Intrinsic Membrane Characteristics Distinguish Two Subsets of Nociceptive Modulatory Neurons in Rat RVM

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Zagon, Aniko, Xianwei Meng, and Howard L. Fields. Intrinsic membrane characteristics distinguish two subsets of nociceptive modulatory neurons in rat RVM. J. Neurophysiol. 78: 2848–2858, 1997. Pain modulating neurons of the rostral ventromedial medulla (RVM) include three physiologically distinct classes of neurons in intact, anesthetized animals: ON and OFF cells that change their activity before the onset of withdrawal reflexes and NEUTRAL cells, which have activity unrelated to withdrawal reflexes. A previous in vitro intracellular study demonstrated that the RVM contains two types of neurons that are distinguished by their action-potential characteristics. The present in vivo intracellular study examined whether these intracellularly recorded action-potential correlates are related with the physiological response properties of RVM neurons recorded. RVM neurons exhibited two distinct types of action potentials in vivo. Fast-spike (FS) neurons (n = 30) had short-duration action potentials (0.27 ± 0.02 (SE) ms at half amplitude) and biphasic afterhyperpolarizations with a characteristic rapid overshooting spike repolarization. Slow-spike (SS) neurons (n = 25) had longer duration action potentials (0.44 ± 0.02 ms at half-amplitude) due to a slower-spike repolarization rate and monophasic afterhyperpolarization. ON and OFF cell classes included both FS and SS neurons. FS ON and OFF neurons had an early onset response to noxious heat stimulation. SS ON and OFF cells showed a delayed onset response to noxious heat. NEUTRAL cells (n = 13) were all SS cells. Among the SS neurons, only NEUTRAL cells had action potentials longer than 0.45 ms (n = 9). FS and SS neurons were intermingled throughout the RVM. The majority of intracellularly labeled cells (n = 15) had fusiform somata with two to five fine caliber primary dendrites and a predominantly mediolateral orientation of the long axis of their dendritic tree. All labeled FS ON cells (n = 5) had large, multipolar somata with four to nine large caliber primary dendrites. The present study defines in vivo membrane and morphological characteristics of RVM neurons that correlate with physiological differences and may be used for identification of nociceptive modulatory RVM neurons in slice preparations.

INTRODUCTION

There are several nociceptive modulatory networks with major connections in the brain stem. An important element of one of these networks is the rostral ventromedial medulla (RVM) oblongata, which comprises the nucleus raphe magnus and part of the adjacent reticular formation (Basbaum and Fields 1984; Besson and Oliveras 1980; Fields and Basbaum 1978; Fields et al. 1991). Electrical or chemical stimulation within the RVM diminishes behavioral responsiveness to noxious stimuli in both awake and anesthetized animals (Fields et al. 1977; Jensen and Yaksh 1984; Oliveras et al. 1975; Proistoff and Anderson 1975; Zorman et al. 1981). This antinociception is the result of the activation of an inhibitory projection to neurons of laminae I, II, and V of the spinal dorsal horn which receive inputs from primary afferent nociceptors (Fields et al. 1977; Light and Kavookjian 1985; Willis 1985). The RVM can also exert a facilitatory effect on withdrawal reflexes (Gray and Dostrovsky 1983; Kaplan and Fields 1991; Mokha et al. 1985; Zhou and Geppert 1992). This bidirectional control of nociceptive transmission is due to the heterogeneity of RVM neurons with regard to their pain modulatory actions (Fields et al. 1991). One class of neurons, the OFF cells, contributes to the inhibitory effect. OFF cells pause before the occurrence of reflex withdrawal from noxious heat and this pause is postulated to permit the occurrence of the reflex (Barbaro et al. 1986; Fields et al. 1983a; Vanegas et al. 1984). A second class of RVM neurons, ON cells, shows a sudden increase in firing that precedes the onset of withdrawal reflexes in response to noxious heat, suggesting that ON cells facilitate nocifensive responses. In addition to OFF and ON cells there are cells that show no change in their firing characteristics during noxious heat stimulation (NEUTRAL cells). The ON, OFF, and NEUTRAL cells are intermingled throughout the RVM. This lack of anatomic segregation of functionally distinct neuronal classes within RVM demands that single unit analysis be used to characterize their pharmacological, histochemical, and physiological properties in vivo.

