Effect of Tactile Inputs on Thalamic Responses to Noxious Colorectal Distension in Rat

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Received 29 November 2001; accepted in final form 22 May 2002

Zhang, Hong-Qi, Elie D. Al-Chaer, and William D. Willis. Effect of tactile inputs on thalamic responses to noxious colorectal distension in rat. J Neurophysiol 88: 1185–1196, 2002; 10.1152/jn.00977.2001. Recent discoveries of visceral nociceptive inputs sharing the classical tactile pathway in the dorsal-column medial lemniscus system have opened a new venue for the investigation of somatovisceral interactions. The current study was designed to determine whether somatic innocuous inputs modulate visceral nociceptive transmission at the thalamic level. The investigation was carried out by means of extra-cellular single-unit recordings in the ventroposterior lateral nucleus of the thalamus in rats anesthetized with pentobarbital. Noxious visceral stimulation was achieved by reproducible colorectal distension (CRD, 20–80 mmHg) with a balloon catheter. Tactile stimulation was delivered by means of a feedback-controlled mechanical stimulator. The response of the neurons to CRD was compared before and after the conditioning procedure by giving tactile stimulation either immediately before CRD or overlapping it. Twenty-five ventroposterior lateral (VPL) thalamic neurons were found among numerous tactile-only neurons to have convergent inputs from both tactile and visceral sources. Their responses to CRD were excitatory (19), inhibitory (4), or bimodal. When cutaneous tactile stimuli were delivered before CRD, the responses were reduced in 18 cases. The reduction, however, was usually short-lasting, immediately following tactile stimulation and could not be enhanced by a prolonged conditioning procedure. It was unlikely to be attributable to neuronal habituation as the inverted procedure, CRD stimulation before tactile, often produced the opposite effect, that is, an enhanced response to skin stimulation. Repeated CRD could bring about sensitization of the responses of thalamic neurons as manifested by increased spontaneous discharge, lowered response threshold, and increased response level. Under such circumstances, the original effect of tactile stimulation on CRD responses could be weakened. In conclusion, tactile stimulation may in most circumstances inhibit thalamic neuronal responses to visceral nociceptive input produced by CRD. However, the effect appears to be mild and short-lasting at the individual neuronal level in the VPL thalamus.

INTRODUCTION

As the highest cognitive center, the brain receives inputs from different sources for integration. In terms of nociception, the spinothalamic tract (STT) has traditionally been viewed as the most important pathway for nociception, including visceral pain, and previous studies of pain mechanisms have focused mainly on this pathway and its related projection areas (for reviews, see Millan 1999; Willis 1979, 1985a–c). There has been ample evidence to show that viscerosomatic convergence is a common phenomenon in this pathway (Foreman 1977, 1984; Gokin et al. 1977; Hancock et al. 1970, 1973, 1975; Ness and Gebhart 1991a,b; Selzer and Spencer 1969a,b; for review, see Gebhart and Ness 1991). Recent studies, however, have found that the dorsal column-medial lemniscus (DC-ML) system may also play an important role in nociceptive processing, in particular for visceral pain (Al-Chaer 1996a–c; Berkley et al. 1993; Rigamonti et al. 1978). A large number of neurons in the dorsal column nuclei (DCN) respond to both visceral and tactile stimulation (for review, see Al-Chaer et al. 1996a; Berkley and Hubscher 1995; Willis et al. 1999). Recent studies (e.g., Al-Chaer et al. 1996a–c, 1997a,b, 1998) have demonstrated that the role played by the DC system in mediating colorectal sensory processing is more important than that of the ventrolateral pathways to the thalamic ventrobasal complex. Most DC-ML neurons that respond to visceral painful stimuli are also sensitive to gentle skin manipulation, such as brushing (Al-Chaer et al. 1996a,b, 1997a, 1998); therefore it is conceivable that light tactile inputs and nociceptive visceral inputs may interact at any of several locations along the DC-ML pathway. The DC-ML pathway, through which light touch information is conveyed, thus appears to be an important system in which interactions between visceral and somatic inputs may take place. As it has been demonstrated that the pelvic visceral inputs to gracile neurons are largely mediated by the postsynaptic DC pathway originating from neurons around lamina X at the L6–S1 level, whereas cutaneous inputs are in part conveyed directly to gracile nucleus by primary afferent fibers (Al-Chaer et al. 1996a,b, 1998), it may be assumed that interactions between the two inputs would most likely take place at or above the level of the DCN.

The hypothesis to be tested in this study is that innocuous tactile inputs may modulate the transmission of visceral pain signals at the thalamic level. This interaction may be mutual in that tactile inputs may inhibit the responses of central neurons to visceral pain, or vice versa; it may also be facilitatory instead of inhibitory. Preliminary results of this study have been reported in abstract form (Zhang et al. 1999a,b).

