Short communication

Viscero-somatic neurons in the primary somatosensory cortex (SI) of the squirrel monkey

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Abstract

Thirty-eight neurons in the primary somatosensory cortex (SI) in α-chloralose/Nembutal, or halothane (in N_2O/O_2) anesthetized squirrel monkeys were tested for responses to distention of the urinary bladder, the distal colon and the lower esophagus. Of the 38 SI neurons studied 13 were classified as visceroceptive. Eight of the 13 visceroceptive neurons responded to stimulation of a single viscus, the other five responded to two viscera. All SI neurons investigated had somatic low threshold type responses. Anesthesia was a critical factor, because 6 of 11 neurons responded to visceral stimulation only under a light halothane anesthetic level, and during moderate halothane anesthesia levels significantly more neurons exhibited visceral inputs than under α-chloralose/Nembutal. The results suggest that the squirrel monkey SI is involved in processing of visceral information.

Keywords: Electrophysiology; Pain; Nociception; Modulation; Visceral sensation; Cortex

A surprisingly high incidence of somato-visceral and viscero-visceral convergence was demonstrated for lateral thalamic neurons of the squirrel monkey, especially in the ventral posterolateral nucleus (VPL) [5]. In the lateral thalamus, 85% of the neurons with somatosensory inputs were found to receive convergent visceral inputs. In contrast to the well-known somatotopy in VPL no viscerotopy was observed. These findings are in keeping with data reported in the rat. In this species neurons located in the gracile nucleus [11] and VPL [4] have convergent inputs from the reproductive organs and the colon onto somatic low threshold type neurons. These results demonstrate that viscero-visceral and viscero-somatic information is conveyed via the dorsal columns to VPL of the rat. Thus, it can be concluded that SI should also have visceral inputs. That different parts of the monkey cortex, including SI, may be involved in autonomic function was shown in the 1950s by Wall and Davis [14], Amassian [1] and in studies performed in the former Soviet Union [7]. Cortical representation of visceral inputs has been abandoned since these early studies. Recently, the existence of individual viscero-ceptive neurons in the rat somatosensory cortex was demonstrated by Follett and Dirks [9].

The aim of the present investigation was to examine the viscero-visceral and viscero-somatic responses of SI neurons in the squirrel monkey to confirm the transfer of visceral information from the somatosensory thalamus to SI. We hypothesized that the somatic and visceral response properties of SI neurons would parallel those of VPL neurons.

The experiments were carried out in three female adult squirrel monkeys. Housing, care and surgical procedures followed the institutional guidelines established by the local Committee for the Humane Use of Animals. Animals were premedicated with atropine (0.5 mg/kg, s.c.), antibiotics (Rocephin 75 mg/kg, i.m.), and dexamethasone (0.25 mg/kg, i.v., twice daily). Anesthesia was induced with ketamine (30 mg/kg, i.m.) and maintained either by intravenous injections of 70 mg/kg α-chloralose followed by smaller additional doses, combined with Nembutal drip (up to 5 mg/kg/h, one experiment), or with halothane (0.5–1% in O_2/NO_2; two experiments; 1.3% during surgical procedures). The anesthetic level was defined as moderate, when either α-chloralose/Nembutal anesthesia was employed, or the halothane concentration was $\geq 0.5\%$. Anesthesia was defined as light, when the applied halothane

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concentration was ≤ 0.3%. All neurons were tested under a moderate level of anesthesia. In addition, some neurons were investigated when the halothane application was interrupted before and during visceral stimulation, to test for anesthesia effects. The depth of anesthesia, however, was always sufficient to suppress withdrawal reflexes when the interdigital skin was pinched.

The right femoral vein and artery were cannulated for drug and fluid administration and blood pressure measurements. The trachea was cannulated to avoid respiratory obstruction due to esophagus catheterization. The lower esophagus and the distal colon were catheterized with double-barreled balloon catheters. Through one of the ports the pressure stimuli were applied and through the second port the intraluminal pressure was measured. The balloons (made from latex condoms) were distendable to a diameter of about 6 cm with minimal increase in intraluminal pressure, so that the pressures measured were due to the intramural tension of the stimulated viscera. The urinary bladder was catheterized via the urethra with a double-lumen catheter without a balloon and stimulated directly. The animal's head was positioned in a stereotaxic holder and a small craniotomy was performed over one hemisphere to access SI. After removal of the dura mater a high resolution polaroid photograph of the region around the central dimple was taken to mark the recording sites.