In recent years, differential responses of ON and OFF cells to putative neurotransmitters, such as opioids, γ-aminobutyric acid (GABA) and norepinephrine, have been demonstrated by using a combination of extracellular unit activity recording, microinjection, and iontophoresis (Fields et al. 1991; Heinricher et al. 1991, 1992, 1994). Differential effects of these putative transmitters on RVM neurons have also been demonstrated in vitro slice preparations (Pan et al. 1990, 1993), where they are correlated with intrinsic membrane characteristics of the cells. In vitro, two classes of cells were distinguished that showed different membrane characteristics and pharmacology. Since the functional classification of RVM cells developed for the in vitro preparations cannot be used in vitro and because there are only two defined in vitro classes compared with at least three in vivo, the correlation of in vitro pharmacological results to specific in vivo classes is unclear. The aim of the present study was to examine whether differences in intrinsic membrane characteristics of RVM neurons correlate with their functional properties. Intrinsic membrane characteristics, distinctive of functionally identified cell classes in vivo, would not only help to elucidate the function of these neurons in operational circuits but would also enable their identification in vitro.
Data acquisition and analysis

Intracellular recordings were made with single barrel glass microelectrodes that were filled with 1.5-M potassium methyl-sulphate and 2% biocytin (R = 40–65 MΩ). The responses to TF tests were assessed in each recorded cell in repeated (at least 3) trials. Only those cells that showed consistent ON, OFF, or NEUTRAL type response to noxious heat stimuli were included in the present study. An intracellular amplifier with an active bridge circuit (Axoclamp 2A, Axon Instruments) was used to record membrane potential and to inject current. The data were stored on magnetic tape (Vetter Instruments) together with the tail temperature and integrated EMG signals. The membrane potential, integrated EMG, and tail temperature data were digitized off-line with the BrainWave data acquisition system (sampling rates: 11 kHz, 100 Hz, and 10 Hz, respectively; BrainWave Systems). Measurements in each selected cell were made automatically from every single action potential with BrainWave Workbench software. The numerical data were loaded into the Quattro statistical package (Borland International) for calculating averages. For each neuron, average action-potential parameters were calculated by using 90% of the spikes. The 10% of spikes with the smallest amplitude were discarded to minimize the error originating from transient instabilities in the recording. The measurements included baseline membrane potential, the maximum rates of spike de- and repolarization, spike peak amplitude, amplitude of afterhyperpolarizations, and their delay from spike peak. Statistical differences between numerical data were determined by using the two-tailed Mann-Whitney U test. The data are expressed as means ± SE. The amplitude of the spikes and afterhyperpolarization were measured from baseline to peak potential. The spike duration was measured at half-amplitude in averages of 12 spikes. Firing threshold was defined as the membrane potential where the fast depolarization was initiated from the preceding EPSP. The current-voltage relationship was determined by using hyperpolarizing constant-current pulses (0.2–1.2 nA) and input resistance was calculated from the linear part of the graph.

Histological procedures

Once a cell was electrophysiologically identified, it was injected with biocytin (−1.2 to −2.5 μA constant current for 2–30 min). Twenty to 60 min after the injection, the rats were perfused transcardially with saline and then with 500 ml of 4% paraformaldehyde, 0.1% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). The medulla was removed, postfixed for 60 min, and put into cryoprotectant (30% sucrose in 0.1 M phosphate buffer, pH 7.4) overnight. Fifty-micron sections were cut on a cryomicrotome, rinsed in Tris-buffered saline (TBS), and treated overnight with 1:1000 solution of ExtraAvidin–horseradish peroxidase (HRP) in TBS. The sections were reacted with tetramethyl benzidine (TMB) and stabilized with cobalt–diamino benzidine (DAB) according to the method of Llewellyn-Smith et al. (1993). The sections were mounted in anatomic order onto gelatin chrome-alum coated glass slides, dehydrated, cleared, and coverslipped. The morphology of the intracellularly labeled, identified cells was reconstructed with the aid of camera lucida drawings. The location of the neurons was determined according to the Paxinos and Watson atlas (1986).

