Morphology and Response Properties of Nociceptive Neurons in the Primary Somatosensory Cortex in Cats

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\begin{abstract}
We recorded 11 WDR neurons from the primary somatosensory cortex in cats. Six WDR neurons were located in lamina III, four in lamina IV, and one in lamina V of area 3b. We identified two types of lamina III WDR neurons: stellate cells with oval somata, many dendrites and axon collaterals and the other neurons were typical pyramidal cells with pyramidal somata, thick apical dendrites and many spines. On the other hand, all lamina IV WDR neurons had small oval somata with many dendrites and axon collaterals. Lamina III WDR neurons had smaller somata and a smaller number of the basal dendrites than lamina IV WDR neurons. The one example of a lamina V WDR neuron recorded in our study had a larger soma, apical dendrites, and a larger number of spines than neurons in other laminae.

\textbf{Key words}: SI, nociceptive neuron, intracellular recording, morphology, cat
\end{abstract}

\section*{INTRODUCTION}
It has been reported that many neurons in the primary somatosensory cortex (SI) receive low-threshold inputs from various body regions including the orofacial skin and mucous membrane\textsuperscript{3,4,12,13,17,20,22}. The cutaneous inputs are represented somatotopically in the SI. Some of these neurons also respond to noxious mechanical or thermal stimulation of the facial skin or
intraoral mucous membrane\textsuperscript{1,2,6,8,9,14--16,23}. Such neurons have been classified into two different types: WDR (wide dynamic range) neurons; showing increasing spike discharges following an increase in mechanical stimulus intensity and NS (noxious-specific) neurons; responding exclusively to noxious mechanical stimulation. Recently we reported the morphology of SI neurons receiving tooth pulp inputs, which have minor morphological differences in comparison with low-threshold mechanoreceptive neurons\textsuperscript{10}. The findings obtained support the idea that studies of the nociceptive SI neurons responsive to noxious stimulation of the skin or oral mucosa would yield more detailed information on the morphological characteristics of nociceptive neurons in the SI. Therefore, we focused on neurons responsive to noxious stimulation of the facial skin and intraoral mucous membrane, and attempted to clarify their morphology.

\textbf{MATERIALS AND METHODS}

Experiments were performed on 28 young adult cats weighing 3.0–3.5 kg. The animals were initially anesthetized with ketamine–HCl (50 mg•kg\textsuperscript{-1}). During surgery, the anesthesia was maintained with a mixture of halothane and oxygen. Gas anesthesia was discontinued after surgery, and the depth of anesthesia was maintained throughout the experiment with \(\alpha\)-chloralose (60 mg•kg\textsuperscript{-1}). During recording sessions, the animals were immobilized with pancuronium bromide (1 mg•kg\textsuperscript{-1}•h\textsuperscript{-1}, iv) and ventilated artificially. Expired CO\textsubscript{2} concentration was monitored and maintained at a level between 3.0\% and 4.0\%. Rectal temperature was maintained between 37 °C and 39 °C by a thermostatically controlled heating pad.

A craniotomy was performed to expose the cerebral cortex over the coronal gyrus. Then glass micropipette electrodes filled with neurobiotin (Vector Lab.) solution with 0.5 M KCl in PBS (pH 8.0) for intracellular recording were advanced through the cortex, and cellular activity was evoked by touching the facial skin or various intraoral structures. Each cell was tested for its response to mechanical and thermal stimuli in the receptive fields. When a neuronal discharge in response to innocuous mechanical stimulation of the receptive fields was obtained, neurons were tested for their responsiveness to thermal stimulation of their receptive fields. A thermal probe was placed in the area of the receptive field most sensitive to innocuous mechanical stimulation. Mechanical stimuli included touching the receptive field with brushes (touch), application of a glass rod (pressure) and application of an arterial clip (pinch). Thermal stimuli consisted of temperatures ranging between 40 °C and 58 °C for each stimulation (duration: 10 to 30 s).

When intracellular potentials were recorded, neuronal activities were fed into a tape recorder (bandwidth DC to 20 KHz) for subsequent analysis of signals. After identification of nociceptive neurons, intracellular injection was done through the neurobiotin-filled electrodes with a depolarizing pulse (3–5 nA, 300 ms duration, 2 cycle/s). After injection, the animals were allowed to survive for 6–10 h and then deeply anesthetized with pentobarbital sodium. They were then perfused transcardially with 500 ml of phosphate-buffered saline (PBS; pH 7.4) followed by 4\% paraformaldehyde in 0.1 M phosphate buffer. The brain was removed and placed in cold fixative for 4 days and then transferred to cold phosphate-buffered 30\% sucrose for 48 h. Serial sections 50 \(\mu\)m thick were cut along the path of electrode penetration. The sections were incubated in peroxidase-conjugated avidin-biotin complex (1 : 100; ABC: Vector Labs.). We used 3,3\'-diaminobenzidine–tetra HCl (DAB: Sigma), nickel ammonium sulfate (0.2 g/100 ml) and 30\% hydrogen peroxide (30 \(\mu\)/
100 m/l) in Tris-buffered saline (0.05 M, pH 7.4) to develop the ABC reaction product, producing a distinctive black chromogen. Every section was counterstained with thionin.

