the SI cortex diminishes after application of the muscarinic receptor antagonist atropine (Alenda and Nuñez, 2004), it is reasonable to believe that the BF, which is the major source of cholinergic afferents to the neocortex, could be involved in the generation of sensory interference.

Cholinergic neurons were selectively destroyed by injection of the immunotoxin 192 IgG-saporin in the BF. As illustrated in Fig. 4, 192 IgG-saporin lesions resulted in extensive cortical cholinergic deafferentation 2 weeks after immunotoxin injection. Each animal with 192 IgG-saporin (8 rats) showed 92% loss of ChAT-positive fibres bilaterally in somatosensory cortical areas in comparison with control rats that only received saline injection in the BF. In control animals ChAT-positive neuronal bodies could be observed (Fig. 4A, short arrow). These neurons were not affected by injection of the immunotoxin 192 IgG-saporin in the BF (Fig. 4B, short arrow).
interference in IgG-saporin treated rats were similar to those tactile responses in neurons that did not show sensory control conditions \((p < 0.001\), Student’s \(t\)-test; Fig. 5C). As indicated above, most of SI neurons affected by an ipsilateral distracter stimulus were also affected by a contralateral distracter stimulus (13 out of 24 neurons; 62%). These data indicate that the decrease in cholinergic projections from the BF sensibly reduced the percentage of neurons displaying sensory interference.

3. Discussion

The present work shows that ipsi- and contralateral distractors induce sensory interference in SI cortical cells. Appearance of a novel characteristic in the test stimulus, such as an increasing stimulation frequency, abolished sensory interference. Although sensory interference was not altered after corpus callosum transection, it was diminished in rats with lesions in the BF cholinergic system. These results suggest that interhemispheric connections are not relevant in inducing sensory interference but that cholinergic inputs from BF may participate in sensory interference generation.

The present study was performed in pentobarbital anesthetized rats in order to obtain stable unit recordings during long sensory stimulation periods. Previous results indicate that sensory responses in the central nervous system are controlled by a balance between excitation and inhibition and that anesthetics can disrupt this balance. Potentiation and agonistic effects of barbiturates have been reported in previous studies of the \(\gamma\)-aminobutyric acid (GABA) receptor channel (e.g. Krampfl et al., 2002). Volatile anaesthetics, such as halothane, mediate their effects through blockade of muscarinic (Anthony et al., 1990; Seeman and Kapur, 2003) and nicotinic mechanisms (Tassonyi et al., 2002) while ketamine depresses NMDA-receptor-mediated glutamatergic transmission (Brockmeyer and Kendig, 1995). Although pentobarbital decreases single unit responsiveness (Alter et al., 1979) it was chosen here because it disrupts fewer glutamatergic and cholinergic interactions than other anesthetics.

Results show that a majority of SI cells decreased their tactile responses when another somatosensory stimulus (distracter stimuli) was simultaneously applied. This decrease is interpreted as sensory interference. The present results extend previous findings (Alenda and Nuñez, 2004) concerning the mechanisms of sensory interference, showing that sensory interference was quickly blocked if a characteristic of the test tactile stimulus was modified (in this case, an increase of the stimulation frequency from 0.5 to 2 Hz). Thus, we can consider that the stimulation frequency increase is interpreted as a novel stimulus and thus it blocks the sensory interference. It is well known that a novelty of the stimulus induces changes in cortical sensory responses (Dias and Honey, 2002; Maatta et al., 2005), and cortical ACh release is increased in comparison with the release observed during the presentation of a familiar stimulus (Miranda et al., 2000). Moreover, it has been suggested that the BF may participate in detecting novel stimuli since neurons in the substantia innominata and in the diagonal band of Broca responded maximally to novel stimuli (Wilson and Rolls, 1990b). It is
reasonable to believe that an increase in the stimulation frequency could raise ACh cortical release, and block sensory interference. Cortical barrels can interact with each other through horizontal connections (Kossut and Juliano, 1999; Urban et al., 2002) that may inhibit adjacent barrels in order to increase the contrast between sensory stimuli (Armstrong-James et al., 1991). There is also evidence from the primary visual cortex demonstrating that intracortical inhibitory mechanisms contribute to the sharpening of tuning functions (Crook et al., 1998; Ringach et al., 2003; Shapley et al., 2003; Thiele et al., 2004). Sensory interference, which also increases the contrast between tactile stimuli, might therefore be explained by cortico-cortical connections that could activate inhibitory GABAergic neurons, thereby limiting neuronal responses in SI cortical neurons (Markram et al., 2004). Moreover sensory interference was blocked by cortical application of the GABA$_A$ receptor antagonist bicuculline (Alenda and Nuñez, 2004).