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METHODS

Preparation and recording procedures

Results were obtained from 13 male Sprague-Dawley rats (body weight 240–300 g) anesthetized with pentobarbital sodium (induction by 50–60 mg/kg ip; maintained with an intravenous infusion of pentobarbital at ~5 mg·kg\(^{-1}\)·h\(^{-1}\)). The trachea was intubated and a jugular or femoral vein was cannulated to allow the infusion of anesthetics. The femoral artery was cannulated in four experiments to monitor blood pressure changes during the experiment. When the femoral artery or vein was cannulated, the groin incision was on the same side as the thalamic recordings to avoid disturbing the cutaneous receptive fields of thalamic neurons from which recordings were to be made. The body temperature was monitored and maintained near 37°C by a feedback-controlled blanket. A craniotomy was performed (right side 12, left 1) above the thalamus.

Single-neuron recordings were carried out extracellularly with the use of tungsten microelectrodes (125-μm shank, 5–12 MΩ). The electrode was advanced stereotaxically into the ventroposterior lateral (VPL) nucleus of the thalamus according to a rat brain atlas (Paxinos and Watson 1998). Thalamic neurons that were well isolated from baseline noise were tested first for their tactile response properties to somatic stimulation, including receptive field location and size, von Frey hair sensitivity, the mechanical stimuli to which they responded best, and the maximum discharge rate (Fig. 1), followed by testing their responses to colorectal distension (CRD) with pressures ranging from 20 to 80 mmHg (Fig. 1C). Only those neurons that responded to both light cutaneous stimuli and CRD were investigated to determine the interactions between the two modalities.

Stimulation and conditioning procedures

To excite neurons that were sensitive to gentle skin touch, such as brushing and the application of von Frey hairs with weak bending forces, mechanical pulses with a consistent displacement were delivered by means of a feedback controlled stimulator (Chubbock 1966). A vibrotactile stimulus with a frequency of 10–200 Hz (amplitudes: 50–500 μm) superimposed on a steady skin indentation of 0.2–1 mm and 20-s duration was delivered via a stimulator probe with a flat tip (≥2 mm in diameter) put on the most sensitive part of the cutaneous receptive field as assessed with von Frey hairs (Fig. 1A). When tactile stimulation was used for the purpose of conditioning, the maximum neuronal discharge was generated with the smallest possible amplitude (typically 100 μm) to avoid spread of skin mechanical pulses to an unnecessarily large area on the body.

Colorectal distension was achieved by means of a balloon catheter inserted 1 cm into the rectum and the descending colon via the anus (Fig. 1C). The balloon catheter was constructed from a latex glove

![FIG. 1. Methods for somatovisceral interactions. A: neuronal response to 10-Hz skin pulses (500-μm amplitude, superimposed on a 20-s steady skin indentation) delivered to the skin receptive field on the tail mapped with a von Frey hair of 2.8 N in force. The effect of conditioning skin stimulus on colorectal distension (CRD) response was tested as shown in B, and analysis of responses and effect of conditioning was based on the formulae shown in C. C: visceral inputs generated by CRD (80 mmHg). The peristimulus time histograms (PSTH) were constructed based on impulse counts for the spike traces shown above, though sometimes there appears to be discrepancy due to closely spaced bursting activity of the neuron.](http://jn.physiology.org/doi/10.1152/jn.00818.2001)
finger tied to a 5- to 6-cm length of tygon tubing, inflated, and left overnight to overcome the tension of the balloon wall (Al-Chaer et al. 1996b). The tubing was connected to a manual pump and, via a T connector, to a pressure transducer. CRD was accomplished by inflation of the balloon by means of a sphygmomanometer to a pressure of 20–80 mmHg for 20 s or longer. Repetition of CRD was at a rate of no more than once every 4 min to avoid over-stimulation and possible sensitization of the colon and rectum (see Fig. 4 and DISCUSSION). CRD stimuli with pressures ≥40 mmHg are considered noxious (Ness et al. 1990).

The standard conditioning procedure employed was designed so that 20-s skin pulses were immediately followed by 20-s CRD at a predetermined intensity (Fig. 1B). Alternatively, the sequence was inverted to test the effect of CRD conditioning on tactile responses (e.g., Fig. 5C) or the two stimuli were given simultaneously (e.g., Figs. 6 and 7).