Precise camera lucida tracings of the stained neurons were drawn at ×400 magnification with a camera lucida drawing tube.

RESULTS

A total of 11 nociceptive neurons were recorded and injected from the primary somatosensory cortex, as shown in Table 1. All of them were classified as WDR neurons, and located in laminae III–V of area 3b. In the present study, we found no nociceptive-specific neurons from the SI. Sample intracellular recordings of WDR neurons responsive to mechanical stimulation of the facial skin are illustrated in Figs. 1A and 2A (a to c). These WDR neurons showed a graded response to an increase of stimulus intensity. In this study, we classified WDR neurons on the basis of their response properties following mechanical stimulation of the receptive field.

Six WDR neurons were located in lamina III, 4 in lamina IV and 1 in lamina V of area 3b, according to the cytoarchitectonic criteria of Hassler and Muhn–Clement (1964)⁶. Two types of lamina III WDR neurons were identified in this study: stellate cells, having oval somata with many dendrites and axon collaterals and typical pyramidal cells with pyramidal somata and thick apical dendrites with many spines. Figure 1 show a sample reconstruction of a typical stellate WDR neuron in lamina III, which had a round soma and spiny dendrites (Fig. 1Ba and b). We did not find axons going deep into the subcortical regions, and some axon collaterals ran through lamina III. However, these collaterals did not extend far from the soma. An interesting morphological characteristic of WDR neurons was the distribution pattern of their dendrites. As shown in Fig. 1B, the dendrites of the lamina III WDR neurons were distributed mainly around the area of the soma, and a small number of apical dendrites extended up to the superficial laminae. On the other hand, lamina III pyramidal WDR neurons had typical pyramidal somata in general and thick apical dendrites with many spines (not shown in this paper).

Table 1 Incidence of WDR neurons with skin and mucosal inputs

<table>
<thead>
<tr>
<th>Laminae</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>WDR</td>
<td>6</td>
<td>4</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
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</table>

Figure 2 shows a sample response and reconstruction of a typical lamina IV WDR neuron. This neuron showed an increase in firing frequency following graded mechanical stimulation of the facial skin (Fig. 2A). Generally, the lamina IV WDR neurons had bipolar somata with dendrites spreading around the soma and axons running deep into the white matter, as illustrated in Fig. 2B. Lamina IV WDR neurons were similar morphologically to LTM neurons, as reported in previous studies⁶,¹⁰,¹¹,²⁶. Basically, lamina IV WDR neurons had dendrites running around the soma, expanding to only a limited extent like those of lamina III WDR neurons and having many varicosities on their dendritic trees. In the present study, we successfully recorded and injected only one WDR neuron from lamina V. This lamina V WDR neuron had morphological characteristics similar to those of lamina V pyramidal neurons reported previously⁶,²³), having a large soma and thick apical dendrites with many spines. The detailed morphological properties of WDR neurons are given in Table 2. Lamina III WDR neurons (mean±SEM, 153.9±37.5, n=6) had smaller somata and a smaller number of basal dendrites (2.6±0.5, n=6) than those of lamina IV WDR.
**Fig. 1** Sample intracellular recording and reconstruction of a lamina III WDR neuron from the SI. The receptive field of this neuron was located in the costralateral upper lip. a: responses to gentle brushing of the receptive fields, b: responses to pressure stimulation of the receptive fields, c: responses to pinching of the receptive fields.

**Fig. 2** Sample intracellular recording and reconstruction of a lamina IV WDR neuron from the SI. The receptive field of this neuron was located in the ipsilateral lower lip. a: responses to gentle brushing of the receptive fields, b: responses to pressure stimulation of the receptive fields, c: responses to pinching of the receptive fields.
Table 2  Incidence of WDR neurons in the SI