Results

Nociceptive modulatory cells

Fifty-five neurons, recorded in 40 animals, were included in the present study. The selected neurons were all spontaneously active with resting potentials more negative than −50 mV and spike amplitude of at least 40 mV. Input resistance in these neurons varied between 11 and 63 MΩ (n = 29). After intracellular penetration, stable intracellular recordings were maintained for up to 45 min. During that time TF tests were performed at least 3 times. Forty-two of the neurons showed a consistent response to noxious stimulation and were classified as ON or OFF cells. Thirteen neurons showed no response during repeated TF tests and were classified as NEUTRAL cells.

Twenty-seven neurons, classified as ON cells, increased their discharge rate prior to the withdrawal response to noxious tail heat. The baseline rate of ongoing activity was 3.3 ± 1.0 Hz (range, 0–24 Hz). During the TF response it increased to 16.3 ± 2.1 Hz (range 2–40 Hz). The increase in activity started up to 3.7 s before the onset of the reflex. In 21 of the cells (78%) the evoked transient depolarization that led to the increase in discharge rate was clearly visible (Fig. 1A).

In the OFF cells (n = 15) the average baseline discharge rate was 7.4 ± 1.4 Hz (ranging from 1.3 to 18 Hz). During the TF response, the discharge rate decreased to 0.9 ± 0.4 Hz (12% of baseline activity; range, 0–5.7 Hz). The onset of this response was up to 4 s before the withdrawal of the tail. In 12 of the 15 neurons (80%), a TF-related, transient hyperpolarization was also observable (Fig. 1B). NEUTRAL cells (n = 13) exhibited a nonrhythmic ongoing discharge. The rate of their discharge varied between 1.7 and 12.4 Hz and therefore either an ON or OFF type response could have been observed had it been present in these cells.

Analyzing the action potentials of the recorded cells, two distinct groups could be distinguished. In one group, the cells had short-duration action potentials with fast repolarization that overshot baseline resting potential, leading to an early, fast afterhyperpolarization (fAHP; Fig. 1). These

Surgical preparations

Sprague-Dawley rats (280–350 g; Bantin and Kingman, Fremont, CA) were anesthetized with pentobarbital sodium (60 mg/kg ip; Nembutal) and the jugular vein was cannulated to allow infusion of anesthetic (20–25 mg/h/animal; Brevital). The animals were placed into a stereotaxic apparatus; after a parietal and occipital craniotomy the cerebellum was removed by suction (to aid the positioning and penetration of microelectrodes). Intracellular recordings were made from cells located in the rostral medulla oblongata (0–10 mm lateral from midline, 2.0–3.5 mm below the ependymal surface of the medulla oblongata). Tail flick (TF) responses to noxious heat stimulation of the tail were tested repeatedly in each penetrated cell. A feedback controlled heat stimulus, linearly increasing at 1.8°C/s over 10 s, was applied to the tail and the latency of reflex withdrawal was determined from the onset of the EMG activity of the longitudinal back muscles. Between trials the temperature of the tail was maintained at 34°C (Heinricher et al. 1992). Core temperature was controlled using a combination of a heating blanket and overhead lamp. The level of anesthesia was assessed by testing responses to noxious pinch of the hindpaw at regular intervals.

In some neurons the effect of intravenous morphine application was also examined. After 10-min control periods, an analgesic dose of morphine (6 mg/kg) was injected at a slow, continuous rate (0.5-ml volume over 1-min period). The activity of intracellularly penetrated cells was monitored for 15 min. During this time, TF tests were carried out at 3-min intervals. After 15 min, 0.4-μg Naloxone hydrochloride (dissolved in 0.2 ml saline) was injected intravenously at the same slow rate and recordings were continued.
neurons were named fast-spiking neurons (FS neurons) following a nomenclature previously established for neurons with similar electrophysiological features (Kawaguchi 1993). In the second group of cells, which we term slow-spike cells (SS cells), the action potentials were wider, their repolarization rate was slower and there was no fAHP (Fig. 2). Physiological characteristics and intrinsic membrane properties of the two cell groups are described below.