Data-acquisition and analysis procedures

The activities recorded from isolated neurons were captured by means of Spike2 software with a CED 1401+ data-analysis instrument. The neuronal responses were analyzed on- and off-line mainly by constructing peristimulus time histograms (PSTH). As there was often moment-to-moment fluctuation in the thalamic neuronal excitability associated with a change in background activity (see Fig. 4; also Al-Chaer et al. 1996b; Berkley et al. 1993) in contrast to tactile neurons (Zhang 1994; Zhang et al. 2001), impulse counts in association with a stimulation procedure were compared with the background activity for the 20-s period immediately before the stimulation took place ("net response", see Fig. 1). If the impulse counts during stimulation were 20% more or less than the background activity, the neuron was then considered to have an excitatory (Fig. 1A) or inhibitory response (Fig. 1C), respectively.

After recording control responses when a skin stimulus or CRD was given alone (Fig. 1, A and C), conditioning was carried out with one stimulus preceding the other to test the interactions between the two responses. The net impulse counts generated by the testing stimulus after the conditioning stimulus were compared with the control, the net impulses taken without conditioning (Fig. 1).

Histological verification of recording sites

A lesion was made at the site of recording or at the end of an electrode track by passing 100- to 500-μA DC current for 10–30 s to identify the locations of neurons recorded in the thalamus by histological examination. A marker electrode was sometimes left in place at known coordinates for the same purpose. The fixed brain was blocked and sectioned at a thickness of 50 μm. The locations of recording sites within the thalamus were verified from nine specimens by reconstruction of microelectrode tracks based on a rat brain atlas (Paxinos and Watson 1998).

RESULTS

Among the numerous thalamic neurons that were isolated using their responses to tactile stimulation, only 27 showed responses to CRD. With exception of two neurons, all of these (93%) had skin receptive fields that were detectable by tapping, brushing, and von Frey hair testing. The 25 neurons with cutaneous receptive fields were studied for their responses to CRD as well as the interaction with tactile responses when CRD was preceded by skin stimulation (Fig. 2). In terms of their responses to CRD, 19 of the 25 thalamic neurons (76%) increased their discharge rate above the background activity by ≥20% and, thus, were considered to have an excitatory response to visceral input. Four neurons (16%) showed a >20% reduction in their responses to CRD in comparison with the background activity and were classified as having inhibitory responses. The remaining two neurons showed variable responses to CRD that appeared to be influenced by fluctuations in background activity, and they were classified as a bimodal group.

Skin receptive fields

The cutaneous receptive fields of these thalamic neurons were located in the caudal parts of the body, on the tail, scrotum, hip region, or hind-limb or -paw (Fig. 2). The sizes of most cutaneous receptive fields were small, and they were sharply demarcated (as shown by the examples in Fig. 1A, inset, and in Figs. 5–7), but three units had large receptive fields, covering an area as extensive as a whole leg and hip. Except for the receptive fields on the tail, most skin receptive fields were located contralateral to the thalamic recording site.

The neurons that responded to CRD were mixed among the abundant neurons that were sensitive to tactile inputs only and therefore in the locus to which the dorsal column-medial lemniscus projects (see Fig. 8). With only one exception, these neurons could be activated by von Frey hairs with an average force of 2.74 ± 0.45 (range 1.65–3.8) Newton (N) and therefore were tactile sensitive neurons. The exceptional neuron was only activated by a stronger, 4.2 N von Frey hair and probably had input from deep tissues. The neurons were all most responsive to low-frequency mechanical pulses and therefore appeared to derive inputs predominantly from hair follicle receptors in the hairy skin or rapidly adapting receptors of the glabrous skin (Leem et al. 1993a,b; Talbot et al. 1968). For the purpose of somatic conditioning, the maximum response was produced by 10-Hz sinusoidal mechanical pulses with an amplitude <500 μm, although other frequencies and steady skin indentation were often tested as well.
Inhibitory effect of tactile stimulation on CRD responses