<table>
<thead>
<tr>
<th>Laminae</th>
<th>Area of somata ($\mu m^2$)</th>
<th>Diameter of apical dendrites ($\mu m$)</th>
<th>Number of spines (/50 $\mu m$ of apical dendrites)</th>
<th>Number of basal dendrites</th>
</tr>
</thead>
<tbody>
<tr>
<td>III</td>
<td>153.9±37.5</td>
<td>2.8±0.9</td>
<td>5.3±5.3</td>
<td>2.6±0.5</td>
</tr>
<tr>
<td>(n = 5)</td>
<td></td>
<td>(n = 5)</td>
<td>(n = 5)</td>
<td>(n = 5)</td>
</tr>
<tr>
<td>IV</td>
<td>227.4±14.5</td>
<td>2.9±0.2</td>
<td>0</td>
<td>6.5±1.3</td>
</tr>
<tr>
<td>(n = 4)</td>
<td></td>
<td>(n = 4)</td>
<td>(n = 4)</td>
<td>(n = 4)</td>
</tr>
<tr>
<td>V</td>
<td>599.7</td>
<td>3.5</td>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td>(n = 1)</td>
<td></td>
<td>(n = 1)</td>
<td>(n = 1)</td>
<td>(n = 1)</td>
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neurons (area of somata: 227.4±14.5, n=4, number of basal dendrites: 2.6±0.5, n=5). Lamina IV WDR neurons had small, oval somata, many dendrites and axon collaterals. Lamina V WDR neurons, of which only one example was recorded in the present study, had larger somata, apical dendrites and more spines than those in other laminae.

DISCUSSION

It has been demonstrated that somatosensory inputs from the body and limbs can be represented somatotopically in the primary somatosensory cortex (SI)\textsuperscript{3,4,12,13}. Many neurons in the SI also receive inputs from the trigeminal system\textsuperscript{1,2,17,20,22} and some receive nociceptive inputs from the skin and oral mucosa\textsuperscript{7-10,14,23}. According to cytoarchitectonic criteria, nociceptive neurons are distributed in areas 1, 2 and 3b of the SI\textsuperscript{7-10,14-16}. Although a huge amount of information about nociceptive SI neurons has been obtained in previous studies using the extracellular recording technique, the morphological characteristics of nociceptive neurons in the SI have not been described. In the present study, we determined the detailed morphology of nociceptive neurons in the orofacial area in the SI and considered the functional and morphological correlation of nociceptive neurons in this area.

In this study we used neurobiotin as an intracellular staining tracer. Labeling with neurobiotin was considered to be satisfactory when the neuronal somata and dendrites were densely stained up to the thinnest, most distant, dendritic tips. For pyramidal nociceptive neurons, which had a triangular somatic profile, spines were seen clearly along even the fine dendritic branches, and the apical dendrites were traceable up to lamina I; for non–pyramidal nociceptive neurons, which had a round somatic profile, the apical dendritic tufts were well filled. Only nociceptive neurons for which intracellular recordings were obtained and were stained were included in the descriptions of morphology.

More than half of the nociceptive neurons we investigated were located in lamina III. It has been reported that in extracellular recording studies, the majority of nociceptive neurons in the SI were distributed in lamina III of areas 1, 2 and 3b\textsuperscript{7-10,14-16}. Cytoarchitectonically, lamina III is thicker than the other laminae and its cells are more densely packed\textsuperscript{23}. Thus the chance of recording nociceptive neuron discharges in lamina III is higher.

It has been reported that lamina III pyramidal neurons have many spines on their apical dendrites and many axon collaterals spreading into laminae III, IV, V and VI\textsuperscript{5,21,26,27}. However, the post–synaptic targets of the boutons of these collaterals are not known. Winfield (1981)\textsuperscript{24}, using electron microscopy found that in the monkey somatosensory cortex, half of the synap-
ses which they analyzed in lamina III were located on the shafts of dendrites of pyramidal neurons, whereas the other half were located on the spines. This suggests that lamina III nociceptive neurons could mediate recurrent excitation and inhibition of other pyramidal neurons.

Previous anatomical studies have revealed that thalamo-cortical projection fibers are more tightly connected in laminae III and IV neurons in the somatosensory cortex than those of cells in other laminae, and a study of the relationship between the laminar distribution of neurons and latencies of the peripheral and thalamic EPSPs of the pyramidal and non-pyramidal neurons in the SI, received that many thalamic EPSPs in laminae III and IV show monosynaptic contact between thalamic axon terminals and SI neurons. These observations suggest that many laminae III and IV non-pyramidal nociceptive neurons receive direct inputs from thalamic relay neurons. Furthermore, non-pyramidal neurons in the SI have been shown to be local circuit neurons, which send their information to adjacent neurons in the same area. These morphological characterizations of laminae III and IV SI neurons support the idea that non-pyramidal laminae III and IV nociceptive neurons receive inputs from the orofacial skin and mucous membrane, and send them to adjacent neurons in the same cortical area. These findings suggest that laminae III and IV WDR neurons receive nociceptive inputs via thalamic relay neurons and relay the nociceptive sensory information to the vicinity of the soma.

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