Fast-spike (FS) cells

Thirty neurons were classified as FS neurons. In FS cells, the duration of action potentials at half-amplitude varied from 0.2 to 0.35 ms (0.27 ± 0.02 ms). They had a fast rate of spike depolarization and a similar, fast rate of spike repolarization (Table 1). During spike repolarization the membrane potential often reached potentials more negative than the resting potential. This overshoot was followed by a transient depolarization then a second hyperpolarization (latter onset afterhyperpolarization; lAHP). The latency to the peak of lAHP was on average 4.6 ± 0.5 ms from the peak of the action potential. Its mean amplitude (7.6 ± 0.5 mV) was not statistically different from that of the fAHP (P > 0.5). The resting potential in FS neurons was 1.9 ± 0.2 mV below firing threshold and excitatory inputs produced a slow, ongoing discharge in most cells. The pattern of this activity was irregular and spikes often appeared in clusters or in short bursts. The mean of the minimum interspike intervals observed between non-burst spikes was 23.8 ± 2.2 ms.

All FS cells were either ON (n = 20) or OFF (n = 10) cells. The parameters of the action potentials (such as resting membrane potential, spike width, spike amplitude, and the ratio of the maximal rate of spike de- and repolarization) were not significantly different between ON and OFF FS cells (P > 0.05; Table 1). The input resistance however was significantly higher in the FS OFF than in the FS ON cells (P < 0.03, n = 14).

SS cells

Twenty-five of the recorded neurons were SS cells. They were defined by their significantly slower rate of spike repolarization compared with the FS neurons (P < 0.02) and their long-duration action potentials (0.44 ± 0.02 ms at half-amplitude; Fig. 3). In SS neurons, the action potentials were followed by a single AHP (medium latency AHP; mAHP) at 2.7 ± 0.2 ms latency to peak from the spike. The average amplitude of this mAHP was significantly larger than the amplitude of fAHP or IAHP in FS cells (P < 0.01).

Comparison of TF-related responses in FS- and SS-type OFF and ON cells

Because SS cells have a more negative resting potential and higher input resistance than FS cells (Table 1), we investigated whether the two types of OFF cells show any differences in their activity during TF tests. We selected FS and SS OFF cells with similar rates of baseline activity during the 5-s periods before the TF tests (Table 2). In FS OFF cells, the TF-related response started at a mean of 1.2 ± 0.4 s before the onset of the tail flick, as determined from the EMG of the tail flick muscles (Grossman et al. 1982). Dur-
FIG. 2. Slow-spiking (SS) neurons. Left: TF responses in SS ON (A), OFF (B), and NEUTRAL (C) cells. Each panel shows activity of the neuron during 10-s TF trial at top and activity of the muscles that control tail movement (EMG) below. Note change in activity pattern of ON and OFF but not NEUTRAL cells before withdrawal of tail (TF). Right: shape of action potentials. Arrow, afterhyperpolarization (mAHP).

TABLE 1. Electrophysiological and morphological properties in functionally identified cell groups

<table>
<thead>
<tr>
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<th>Fast-Spike Cells</th>
<th>Slow-Spike Cells</th>
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<tr>
<td></td>
<td>ON</td>
<td>OFF</td>
<td>ON</td>
</tr>
<tr>
<td>Resting potential, mV</td>
<td>60 ± 1.8</td>
<td>59 ± 2.1</td>
<td>64 ± 2.7</td>
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<td>Input resistance, MΩ</td>
<td>18 ± 2</td>
<td>29 ± 4</td>
<td>34 ± 6</td>
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<td>Discharge threshold, mV</td>
<td>58 ± 1.5</td>
<td>58 ± 2.0</td>
<td>62 ± 2.0</td>
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Spike parameters

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<th>Slow-Spike Cells</th>
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<tr>
<td>Spike amplitude, mV</td>
<td>56 ± 2.3</td>
<td>54 ± 2.3</td>
<td>63 ± 3.3</td>
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<tr>
<td>Spike width,* ms</td>
<td>0.27 ± 0.01</td>
<td>0.26 ± 0.02</td>
<td>0.38 ± 0.02</td>
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<tr>
<td>Max. depolarization, V/s</td>
<td>280 ± 16</td>
<td>287 ± 27</td>
<td>275 ± 20</td>
</tr>
<tr>
<td>Max. repolarization, V/s</td>
<td>270 ± 13</td>
<td>287 ± 29</td>
<td>206 ± 12</td>
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<tr>
<td>Ratio of de-repolarization</td>
<td>1.0 ± 0.03</td>
<td>1.0 ± 0.02</td>
<td>1.4 ± 0.05</td>
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<td>Latency to first AHP, ms</td>
<td>0.6 ± 0.02</td>
<td>0.6 ± 0.1</td>
<td>2.8 ± 0.3</td>
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<tr>
<td>Amplitude of AHP, mV</td>
<td>5.9 ± 0.7</td>
<td>5.9 ± 1.0</td>
<td>11.2 ± 1.6</td>
</tr>
</tbody>
</table>