Conditioning tactile stimulation had a predominantly inhibitory effect on later neuronal responses to noxious CRD, manifested as a reduction in impulse counts in response to CRD with preceding tactile response in comparison with the control responses. Of the 19 neurons that were excited by CRD, a reduction in the visceral response was brought about by the preceding skin stimulation in 16 units for which two examples are shown in Figs. 3 and 5. The strongest reduction was often immediately after skin stimulation and lasted for a short time, <10 s (e.g., Figs. 3 and 5). The magnitude of the inhibitory effect ranged from mild in the majority (e.g., Figs. 3 and 5) to nearly 100% in a rare case. The remaining three neurons either showed no effects (2) or inconsistent results in association with the skin conditioning stimulation. For the four neurons that initially had an inhibitory response to CRD, the preceding excitatory tactile response enhanced the inhibition further (i.e., the impulse counts were further reduced, see Fig. 1) in two units or had an inconsistent/complex effect (Figs. 6 and 7). An example of an excitatory neuronal response to mechanical stimulation of the skin and inhibitory response to CRD is shown in Fig. 1. When a mechanical stimulus (10 Hz, 500 μm for 20 s) was delivered to the receptive field on the tail, the neuron clearly changed its discharge rate, as illustrated by the well-isolated spikes and the PSTH. In comparison with a background activity of 45 spikes for a 20-s period before the stimulation (A1 segment), the neuron had 149 discharges during the 20-s mechanical stimulus (A2), and thus it was excited by the tactile stimulus, with a net response (A2-A1) of 104/20 s. However, when the noxious CRD (>40 mmHg) was applied, the same neuron responded with fewer impulses in comparison with the spontaneous background activity. Thus it was considered to have an inhibitory response (Fig. 1C), with net responses of −8, −7, and −19 to 20 s of 40, 60, and 80 mmHg CRD, respectively. When the 80 mmHg CRD was preceded by the skin stimulation to test for an interaction, as shown in Fig. 1B, the initial inhibitory response to CRD was further enhanced, as the net response now was −33 in comparison with −19 spikes/20 s generated before the conditioning procedure. Because there was a 42% change associated with the preceding tactile stimulation (B) in comparison with the control (C), it appeared that the neuron’s response to nociceptive CRD was strongly affected by the somatic conditioning.

Neurons that had excitatory responses to CRD under control conditions were excited by noxious CRD (≥40 mmHg), and the response was a function of the CRD stimulus intensity (● in A). It had a tactile receptive field on the lateral part of the heel and could be activated by a 3.2 N von Frey hair. The best response to skin stimulation was generated by 10-Hz, 500-μm mechanical pulses (C). When the CRD was after the skin stimulation, the response to CRD (● and ○ in A) was lower in comparison with the control (● in A, a sample shown in C). ● and △ in A plot the net response to CRD for the whole 20-s period. However, the conditioning effect was most obvious immediately following the skin pulses, as shown by the ○ and △ that plot the first 10-s CRD net response. The short inhibition was unlikely attributable to the fatigue or habituation of the neuron, as the longer skin stimulation for 50 and 90 s, as shown in D, did not bring about a systematic increase in the effect (see Discussion). The numbers in each histogram are impulse counts for the relevant 20-s period, and the values with smaller fonts (being subtracted in D) indicate the background activity level.
circumstances had their noxious visceral responses inhibited by preceding tactile inputs in 16 of 19 (84%) cases. One example is shown in Fig. 3 where the neuron’s net responses to CRD at different pressures under control circumstances are plotted in A (—) and shown by the example trace and PSTH in B. The excitatory response of the neuron to the visceral nociceptive input was a function of the stimulus intensity, increasing from the near-zero background activity to ~80 imp/20 s in response to 80 mmHg CRD. However, when the CRD response was preceded by tactile input, 10-Hz mechanical pulses applied to the skin receptive field on the contralateral heel, the response to CRD was systematically reduced (Fig. 3A) in comparison with the control.

It is worth pointing out that the reduction was the strongest during the initial part of CRD following the somatic stimulation in the majority of the cases (with one exception), as illustrated by the sample traces in Fig. 3. This effect is shown in Fig. 3A where the net responses to CRD for the first 10 s are plotted as △ and ○. The reduction at 60 mmHg CRD, for example, was 59% for the first 10 s in comparison with 21% for the whole 20 s (for 80 mmHg, 43% vs. 12% reduction). It is unlikely, however, that such a short-lasting inhibition is attributable to habituation or fatigue of the neuron as, if it were, the effect on the CRD response would be more or less a function of the strength and/or duration of the preceding tactile stimulation. However, this was not the case, as illustrated in Fig. 3D. When the tactile stimulation was prolonged from the standard 20 s to a longer period, 50–90 s, the inhibitory effect was not further enhanced systematically. Although there was a more obvious reduction in impulse counts after 50 s of skin vibration, the same was not seen when the vibration was even longer, for example 90 s. The reason for the smaller conditioning effect after the longer somatic stimulation is not altogether clear, but it may be related to a change in the neuron’s sensitivity to the nociceptive inputs. It was noted that the background, spontaneous activity of the neuron increased gradually after repeated CRD stimulation, and this change was often found to be associated with an increase in sensitivity of the neurons to nociceptive inputs (see Fig. 4). In this case, the background activity of the neuron was low, 4 imp/20 s at the start of the test, but it gradually increased with repeated CRD stimulation and reached 17 imp/20 s when the 90 s trial of skin vibration as the conditioning stimulus took place. The neuron’s sensitivity may have been altered by this time and the weak inhibitory effect may have been obscured or offset by the high afterdischarge of the sensitized neuron (see the next 2 subsections and discussion).