Thus, sensory interference induced by a distracter applied to the same hindlimb as the tactile test stimuli may occur through activation of local horizontal connections between different SI areas with specific RFs. If a distracter is applied to the contralateral hindlimb, interhemispheric connections through the corpus callosum would be responsible for sensory interference. Electrical stimulation of the contralateral SI cortex induced an early excitation followed by a long-lasting inhibition that decreased tactile responses for 500 ms. However, the number of SI cortical neurons expressing sensory interference from contralateral distracters did not decrease significantly after corpus callosum transection, suggesting that interhemispheric connections through the corpus callosum are not the major anatomical substrate responsible for the sensory interference evoked by a contralateral distracter.

Previous results showed that sensory interference is blocked by cortical application of the muscarinic receptor antagonist atropine (Alenda and Nuñez, 2004), indicating that
a cholinergic pathway may be involved in sensory interference. Injection of 192 IgG-saporin in the BF produced a selective lesion of cholinergic neurons (Berger-Sweeney et al., 1994; Torres et al., 1994). In the present study, 192 IgG-saporin injections in the BF significantly decreased the number of neurons showing sensory interference. However, the reduction of tactile responses to distractor stimuli was not altered in SI cortical neurons of animals with lesions, probably because the inhibition provoked by the distractor stimuli was due to the activity of GABAergic neurons, which were not affected by 192 IgG-saporin injections. Our data agrees with previous reports that suggest an important cholinergic role of the BF, modulating the efficacy of cortical sensory information processing (Sillito and Kemp, 1983; Metherate et al., 1987; Tremblay et al., 1990; Himmelheber et al., 2001; Roberts et al., 2005; Zinke et al., 2006) or in attentional processes (Everitt and Robbins, 1997; Sarter and Bruno, 1997; Miranda et al., 2000; Dalley et al., 2004).

The fact that rats with 192 IgG-saporin lesions displayed lower tactile responses in neurons expressing sensory interference compared to those that did not show sensory interference or to neurons recorded in control rats suggest that there are heterogeneous neuronal populations in the SI cortex and that only some of them express sensory interference or, more probably, that the SI cortical neurons expressing sensory interference are more affected by cholinergic inputs. ACh may modulate cortical responses through a variety of presynaptic and postsynaptic mechanisms and receptors (Kimura and Baughman, 1997; Alkondon et al., 2000; Kimura, 2000). In vitro studies suggest that a key function of cortical ACh may be to control the flow of neuronal information by selectively suppressing lateral intracortical synapses via a muscarinic mechanism, at the same time as it boosts the efficacy of thalamocortical/feed-forward connections via a nicotinic mechanism (Gil et al., 1997; Kimura et al., 1999; Hsieh et al., 2000; Metherate and Hsieh, 2004). The kind of cholinergic receptors activated and the neuronal population affected may be crucial in determining whether SI cortical neurons may increase or decrease tactile responses.