Morphology (note lower cell counts), µm

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<tr>
<th></th>
<th>Fast-Spike Cells</th>
<th>Slow-Spike Cells</th>
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<tbody>
<tr>
<td>Long soma axis</td>
<td>42 ± 2.5</td>
<td>23 ± 1.7</td>
<td>27 ± 1.5</td>
</tr>
<tr>
<td>Short soma axis</td>
<td>26 ± 1.9</td>
<td>15 ± 1.6</td>
<td>16 ± 2.1</td>
</tr>
<tr>
<td>Primary dendrite diameter</td>
<td>5.6 ± 0.6</td>
<td>2.6 ± 0.2</td>
<td>2.0 ± 0.0</td>
</tr>
</tbody>
</table>

Values are means ± SE; number of events for fast-spike (FS) ON cells was 20, for FS OFF cells was 10, for slow-spike (SS) ON cells was 7, for SS OFF cells was 5, and for SS NEUTRAL cells was 13. AHP, afterhyperpolarization. * Measured at half amplitude. † The number refers to cells where the quality of intracellular labeling was suitable for morphological measurements.
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FIG. 3. Intrinsic membrane characteristics in FS and SS neurons.

The characteristics of the TF-related responses were also compared between the two subtypes of ON cells. The onset of the TF response occurred earlier in FS than in SS ON cells, similar to OFF cells \( (P < 0.05; \text{Table 2}) \). The duration, but not the pattern, of the evoked responses was similar in FS and SS ON cells. The pattern of their TF-related discharge...
was similar in character to their ongoing discharge (clustered (bursty) in FS cells and regular in SS cells).

**Effect of intravenous morphine**

In previous in vitro experiments by Pan et al. (1990), neurons of the RVM with different electrophysiological characteristics responded differently to bath application of morphine. The so-called “secondary” cells but not the “primary” cells were directly sensitive to opioids. Because only ON cells are directly sensitive to opioid agonists in the in vivo preparation (Heinricher et al. 1992), in the current study we examined the response of ON cells to systemic application of morphine (n = 4). These experiments, where stable intracellular recordings for >30 min are required despite the drug-induced changes in cardiovascular and respiratory parameters, were difficult to execute. The effect of intravenous morphine was examined in two FS- and two SS-type ON cells.

After intravenous injections of morphine, the TF response stopped within 6 min. TF-related firing also diminished or ceased in all four ON cells. Fifteen minutes after morphine injection, Naloxone was injected, which promptly reversed the analgesic effect of morphine and restored the TF response at latencies close to or shorter than during the control period.

**FS ON CELLS.** After intravenous injection of morphine, a hyperpolarization of up to 7 mV accompanied the morphine-induced TF inhibition in both FS ON cells (Fig. 5A). These cells remained hyperpolarized for >10 min after which time a slow depolarization started. At this time, the TF responses were still diminished. In both cells, a depolarization induced by application of noxious heat to the tail was still observable during morphine analgesia. However, because of the morphine-induced hyperpolarization, fewer action potentials were elicited by tail heat. The input resistance, as determined from the linear phase of the current-voltage curve, decreased in both cells during the morphine effect. The amplitude of depolarizing current pulses that were required to induce spiking increased several fold (from 0.1 and 0.3 nA to 0.8 and 1.3 nA, respectively, in the 2 cells). The mean duration of the spikes decreased by about one-third (from 0.35 ms to 0.2 and 0.23 ms) and the amplitude of the fAHP increased on average by 16% (Fig. 5B). After Naloxone injection, spontaneous activity reappeared in both cells (Fig. 5C). A tendency to return to control values was also observed in the resting potential, input resistance, and spike parameters.