For the four neurons that had an inhibitory response to CRD, the preceding tactile stimulation further enhanced the inhibitory response in two cases; that is, the impulse rate in response to CRD was further reduced following tactile stimulation (Fig. 1). The other two cells had inconsistent, or rather, more complicated effects from the somatic conditioning. The neuron whose data are shown in Fig. 6 had a skin receptive field on the medial side of the hind-paw (D) and could be excited by a von Frey hair that bent at 2.8 N as well as by a controlled mechanical pulse train (C). The same neuron had an inhibitory response to CRD ≥40 mmHg, as shown by the sample trace in B and the stimulus-response curve in A (top). When CRD was preceded by a skin pulse train, the net response to the visceral

![FIG. 4. Sensitization of thalamic neurons in response to CRD. A: the neuronal response to CRD (60 mmHg) at the beginning of the recording session when the neuron had low spontaneous activity and high threshold for response. The neuron’s response to CRD (C) as a function of the stimulus intensity is plotted in the D, bottom curve, along with the averaged (± SD, n = 4) background/spontaneous activity when the CRD intensity was 0. After CRD was repeated 15 times, the neuron’s spontaneous activity increased, from 50 to 110 imp/20 s as shown by the sample trace in B and the D, top curve. The threshold for response to CRD was lowered from the 60 mmHg at the beginning to 20 mmHg in the end, the end response level was higher than the initial records. The inset in D shows the well-isolated neuronal spikes.](http://jn.physiology.org/)

Sensitization of thalamic neurons by repeated CRD

An example of the sensitization of the responses of thalamic neurons after repeated CRD is shown in Fig. 4. When the CRD was delivered, the response threshold at the beginning was high and the neuron only showed a clear response when the strength of CRD reached 60–80 mmHg, as shown by the trace in A and by impulse counts as a function of CRD intensity in D. After CRD was repeated for 15 times, the neuron’s responses appeared to have been sensitized because its spontaneous activity increased dramatically, more than doubling (comparing the averaged background activity, 50 ± 6.04 vs. 110 ± 14.2 imp/20 s, when CRD intensity was 0 in Fig. 4D), the threshold for response to CRD was lowered (from 60 to 20 mmHg), and the response level was higher than that at the beginning. It was noted in our experiments that an increased background activity was often associated with an increased sensitivity to noxious visceral input (see Discussion). It was further noted that the sensitized neuron often showed sustained high activity after CRD had ceased (afterdischarge; Fig. 4B).
Effect of CRD on skin responses

If the short-lasting suppression of action potentials in response to visceral inputs were due to habituation, the same could be expected to occur whenever a vigorous excitatory response of a neuron occurred. However, when the conditioning procedure was inverted (skin vibration after CRD), a reduction in the response to skin vibration was never seen. In 19 cases tested in this arrangement, 8 neurons had their response to skin stimulation increased, with the remainder showing either inconsistent and small effect (4) or no change (7) in association with the preceding CRD. In the example shown in Fig. 5, the response to CRD was affected in particular during the first 10 s by the conditioning somatic stimulation (comparing A with B of Fig. 5), but the inverted procedure (CRD before skin stimulation) brought about an elevation rather than a reduction in response to skin pulses (Fig. 5C). Furthermore, the increased activity was most intense immediately following a vigorous response to CRD. This observation provides further support for the view that the suppressive effect of tactile stimulation on the CRD response is a genuine inhibition rather than habituation.

Responses to overlapping stimuli

In two experiments, when the standard conditioning procedures were completed, both somatic and visceral stimuli were delivered simultaneously in an overlapped manner. One example is shown in Fig. 6 where, when the excitatory somatic response overlapped with the inhibitory visceral one, the new response level, 84 (=255–171) was lower than that of the purely somatic net response (182 in C). However, the inhibitory effect associated with the skin conditioning stimulation appeared to be weaker, although a trough is still visible in the histogram in F, as the net response to CRD alone following the overlapped stimulation was $-11$ in contrast to $-74$ imp/20 s in E.

Figure 7 shows another example in which a conditioning effect on an inhibitory CRD response was complex. The response of the neuron to CRD was inhibitory, although the stimulus-response curve was nonlinear (A, top curve for control CRD response). In this case, the excitatory response to tactile stimulation did not systematically alter the inhibitory CRD response that followed as shown by comparing the bottom curve with the top one in A and the sample traces in B and D (D, net response $-48$ imp/20 s, in comparison with the control, $-61$ imp/20 s in B for CRD 60 mmHg). Instead the inhibitory response to CRD appeared to be potent enough to affect the neuron’s response to somatic stimuli when both stimuli overlapped, as shown in E. The net excitatory response to skin vibration was reduced practically to zero when the tactile and visceral stimuli overlapped as shown in E in comparison with C and D. It thus appears that the inhibitory response to CRD was a strong and dominant drive to this particular neuron. However, the inhibitory effect on the CRD response was somehow enhanced by the overlapping stimuli because the net response to CRD alone was $-158$ in E in comparison with that in D, $-48$ imp/20 s.