If the BF contributes to increasing sensory responses to highly salient stimuli or to decreasing tactile responses when a distracter stimulus is simultaneously applied, it should receive sensory information. Visual and auditory responses were recorded in the BF of freely moving rats, waking monkeys and guinea-pigs (Santos-Benitez et al., 1995; Chernyshev and Weinberger, 1998; Wilson and Ma, 2004). Sensory inputs may arrive at the BF from sensory cortical areas throughout the prefrontal cortex (Wilson and Rolls, 1990a; Zaborszky et al., 1997; Sarter et al., 2005). Electrophysiological recordings have described two different areas in the prefrontal cortex of rats that responded to electrical stimulation of the SI cortex or the visual cortex, indicating that sensory inputs are sorted in the prefrontal cortex (Golmayo et al., 2003). Sensory segregation is also maintained in the projection from the prefrontal cortex to the BF in which some neurons (33%) responded only to electrical stimulation of the ‘somatosensory-responsive’ prefrontal area and other neurons (42%) only responded to ‘visually-responsive’ prefrontal area. Thus, the topographical organization of the projections from the sensory cortex to the prefrontal cortex-BF and those back to the sensory cortex may have an important role in selective modulation of sensory processes via a cholinergic mechanism (Golmayo et al., 2003). In agreement with this hypothesis, it has been demonstrated that stimulation of cholinergic and glutamatergic receptors in the prefrontal cortex is sufficient to increase ACh release in the posterior parietal cortex, via the activation of the BF (Nelson et al., 2005; Sarter et al., 2005). Also, it has been suggested that short-term plasticity of SI cortex may be regulated by a prefrontal–cortical sensory gating system (Schafer et al., 2005). In accordance with these data, it seems that sensory interference could be a function of the associative cortices – BF – sensory cortices network. It is reasonable to believe that this neuronal network may also contribute in attentional processes that occur in behavioral animals.

4. Experimental procedures

Experiments were performed on 52 pentobarbital-anesthetized (33 mg/kg i.p.) adult Wistar rats (from Iffa-Credo, France), weighing 190–230 g. Animals were placed in a Kopf stereotaxic device in which surgical procedures and recordings were performed. The body temperature was maintained at 37 °C. Supplemental doses of anesthetic were given to maintain areflexia. Local anesthetic (lidocaine 1%) was applied to all skin incisions. Experiments were carried out in accordance with the European Communities Council Directive (86/609/ EEC), and every effort was made to minimize animal suffering and the number of animals used.

4.1. Surgical and recording procedures

An incision was made exposing the dorsal aspect of the skull and a small hole was drilled in the skull over the SI cortex. The dura mater was removed, and the cortical surface was covered with mineral oil. Single unit recordings in SI cortex (A 1–3 mm, L 2.5–4 mm, from bregma) were made at 900 to 1500 μm below the surface (Paxinos and Watson, 1998), using tungsten microelectrodes (2–5 MΩ; World Precision Instruments, Sarasota, FL, USA). Unit firing was filtered (0.3–3 kHz), amplified via an AC preamplifier (DAM80; World Precision Instruments) and fed into a personal computer (sample rate: 8 kHz) with the temporal reference of the stimuli for off-line analysis using a Spike 2 software (Cambridge Electronic Design, Cambridge, UK). The spike amplitude and shape were continuously monitored on-line in an analog oscilloscope. Additional off-line spike amplitude and shape analyses were performed by computer to ensure that the same cell was recorded during the entire experiment and to transform spikes into time point-processes for further data analysis (see below).

In some animals (n = 9), the corpus callosum was completely transected with a thin stainless steel knife that was inserted 5 mm into the midline and displaced 7 mm caudally from the bregma. Unit recordings in SI cortex were performed before (control) and immediately after the corpus callosum lesion using the same recording technique indicates above.

Animals (n = 8) designated for cholinergic lesions received infusions of the cholinergic immunotoxin 192 IgG-saporin...
(Chemicon International, Temecula, CA, USA), which consists of a ribosome inactivating enzyme conjugated with a monoclonal antibody targeted to the p75 nerve growth factor receptor expressed by cholinergic neurons. Bilateral injections (0.3 µg/µl in saline and 0.5 µl per hemisphere) were made in the BF (from bregma: AP—0.8 mm, L 2.5 mm and V 7.5 mm), using a 1 µl Hamilton syringe. Control animals received infusions of saline. The needle was left in place from 30 s before until 3 min after injections. Rats were monitored during recovery in their home cage with food and water available ad libitum before unit recordings in the SI cortex of injected rats were performed 2 weeks later.

4.2  Sensory and electrical stimulation

Tactile stimulation was performed by an electronically gated solenoid with a probe of 1 mm in diameter that produced <0.5 mm skin deflections. The SI cortex was carefully mapped with a small hand-held brush to locate neurons of primary somatosensory cortex responding to light mechanical stimulation of the hindlimb, as detected with an audiometer driven by the amplified neuronal activity. RFs were defined by the limits at which stimuli elicited changes in the unit activity. Control tactile stimulation consisted in tactile pulses lasting 20 ms and delivered at the RF at 0.5 Hz during 60 s.

