Primary- and Secondary-Like Jaw-Muscle Spindle Afferents Have Characteristic Topographic Distributions

DEAN DESSEM, REVERS DONGA, AND PIFU LUO
Department of Physiology, University of Maryland Dental School, Baltimore, Maryland 21201-1586

Dessem, Dean, Revers Donga, and Pifu Luo. Primary- and secondary-like jaw-muscle spindle afferents have characteristic topographic distributions. J. Neurophysiol. 77: 2925 ± 2944, 1997. Single-jaw-muscle spindle afferent axons were characterized physiologically and intracellularly stained to determine whether particular physiological types of spindle afferent showed distinctive morphologies. Microelectrodes filled with either horseradish peroxidase (HRP) or biotinamide (Neurobiotin) were advanced into the mesencephalic trigeminal nucleus (Vme) in anesthetized rats. Intracellular recordings then were characterized by their response to palpation of the jaw muscles; when pressure was applied to the teeth and during passive ramp and hold and sinusoidal jaw movement. Seventy-one afferents were characterized physiologically and injected with HRP; an additional 61 afferents were typed and injected with biotinamide. The response of 43 stained neurons was recorded in the presence of suxamethonium. The major projection areas of these afferents were the: trigeminal motor nucleus (Vmo); region dorsal to Vmo; reticular formation, spinal trigeminal nucleus, superior cerebellar peduncle and Vme. One afferent type was modulated strongly during stretching of the jaw-elevator muscles. Based on their high sensitivity during stretching of the jaw muscles and/or their silencing during the release phase of muscle stretch, these afferents were classified as primary-like spindle afferents. These afferents projected most strongly to Vmo. A second type of afferent was modulated only modestly during stretching of the jaw-elevator muscles. These tonic afferents were classified as secondary-like spindle afferents because of their low dynamic sensitivity during ramp muscle stretch and their continued discharge during the release phase of muscle stretch. Secondary-like afferents projected most strongly to the region dorsal to Vmo. Boutons (n = 3,834) from 11 afferents were studied in detail. Secondary-like afferents had statistically larger boutons within Vmo. In both secondary- and primary-like spindle afferents, only a small number of boutons were associated closely with the somata and proximal dendrites of trigeminal motoneurons. In these cases, however, two to five boutons appeared to contact individual motoneurons, implying multiple monosynaptic inputs to a selective subset of jaw-elevator motoneurons. Some “giant” boutons were present dorsal to Vmo and in Vme. These results demonstrate that dynamically sensitive and nondynamically sensitive jaw-elevator muscle spindle afferents project preferentially to different regions. Primary-like spindle afferents are capable of providing feedback related to the dynamic phases of muscle stretch and project most heavily to Vmo. Secondary-like spindle afferents can transmit a feedback signal associated with muscle length and project most strongly to the supratrigeminal region. Both types of afferent have projections caudal to Vmo that may serve longer latency jaw-muscle stretch reflexes and/or the projection of proprioceptive information to the thalamus and cerebellum.