FIG. 5. Effects of tactile stimulation on the CRD response and vice versa. The neuron displayed an excitatory response to the CRD (A). It also had a localized skin receptive field on the heel of the contralateral foot, which was sensitive to a von Frey hair of 3.2 N (=170 mg; inset in A). B: when the excitatory skin response preceded the CRD, it affected the latter in particular during the early part of the CRD response. The net response to the 20-s CRD was reduced from 67 to 53 imp, representing a 20% reduction comparing B with A. The reduction in the first 10 s was most severe, with a net response of only 11 spikes (18 spikes in the first 10 s of CRD, minus 7 spikes in the last 10 s before skin stimulation). This suppression of the CRD response was not attributable to habituation of the neuron, as evidenced when the sequence of the stimuli were inverted, CRD before tactile stimulation where the CRD response did not prevent the skin response at all. Instead, the response to skin stimulation, in particular the early part, was enhanced by the preceding CRD (C).
Locations of the neurons in the thalamus

Of the nine brain specimens examined histologically, the 22 neurons that showed responses to and interactions between somatic and CRD stimuli were found to be located in the lateral part of the ventroposterior lateral (VPL) nucleus, consistent with previous findings (Al-Chaer 1996b). An example is shown in Fig. 8 in which two electrode tracks were identified. Three thalamic cells were recorded in these two penetrations. Neuron 1 in the lateral track had a skin receptive field on the proximal part of the tail (sensitive to 2.8 N von Frey hair). Neuron 2 in the more medial track in VPL had a large receptive field on the hip and proximal leg. Both neurons 1 and 2 also responded to convergent inputs from CRD. Neuron 3, also in the more medial track, could be activated by a 3.6 N von Frey hair applied on the foot but did not respond to CRD ≤ 60 mmHg. The depths of the cells shown in Fig. 8 are representative for neuronal locations in the thalamus in the current study, that is, between 5.5 and 7.0 mm (6.5 ± 0.48 (SD) mm) from the cortical surface. As neurons with convergent somatic and visceral inputs were mixed among the abundant cells that responded to light tactile stimuli alone, they appear to be located at the thalamic center to which the dorsal column-medial lemniscus projects as verified in the brain specimens examined and shown in previous studies as well (e.g., Al-Chaer et al. 1996b; Berkley et al. 1993). From our limited data, there was no clear tendency for inhibitory neurons to cluster or to be separate from those that had excitatory responses.

DISCUSSION

In the current study, we used controlled and reproducible tactile and visceral nociceptive stimulation to investigate somatovisceral interactions in the thalamus to enhance our understanding of central pain mechanisms. In essence, the tactile stimulation preceding CRD either caused a reduction in the thalamic neuronal activity (in the majority of cases) or had no convincing effect (in the remaining cases; Fig. 2). In no case did the tactile stimulation increase or exacerbate the CRD response. This was in contrast to B and E where there was no such a change when the background activity was low and when the tests were conducted earlier.

FIG. 6. Effect of somatic conditioning on inhibitory CRD responses. The neuron had a skin receptive field on the hind-paw that could be excited by a light von Frey hair of 2.8 N (D) and controlled mechanical pulse train (C; 10 Hz, 250 μm, superimposed on 0.5-mm step indentation). The response to CRD ≥ 40 mmHg was inhibitory, as shown by the sample traces in B, and plotted with △ in the top curve in stimulus-response relation in A (counting the net response levels for the 1st 10-s CRD response vs. 10-s background activity immediately before the stimulation). When the CRD was preceded by a skin pulse train in E, the net response to the visceral inputs was lower than that without the conditioning (A, bottom, ○). When the excitatory somatic response overlapped the inhibitory visceral one in F, the new response level, 84 imp/20 s (= 255–171) was lower than that of control excitatory skin response (182 in C and 91 imp/20 s in E) but higher than the inhibitory response to CRD alone in G (−77 imp/20 s). Furthermore, the impulse count for the after-stimulation period is 217/20 s in comparison with the background activity of 171 (representing 27% change). This is in contrast to B and E where there was no such a change when the background activity was low and when the tests were conducted earlier.
Methodological concerns