All animals breathed spontaneously. End-tidal CO_2 concentration and mean arterial blood pressure were continuously measured and were between 3.5% and 5.5%, and 100–150 mmHg, respectively. Body temperature was monitored and maintained around 38°C with a feedback controlled heating pad.
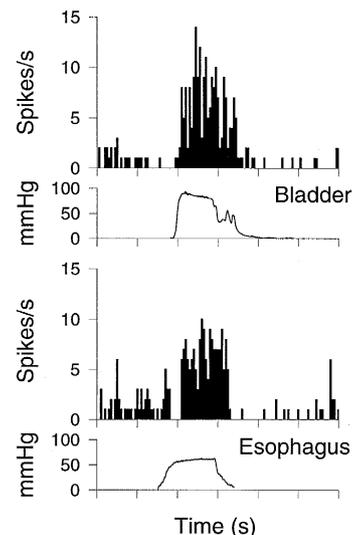
Tungsten microelectrodes were used to explore the

Fig. 1. Peristimulus–time histograms of the responses of an SI neuron to distention of the urinary bladder (upper panel), and distention of the esophagus (lower panel). The lower trace under each histogram represents intraluminal pressure in each viscus.

rostral parietal cortex to study SI. Isolated single neuronal action potentials were displayed on an oscilloscope, window discriminated, and fed into a computer to generate peristimulus time histograms (PSTHs; bin width: 1 s). Intraluminal pressures were generally increased to 60 mmHg (range 50–85 mmHg) over a 30–60 s time period. In addition, neurons were tested with innocuous and noxious mechanical stimuli applied to the skin. Interstimulus intervals between somatic noxious and visceral stimuli, respectively, were at least 5 min.

All data were stored on tape and analyzed on- and off-line. Neuronal discharge rates before and during stimulation were compared. The mean change in activity was considered a response when the unit activity increased or decreased and this change was greater than 30% from baseline, and statistically significant (t-test, $P < 0.05$). To be classified as visceroceptive, neurons had to respond at least twice to at least one of the three visceral organs.

Of 38 neurons tested in SI, all had low threshold type somatic receptive fields, and in addition 13 responded to distention of the urinary bladder, the lower esophagus and/or the distal colon, and were classified as somato-visceroceptive. The visceral responses were increases (+; $n = 4$), decreases (−; $n = 7$), or changes in neuronal activity in both directions, i.e., excitation followed by inhibition or vice versa (±; $n = 7$). With bladder distention 3 of 38 SI neurons were inhibited, and one was excited, with colon distention 3 of 37 neurons were inhibited, one was excited, and one showed a mixed response, and with esophagus distention 1 of 32 neurons was inhibited, two were excited, and six had mixed responses. Eight of the 13 visceroceptive neurons responded to stimulation of only one viscus, and five responded to two viscera (for details see Table 1).

Table 1
Viscero-visceral and somato-visceral convergent response types of SI neurons in the squirrel monkey

Cell no.	Bladder			Colon			Esophagus			Som. RF
	+	−	±	+	−	±	+	−	±	
1		X								hand, deep
2				X						hand, hair
3					X					hand, hair
4							X			pelvis, touch
5							X			pelvis, touch
6								X		hand, deep
7									X	hand, hair
8									X	hand, hair
9	X								X	hand, touch
10		X		X						hand, deep
11		X							X	hand, touch
12					X				X	hand, hair
13						X			X	hand, hair

The stimuli were distention of the urinary bladder, distal colon and lower esophagus, and innocuous and noxious stimulation of somatic structures. All somatic responses were low threshold type (+, excitation; −, inhibition; ±, mixed responses; Som. RF, location and type of the somatic receptive fields).

Fig. 1 shows the visceral responses of a visceroceptive SI neuron with a somatic receptive field on digits 2–4 of the contralateral hand. The neuron was somatic low threshold type. Under moderate gas anesthesia, bladder stimulation did not drive the unit, but under light anesthesia the unit increased its activity with bladder distention (upper panel). The unit's responses to the first two esophagus distentions were excitatory (see example in Fig. 1, lower panel). The following three esophagus distentions, however, reduced the activity of the unit to zero spikes/s. Excitatory and inhibitory responses to esophagus distention were seen under both light and moderate gas anesthesia, thus the level of anesthesia had only an effect on the bladder inputs, but apparently not on the esophagus inputs.

Under α-chloralose/Nembutal anesthesia 2 of 20 cortical neurons were visceroceptive, while under halothane anesthesia 11 of 18 were visceroceptive, which is statistically significantly different (Fisher's exact test, $P \leq 0.025$). Of 11 neurons tested under different levels of halothane anesthesia, six exhibited dependence on depth of anesthesia: 2 of 4 neurons with bladder inputs, 3 of 5 neurons with colon inputs, and 4 of 6 with esophagus inputs responded to visceral stimulation only under light anesthetic conditions. Taking only the level of anesthesia into account, 2 of 20 neurons tested under moderate chloralose/Nembutal anesthesia exhibited visceral responses, while 5 of 18 did so under moderate gas anesthesia. This is statistically different (Fishers Exact test, $P < 0.04$). These data indicate that kind and depth of anesthesia critically influence the visceral responsiveness of cortical neurons. Simplified stimulus response curves for excitatory and inhibitory esophagus responses are shown in Fig. 2. Similar responses were observed for colon and bladder distentions (not shown). The magnitudes of the visceral responses were comparable to the somatic responses.