**SS ON CELLS.** During morphine analgesia, hyperpolarization was also apparent in the two SS ON cells (from −58 and −64 mV to −70 and −67 mV, respectively). In parallel with the hyperpolarization, the spontaneous firing and the TF-related response diminished in both cells (Fig. 6A). The amplitude of depolarizing current pulses required to induce action potentials increased only approximately twofold in the SS ON cells (from 0.2 nA to 0.3 and 0.5 nA, respectively). The input resistance of the neuron, the spike duration, and the amplitude of the mAHP also remained close to the control in both cells during morphine analgesia (Fig. 6B). After injection of Naloxone, the resting potential and discharge threshold rapidly returned to control and the ongoing discharge rate increased (Fig. 6C).

**Morphology of physiologically identified cells**

Twenty-one of the above physiologically identified cells were labeled intracellularly and recovered for anatomic reconstruction and analysis. In the majority of these cells, extensive parts of the dendritic arbor as well as the origin of the axonal shaft were visualized. The labeled cells were within the nucleus raphe magnus or the adjacent medial region of the gigantocellular nucleus pars-α, at the rostrocaudal level of the facial nucleus. According to the size of the somata and the characteristics of the dendritic tree, there appeared to be two morphologically distinct cell types in coronal planes of the medulla oblongata (Fig. 8). Cells with the two different morphological features were intermingled throughout the RVM.

Fifteen of the labeled cells were small-to-medium sized cells with elongated somata (Table 1, Fig. 7, ▲). The size of the somata varied from 17 × 10 to 33 × 25 μm (as measured along the longest axis and at a right angle to it in 13 cells). They had two to five (average of 4) small-caliber (2–3 μm) primary dendrites. The primary branches frequently branched at ~50 μm from the soma while branching further distally was rare. The orientation of the dendritic trees was predominantly mediolateral. Ten of these neurons were SS cells (5 NEUTRAL, 4 ON, and 1 OFF) and five were FS OFF cells (Fig. 8A and B).

Six cells had large, triangular- or multipolar-shaped somata (sizes ranged from 40 × 15 to 65 × 30 μm, n = 5) (Fig. 7, ▐). They had four to nine (average of 6) large caliber (4–8 μm) primary dendrites. The short and long axes of the cell bodies as well as the diameter of primary dendrites were significantly larger in these neurons compared with cells of the previous type (P < 0.01 in all cases). The

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**TABLE 2. Properties of TF response in the five neuronal groups**

<table>
<thead>
<tr>
<th></th>
<th>Fast-Spike Cells</th>
<th>Slow-Spike Cells</th>
<th>Mechanical Parameters</th>
<th>Basal Characteristics</th>
<th>Firing Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latency of TF, s</td>
<td>5.8 ± 0.4</td>
<td>5.0 ± 0.2</td>
<td>0.6 ± 5.0</td>
<td>2.8 ± 1.3</td>
<td>4.6 ± 1.3</td>
</tr>
<tr>
<td>Basal firing rate, Hz</td>
<td>2.8 ± 1.3</td>
<td>4.6 ± 1.3</td>
<td>7.3 ± 1.8</td>
<td>7.6 ± 2.8</td>
<td>4.5 ± 1.0</td>
</tr>
<tr>
<td>Firing rate during TF, Hz</td>
<td>15.1 ± 2.6</td>
<td>24.1 ± 4.6</td>
<td>0.6 ± 0.2</td>
<td>1.4 ± 1.1</td>
<td>N/A</td>
</tr>
<tr>
<td>PSP onset prior to TF, s</td>
<td>4.7 ± 0.6</td>
<td>5.7 ± 1.1</td>
<td>3.5 ± 0.5</td>
<td>1.7 ± 0.3</td>
<td>N/A</td>
</tr>
<tr>
<td>Duration of PSP, s</td>
<td>1.4 ± 0.3</td>
<td>0.5 ± 0.1</td>
<td>15.2 ± 0.4</td>
<td>0.4 ± 0.4</td>
<td>N/A</td>
</tr>
<tr>
<td>Amplitude of PSP, mV</td>
<td>4.7 ± 0.6</td>
<td>1.2 ± 0.6</td>
<td>5.0 ± 0.8</td>
<td>2.7 ± 1.1</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Values are means ± SE; number of events for fast-spike (FS) ON cells was 20, for FS OFF cells was 10, for slow-spike (SS) ON cells was 7, for SS OFF cells was 5, and for SS NEUTRAL cells was 13. TF, tail flick; PSP, postsynaptic potential.
FIG. 5. A: response of FS-type ON cells to morphine analgesia. Changes in TF response in an FS type ON cell during morphine-induced analgesia (A2), then during Naloxone-induced reversal of morphine analgesia (A3). B: shape of action potentials during spontaneous activity (C), during morphine analgesia (M), and after Naloxone administration (N). C: chart record of resting potential in the same cell. Arrow, start of morphine (M) and of Naloxone (N) injections. Numbered arrows point to the location where 3 inserts, shown at a faster time base in A, were taken from. D: anatomic location and morphology of the cell.