Although many studies have demonstrated somatovisceral interactions in the CNS (e.g., Chung et al. 1984; Gerhart et al. 1981), our study used innocuous vibrotactile pulses for the somatic stimulation and thus differed from most previous ones where inhibition was brought about by nocigenic stimuli. We have demonstrated that gentle manipulation of the skin surface may have a limited effect on thalamic neuronal responses to visceral nociceptive inputs. For tactile conditioning, we specifically chose mechanical vibrotactile stimulation because it can be quantitatively reproduced by feedback control, and no nociceptive inputs are elicited at the amplitudes used in this study. Although different frequencies were tested at the beginning of each trial, we found that 10-Hz mechanical pulses were usually the best for the generation of a maximal skin response. This is perhaps due to the fact that most if not all thalamic neurons receive convergent inputs from many types of cutaneous receptors (Leem et al. 1993a,b; Talbot et al. 1968; Zhang 1994; Zhang et al. 1996, 2001). Interestingly, several previous studies have also reported that the best pain-relieving effect was produced by low frequency vibration (Lundeberg et al. 1984; Ottoson et al. 1981). Electroacupuncture at 1–4 Hz, with or without small trains of 100-Hz stimuli, often produces the best pain relieving effect (Eriksson et al. 1979).

The current study used CRD to generate noxious visceral inputs. In comparison with chemical methods, such as injecting mustard oil into the colon, this method has the advantage of being more natural (mimicking lower bowel obstruction that may elicit pain), reversible and reproducible, which is crucial for an interaction study. As this method involves CRD, mechanical stretch of the skin may be a concern. However, there was no visible abdominal wall movement with CRD up to the maximum strength used (80 mmHg) unless the balloon was damaged. Furthermore,
as shown in Fig. 2, most cutaneous receptive fields observed were located on the tail, scrotum, leg, and foot, regions that are not near the abdominal area and therefore unlikely to be affected by any abdominal movement.

The inhibitory effect of conditioning stimulation of the skin on CRD responses observed in the current study is unlikely to be attributable to a systemic effect, such as by autonomic reflexes. First, in those four experiments where blood pressure was continuously monitored, the change in blood pressure, if any, in association with the CRD was small, <10 mmHg. Second, there was no blood pressure change in association with somatic stimulation, and yet the interaction effect on CRD response was most obvious immediately following tactile conditioning. As any blood pressure change due to reflex in association with nociceptive CRD only occurs later than the neuronal responses (see also Fig. 9 of Jänig 1993), the interaction effect is unlikely to be a reflex effect attributable to the activation of baroreceptors.

Mechanisms of somatovisceral and tactile-nociceptive interactions

Numerous studies have shown that viscerosomatic convergence appears to be the rule rather than an exception at both the spinal and supraspinal levels, as has been demonstrated by many electrophysiological studies (for review, see Ness and Gebhart 1990; Willis and Coggeshall 1991). In this study, the majority of central neurons responsive to colorectal inputs did have convergent inputs from the body surface, and almost all thalamic neurons that responded to colorectal distension had tactile receptive fields. This is consistent with previous reports in which the majority of somatovisceral sensitive neurons had low-threshold responses to skin inputs in monkeys (Al-Chaer et al. 1998; Brüggemann et al. 1994; Chandler et al. 1992) or in rats (56%) (Al-Chaer et al. 1996b; Berkley et al. 1993).

In this study, the effect of skin tactile conditioning stimulation on the CRD responses at the single thalamic neuron level is predominantly inhibitory (Figs. 1, 3, and 5–7). This observation is in general agreement with some of the previous electrophysiological studies in the dorsal horn of the rat (Ness and Gebhart 1990; Willis and Coggeshall 1991), the thalamus of the squirrel monkey (Brüggemann et al. 1998), and psychophysical observations that somatic stimulation reduced perception of gut distension in humans (Coffin et al. 1994). There were also reports that vibrotactile treatment may have a relieving effect for toothache and other orofacial pains (Ottoson et al. 1981) as well as acute and chronic musculoskeletal pain (Lundeberg et al. 1984).
The suppression of visceral responses observed in this study is unlikely to be attributable to habituation based on three arguments. First, the suppression to the same extent of spontaneous neuronal activity was not seen when skin stimulation was used alone (Figs. 1, 6, and 7) and only occurred when the CRD response followed. Second, when the conditioning procedure was inverted, CRD before the skin stimulation, the effect was often (8/19) opposite, that is, the response to the somatic stimulus that followed was enhanced (Fig. 5). Third, prolonged skin conditioning does not necessarily produce more effect, as shown in Fig. 3. If the reduction in CRD response was due to habituation, it ought to occur after any vigorous neuronal discharge, but this was not the case. Thus the suppressing effect of the skin stimulation on the CRD response appears to be a genuine inhibitory effect in which an inhibitory neuronal circuit is involved.