In order to induce sensory interference, a gentle non-rhythmic tickling was applied with a paintbrush continuously for 60 s at a separate RF that was located on the same limb of the recorded neuron RF (ipsilateral distractor stimulus) or at the same RF of the recorded neuron but on the contralateral limb (contralateral distractor stimulus). Unit activity was recorded during application of tactile pulses delivered at its RF (control) and during the application of ipsi- or contralateral distractor stimulus.

Electrical stimulation of the SI cortex was performed through bipolar electrodes (100 µm diameter blunt cut insulated stainless steel wire; World Precision Instruments) placed symmetrically in the contralateral hemisphere from the cortical unit recording. Brief rectangular pulses lasting 0.3 ms and at intensities of 50–500 µA were produced by a Grass S88 stimulator coupled to photoelectric stimulus isolation unit.

4.3  Histological verification of electrodes and lesions

Upon completion of the experiments, animals were deeply anesthetized with sodium-pentobarbital (50 mg/kg) and then perfused transectually with saline followed by formalin (4% in saline). The brains were removed, stored in 30% sucrose saline and cut on a freezing microtome. Coronal sections 50 µm thick were stained with the Nissl method to locate the recording and stimulation sites or corpus callosum lesion. The recording and stimulating electrodes had been positioned within their target structures in all the analyzed animals.

4.4  Immunohistochemical procedure

To verify the cholinergic denervation of the cortex after 192 IgG-saporin injections in the BF, brain choline acetyltransferase (ChAT) was immunostained. After completing the extra-cellular recordings, 16 rats were deeply anaesthetized and transectually perfused with 0.9% heparinized saline followed by a mixture containing 4% paraformaldehyde, 15% picric acid and 0.1% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4 and then by increasing concentrations of sucrose in phosphate buffer for cryopreservation. After removal, brains were stored in 30% sucrose for at least 5 days before undergoing frozen serial sectioning at 60 µm in a coronal orientation. Sections were immersed in phosphate buffer of 0.3% Triton X-100, 2% bovine serum albumin and 3% normal rabbit serum for 60 min at room temperature. They were then reacted with a 1:100 dilution of a goat polyclonal antibody for ChAT (AB144P; Chemicon, Temecula, CA, USA) overnight at 4 °C. After repeated washes with phosphate buffer, sections were incubated with a 1:500 rabbit antigoat secondary antibody (AP106B, Chemicon) for 90 min. at room temperature. Immunohistochemical detection of ChAT in the cortex was carried out using the avidin–biotin–peroxidase technique with the Vectastain ABC Ellite kit (Vector Laboratories, Burlingame, USA) and DAB substrate.

The effect of 192 IgG-saporin injection in the BF was assessed by counting the number of ChAT immunoreactive fibers in the SI cortical area per slide from two sections of 60 µm taken at between −1.0 and −2.0 mm from the bregma in each rat. The effect of 192 IgG-saporin injection was expressed as a percentage of control values.

4.5  Data analysis

Recordings were accepted for statistical analysis when the fluctuation of the unit amplitude was lower than 10% throughout the experiment. Summed peristimulus time histograms (PSTHs; 2 ms bins) were calculated from 30 stimuli using the Spike 2 software. The mean tactile response was measured from the PSTH as the number of spikes evoked at 10–50 ms after stimulus onset divided by the number of stimuli. We considered that neurons responded to tactile stimulation when the cell discharged at least one spike per two stimuli. Neurons with sensory responses that were less than one spike per two stimuli were not analyzed. During the electrical cortical stimulation protocol, the ratio between tactile responses preceded by cortical stimulus and tactile responses without cortical stimulus was calculated and plotted according to the delay between the cortical and the tactile stimuli. All data are shown as mean ± standard error. The two-tailed Student’s t-test or Wilcoxon matched-pairs analyses were used for comparisons. The Student’s t-test was used to compare data from different neuronal populations while the Wilcoxon matched-pairs statistics was used to compare data from the same neuron in different conditions. The frequency distribution of neurons displaying sensory-interference was analyzed using the chi-square test. Differences were considered statistically significant at the 95% level (p < 0.05).

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