dendritic tree showed a symmetrical, radial appearance resulting from frequent dichotomous branching. Input resistance in these cells was relatively low (13–22 MΩ, n = 5). All five labeled FS ON cells were of this morphological class (Fig. 8, C and D). The sixth neuron was a NEUTRAL cell.

DISCUSSION

The present study demonstrates two physiologically distinct RVM neuronal groups that can be differentiated in vivo on the basis of their intrinsic membrane properties. The FS neurons have a relatively short-duration action potential with a fast repolarization phase. The SS neurons lack this feature. There is a partial correlation of this categorization with the previously defined functional division of RVM neurons into ON, OFF, and NEUTRAL cells. RVM NEUTRAL cells were all in the SS category and were further distinguished from SS ON/OFF neurons by their significantly longer duration action potentials. Among FS cells, ON and OFF cells showed morphological differences. The present results also demonstrate the existence of two distinct subtypes of RVM ON and OFF cells. Thus the onset of the ON cell burst and OFF cell pause, which respectively facilitate and inhibit nocifensive responses, occurs significantly earlier in the FS than the SS neurons.

It could be argued that differences between FS and SS neurons in vivo are the result of the damage caused by the intracellular recording electrode. Such damage is expected
Comparison with previous in vitro data

Neurons have been classified previously based on the characteristics of their action potentials in RVM slice preparations (Pan et al. 1990). The in vitro primary cells exhibited a wide action potential followed by a monophasic AHP. In the secondary cells, a relatively brief action potential was followed by a biphasic AHP. The similarity between their spike characteristics suggests that primary cells recorded in vitro correspond to the SS neurons found in the present study. Secondary and FS neurons presumably are another corresponding population. The dendritic branching pattern and orientation described in primary cells corresponds well to the characteristics observed in vivo in 10 of 11 of the electrophysiologically similar SS cells. The secondary cells labeled by Pan et al. were morphologically similar to the FS OFF cells of the current study. Morphological features of in vivo and in vitro labeled cells must however be compared with caution because the conditions of fixation differ between in vivo and in vitro labeled neurons.

Differences in membrane properties between functionally distinct classes of cortical neurons have been studied extensively for over a decade (Baranyi et al. 1993; Chen et al. 1996a; Cheney and Fenz 1980; McCormick et al. 1985; Nunez et al. 1993; Sutor and Hablitz 1989). Intrinsic membrane properties, similar to those described in the present study, have been used to differentiate cortical neurons with distinct functional characteristics (Chen et al. 1996a; Cheney and Fetz 1980). For example, three types of corticospinal neurons are classified on the basis of their action potential properties in the rat (Tseng and Prince 1993). The duration of the action potential has also been proposed as a reference to cause a depolarization of the membrane and a decrease in input resistance together with a slowing of the speed of spike de- and repolarization. No differences consistent with such a “damage” effect were observed, as neurons that exhibited wide spikes and relatively slow spike repolarization (SS cells) had resting potentials more negative than −60 mV and a relatively high-input resistance. Cells, with more depolarized resting potential and lower input resistance, on the other hand, showed a rapid rate of spike de- and repolarization (FS cells). The differences in electrophysiological characteristics between SS and FS cells are more likely to reflect differences in their intrinsic membrane properties and thus may be useful for electrophysiological identification of RVM neurons that exhibit early and late onset responses to noxious heat stimulus.

Labeled FS ON and OFF cells were distinguished from each other by differences in their soma size and dendritic morphology. Large, multipolar neurons with large caliber primary dendrites were typical of FS ON cells, whereas smaller size neurons were more common among all other neuronal types. Identical morphological features of in vivo labeled RVM cells were described previously. Mason et al. (1990) found that 88% of OFF cells and 35% of ON cells had small, usually fusiform somata, small caliber dendrites, and a predominantly medio-lateral orientation of their dendritic trees— a morphology, similar to the 100% of OFF cells and 44% of ON cells, found in the present study. Mason et al. (1990) also reported a population of ON cells with large cell bodies and large caliber dendrites, similar to those observed among the FS ON cells.