The 13 visceroceptive neurons were found in SI intermingled with the non-visceroceptive neurons. All visceroceptive neurons had somatic low threshold type responses (for details see Table 1). Although the numbers are small, it is striking that for the visceroceptive neurons the somatic receptive fields were located on the hand (and not the leg; 11 vs. 0), while those of non-visceroceptive neurons were more often located on the leg (4 vs. 12).
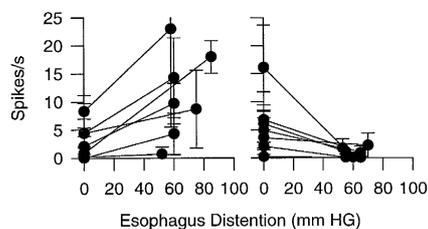

Fig. 2. Simplified stimulus–response curves for visceroceptive neurons in SI are shown. For each neuron the largest changes in firing rate from ongoing activity are illustrated due to distention of the esophagus. Variability in firing rate is indicated by the standard deviations. The left panels show excitatory, and right panels inhibitory, responses.
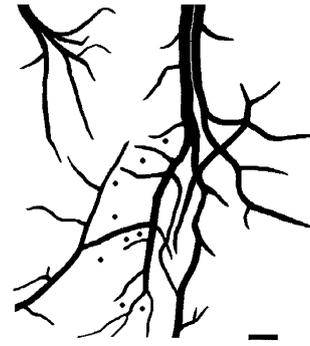

Fig. 3. Recording sites where visceral responsive neurons were found in the hand SI region in one squirrel monkey. The line drawing shows the cortical surface around the central fissure, dorsal is up, and posterior is left. The two vertical vessels run within the fissure and approximate the border between the somatosensory and motor cortices. The dots mark penetrations where visceral inputs were found. Scale bar = 1 mm.

Like the visceroceptive neurons, the non-visceroceptive neurons ($n = 25$) had small, well-defined and somatotopically arranged receptive fields on the skin of the lower extremities (13), upper extremities (10), and thorax (2). These neurons responded to hair movement (7), deep structures (7), touch (6), and to hair and touch (5) stimuli.

The visceroceptive neurons were located in a depth ranging from 255 to 984 μm (mean $\pm$ S.D.; 540 $\pm$ 245 μm; $n = 13$), while the non-visceroceptive neurons were located deeper: from 111 to 1648 μm (878 $\pm$ 496; $n = 25$). The cortical depth difference between visceroceptive and non-visceroceptive neurons was statistically significant (Mann–Whitney Rank Sum test; $P < 0.04$).

In Fig. 3 the cortical surface of the region surrounding the central fissure (dimple) of one of the animals is shown. The nine marks (of 16 examined) indicate the sites where visceroceptive neurons were found, in the hand representation region of SI. Similar and more superior (corresponding to the hindlimb and foot representation areas), regions were explored in this animal and the other two monkeys. Although the locations of the cortical neurons were not verified histologically, the majority of the neurons examined were thought to be in areas 3b and 1 since most had small receptive fields on the skin and responded to touch or hair displacement [10,13].

The results demonstrate that neurons with somato-visceral and viscero-visceral convergent inputs exist in the squirrel monkey SI. The SI visceral responses were all nociceptive and converged on neurons with somatic low threshold type responses.

There are only a few observations regarding the single unit visceral responses of SI neurons. The older literature has examined the involvement of the cortex in visceroception by evoked potentials, or by monitoring autonomic responses to electrical stimulation of the cortical mantle. In spite of the technical limitations, these early studies repeatedly show that the somatosensory cortex receives visceral inputs, for a review see Chapters 8 and 9 of Chernigovskiy [7]. More recently, Follett and Dirks [9] demonstrated

colorectal distention responsive neurons in SI in pentobarbitone anesthetized rats, and Chandler et al. [6] showed that thalamic visceroceptive neurons can be antidromically activated from SI in the macaque monkey.

There seems to be a large difference in the incidence of neurons responding to visceral stimuli between the lateral thalamus and SI (89% vs. 34%) in the squirrel monkey. This difference in incidence may be primarily due to different effects of anesthesia on the two regions, especially because approximately 50% of the cortical responses occurred only under light levels of anesthesia. That anesthesia is a critical factor which influences cortical responsivity is also illustrated by the fact that most visceral responses were seen under halothane, and not under α-chloralose/Nembutal anesthesia. Thus it is possible that in an awake animal the incidence and coding properties of cortical neurons would more closely resemble those observed in VPL.

Based on our interpretation of the studies examining SI nociceptive cells in the awake or anesthetized macaque [8,12], the incidence of somatic nociceptive cells in macaque SI is approximated to be in the range of 0.1–1%. Given the incidence of nociceptive cells in squirrel monkey VPL [2], the maximum incidence of SI somatic nociceptive cells in this primate can be around 10%. Compared to these incidences (0.1–10%), the incidence of visceral nociceptive neurons in SI (34%) is very high. It is generally accepted that SI plays an important role in the discriminatory aspects of somatic nociception [8,12], and recent brain imaging studies in humans have implied the involvement of the region in somatic pain perception [3]. The higher incidence of visceral nociceptive neurons in SI, as compared to somatic nociceptive neurons, forces the conclusion that SI must be involved in processing visceral nociceptive information.

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