Excitatory tactile inputs from the body surface may activate inhibitory interneurons along the pathway to the thalamus, and they in turn may suppress the responses of target neurons to the visceral nociceptive inputs that followed. The locations in which the inhibitory interneurons reside may include the nucleus gracilis and/or the ventral posterolateral nucleus of the thalamus itself. Because it is reported that the rat VPL nucleus, unlike those of primates, lacks inhibitory GABAergic interneurons in the ventrobasal complex (for review, see Paxinos 1995), it is possible that the inhibition observed in this study takes place mainly at the levels below the thalamus, in particular the dorsal column nuclei (currently under investigation). Alternatively the skin manipulation may activate the endogenous pain control system by activating opiate receptors (Yang et al. 1999; for review see Han 1999); but because the inhibitory effect observed in this study was usually immediate and short lasting, this mechanism is less likely the explanation.

The current study also demonstrated that the inhibitory effect on the CRD response was generally weak and short lasting (Figs. 1, 3, and 5–7) and cannot be improved by prolonged tactile stimulation (Fig. 3). However, this is consistent with previous reports that the strongest inhibition from skin only occurs when the stimulation reaches noxious intensities (Chung et al. 1984; Gerhart et al. 1981). In monkeys, although some inhibition can be evoked by stimulation of large myelinated axons of a peripheral nerve, the inhibition is much more powerful if small myelinated or unmyelinated afferents are stimulated as well (Chung et al. 1984). In Selzer and Spencer’s study (1969b) in which electrical stimuli were used for interactions between visceral and cutaneous afferents in the spinal ventrolateral column, the inhibition of the visceral response did not appear until the conditioning cutaneous stimulus reached 1.8–2.0 times threshold, when Aδ fibers were recruited, and only continuous pinching (not brushing) of the skin over the lateral thigh strongly inhibited the visceral response. In transcutaneous electrical nerve stimulation (TENS), the stimulus intensity also needs to be high to generate the best inhibitory effect (e.g., Lee et al. 1985). Thus it appears that the nociceptive suppression may still largely depend on a counter-irritation mechanism rather than on the weak inhibitory effect generated by the light touch as shown in the present study.

The short-lasting nature of the inhibition, which is not prolonged by a long conditioning stimulus, is perhaps determined by the neuronal circuitry. Of interest is that at the cortical (SI) level, the inhibition by the preceding conditioning stimulation, either somatic or visceral, on the following test response was also reported to be short-lasting, <100 ms (Chernigovskii et al. 1978). At the spinal level, when electrical stimuli were used for interactions between visceral and cutaneous afferents to modify activity in the ventrolateral column, mutual inhibition was also short-lasting (<300 ms) as assessed by means of cord dorsal potentials and single-unit discharges (Selzer and Spencer 1969b).

**Influence of background neuronal activity**

Fluctuations in the background activity appear to reflect changes in the responsiveness of the neuron as often an increased ongoing activity is associated with a reduced threshold and increased response magnitude (Fig. 4) as well as the appearance of a prominent afterdischarge (Figs. 4 and 6). This finding is consistent with the reports in which background activity increased along with decreased threshold and increased response level in neurons sensitized by inflammation in the majority of units (e.g., Al-Chaer et al. 1996b; Schaible et al. 1987). In the case of colorectal nociception, inflammation of the colon with mustard oil could induce an increase in the background activity and facilitate the responses to CRD in central neuron recordings, including postsynaptic dorsal column and gracile neurons, as well as neurons in the VPL nucleus of the thalamus (Al-Chaer et al. 1996a–c, 1997b). To overcome the problem of changing responsiveness of the neurons in association with the background change, we always subtracted the background activity from the response impulse counts to derive the “net response” to compare the effects of different trials, not withstanding that the response change itself may not necessarily be in proportion to the changes in the background activity.

**Skin receptive fields of visceral nociceptive neurons**

The current study has shown that the skin areas that may interact with colorectal nociception at the thalamic level are located in the caudal part of the body including the tail, leg and foot, and perineal and hip regions. In terms of the spinal segmental arrangement, they are within the lumbar and sacral dermatores (Takahashi et al. 1994), the same segments that innervate the hindgut (L2–S3). Despite having generally smaller receptive fields on the extremities than those on the abdominal wall, there was no clear correlation between the type of interactions, whether excitatory or inhibitory, and the skin receptive fields from our limited data. Whether the skin spots, in particular the tender points, where the interactions take place are related to acupoints and the meridians of traditional Chinese medicine remains to be investigated further, preferably in primates.

The authors thank G. Gonzales and P. S. Chen for technical assistance. This work was supported by Hong Kong CERG Grant HKBU2093/01M, University of New South Wales Faculty of Medicine postdoctoral fellowship.
to H. Q. Zhang and National Institute of Neurological Disorders and Stroke Grants NS-11255 and NS-09743.

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