Effect of morphine

Previous extracellular studies have shown that systemically administered opioids inhibit ON cells, suppressing both tonic and TF-related activity (Heinricher et al. 1992). Heinricher et al. found a 34–100% depression of TF-related dis-
A: response of SS-type ON cell to morphine analgesia. A2: changes in TF response in a SS-type ON cell during morphine induced analgesia. B: shape of action potentials during spontaneous activity (C), during morphine analgesia (M), and after Naloxone administration (N). C: chart record of resting potential in same cell. Numbered arrows point to the location where the 2 inserts, shown at a faster time base in A, were taken from.

Opioid activation of OFF cells is indirect, either via presynaptic inhibition of inhibitory afferent terminals or by postsynaptic inhibition of inhibitory neurons (Fields et al. 1991; Heinricher et al. 1994). In slice preparations of the RVM, µ-opioid receptor agonists hyperpolarized 16 of 24 secondary cells by increasing potassium conductance, while eliciting no direct changes in any of the primary cells (Pan et al. 1990). These in vivo and in vitro studies suggest that primary cells include mainly OFF cells whereas secondary cells correspond largely to ON cells. In the current study, systemic morphine inhibited both FS- and SS-type ON cells; however, only in FS cells did this inhibition show features

FIG. 7. Anatomic localization of FS and SS neurons. Location of 21 labeled neurons is shown comprised into 4 coronal sections of medulla oblongata. Sections were taken 0.8-, 1.5-, 2.0-, and 2.6-mm caudal to interaural level. FS and SS neurons were quite widely distributed both rostrocaudally and laterally within RVM. Highest density of labeled cells was at level with the caudal part of facial nucleus. ▲, small- to medium-sized neurons with horizontally oriented dendritic tree. □, large-size multipolar neurons with radially arranged large-caliber dendrites. 7, facial nerve; CN, cochlear nucleus; GiA, gigantocellular nucleus pars α; n7, facial nucleus; py, pyramidal tract; RPs, raphe pallidus; RMg, raphe magnus; SnT, principal sensory trigeminal nucleus; SpT, spinal trigeminal nucleus; SO, superior olive.
similar to the morphine-induced direct inhibition that was described in secondary cells (Pan et al. 1990). Furthermore, the proportion of ON cells encountered among FS neurons (20 of 30 cells) was similar to the proportion of morphine-sensitive neurons found among secondary cells in vitro (16 of 24).

Possible functional role of FS- and SS-type nociceptive modulatory cells

In the present study, a significant difference in the time of onset of TF-related responses was observed between FS- and SS-type nociceptive modulatory neurons. FS-type ON and OFF cells showed significantly earlier activity changes than SS-type cells prior to the TF. The difference in the onset of TF-related responses between neurons of the FS and SS types may be the result of differences in their activation threshold or differences in their afferent input. Because FS ON and OFF cells respond to noxious stimuli earlier than SS cells, it is possible that they provide input to SS-type ON or OFF cells. This proposal is in keeping with evidence of intrinsic connectivity in the RVM. Intracellular labeling and tract-tracing experiments have demonstrated intrinsic projections between RVM neurons (Zagon 1995). Axon collaterals of OFF cells and to a lesser extent from ON cells project throughout the RVM (Mason and Fields 1989). The TF-related pause in OFF cell activity is mediated by GABA (Heinricher et al. 1991). Because GABA immunoreactive neurons are present within the RVM (Jones et al. 1991), the GABAergic input to OFF cells may in part be of local, RVM origin. If this is the case, because of their early discharge onset, FS ON cells are potentially attractive candidates to provide GABAergic input to RVM OFF cells.

In summary, the present findings demonstrate two physiologically significant subtypes of ON and OFF cells: FS and SS. Compared with SS neurons, FS cells have an earlier onset response to noxious heat stimuli. FS and SS neurons are distinguished by the characteristics of their action potentials. These characteristics can also be recognized in a slice preparation and therefore may be useful for identification of different types of RVM neurons in vitro. The contribution of these different categories of RVM neurons to pain modulation remains to be elucidated.

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