INTRODUCTION

Different types of afferent termination within the mammalian muscle spindle are known to be correlated with different physiological responses (Boyd and Ward 1975). Much less is known, however, about what correlations may exist between the types of peripheral afferent termination and the axonal trajectory and terminations of these neurons within the CNS. In the spinal cord, studies using intracellular staining (Brown and Fyffe 1978, 1981; Burke et al. 1979; Conradi et al. 1983; Fyffe and Light 1984; Ishizuka et al. 1979; Keirstead and Rose 1988a, b; Rose and Keirstead 1986) have shed light on the central distribution of primary spindle afferents. Additional information about the distribution of primary afferents in the spinal cord comes from studies in which the extracellular field potentials generated by single Ia afferents have been mapped (Munson and Sypert 1979). The relationship between primary spindle afferents and spinal motoneurons has been examined using both electrophysiological and intracellular staining techniques (Brown and Fyffe 1981; Burke et al. 1979; Mendell and Henneman 1971; Watt et al. 1976). Much less is known about the central distribution of secondary muscle spindle afferents. Only one small study (Fyffe 1979) subsequently discussed by Brown (1981) has intracellularly labeled secondary muscle spindle afferents. Additional information on the distribution of secondary spindle afferents in the spinal cord comes from extracellular electrophysiological studies (Edgley and Jankowska 1987) and studies in which the synaptic inputs from secondary spindle afferents to motoneurons were examined (Kirkwood and Sears 1982; Munson et al. 1982; Stauffer et al. 1976). In contrast to the spinal cord, there is no convincing information demonstrating the central distribution of different jaw-muscle spindle afferent types. Studies in which retrograde neuroanatomic tracers have been injected into the muscles of mastication provide a macroscopic map of the distribution of jaw-muscle spindle afferents (Capra and Wax 1989; Gottlieb et al. 1984; Nomura and Mizuno 1985; Raapana and Arvidsson 1993; Rok et al. 1985) but provide no physiological information. Intracellular labeling studies in the rat (Appenteng et al. 1985; Dessem and Taylor 1989; Lingenhölzl and Friauf 1991; Luo and Dessem 1995; Luo et al. 1991, 1995a) provide more detailed information about the distribution of single jaw-muscle spindle afferents, but none of these studies have characterized the responses of these afferents in enough detail to distinguish between muscle spindle afferent types. Additional information concerning the central distribution of jaw-elevator muscle spindle afferents can be gained from electrophysiological mapping studies in which jaw-elevator muscle spindles are characterized and their central distribution is inferred from unitary spike-triggered averaging (Appenteng et al. 1978, 1989; Taylor et al. 1993a). Although these studies may provide a gross estimate of the afferent distribution, they cannot provide...
detailed information about the branching of the axon, the size and number of boutons, and the relationship between the terminations of the afferents and the distribution of trigeminal motoneurons.

In the cat, Shigenaga and co-workers (1988, 1990), have attempted to classify primary and secondary jaw-muscle spindle afferents based on their response during a 1-s step muscle stretch. Using this unconventional criterion, these authors concluded that there was no correlation between the afferent response and central trajectory of jaw-muscle spindle afferents. The experiments described in this paper were carried out to examine the relationship between jaw-muscle spindle afferents identified by classical physiological methods with their central distribution. Jaw-muscle spindle afferents were impaled intracellularly, physiologically classified using controlled stretching of the jaw muscles, and intracellularly stained with either horseradish peroxidase (HRP) or biotinamide to determine the central distribution of jaw-muscle spindle afferent types.

METHODS

Male Wistar rats (300–350 g) were anesthetized initially with pentobarbital sodium (20 mg/kg ip) supplemented with additional injections (8 mg/kg iv) every hour to maintain adequate anesthesia. To reduce secretions in the airways and trachea, atropine (1 mg/kg sc) was administered after the induction of anesthesia. The femoral vein and artery then were cannulated, and systemic arterial blood pressure was monitored for the duration of the experiment. Body temperature was maintained at 37°C by means of a thermostatically controlled heating pad. The animals then were placed in a stereotaxic frame and ventilated (2 cm³, rate: 100/min) for the duration of the experiment with humidified air while maintaining a positive end expiratory pressure of 1 cm H₂O to prevent lung collapse. Animals used for intracellular HRP labeling were paralyzed with gallamine triethiodide (20 mg/kg). Anesthesia in these animals was maintained during paralysis by regular supplements of anesthetic that were determined to be sufficient to prevent a limb-withdrawal reflex before paralyzing the animal. The plane of anesthesia also was checked periodically by allowing the paralysis induced by gallamine to wear off. Rats used for intracellular biotinamide staining were not administered gallamine. All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Maryland before the onset of experiments. To gain access to the mesencephalic trigeminal nucleus, the bone, dura, and pia mater overlying the dorsal and posterior surfaces of the cerebellum were removed, and warmed mineral oil was applied to the surface of the brain stem and cerebellar cortex. Before electrophysiological recording, chlorpromazine (150 mg/kg) was administered to suppress background fusimotor activity (Cody et al. 1972), and a pneumothorax was performed to enhance intracellular recording stability. Microelectrodes then were advanced via a stepping motor rostroventrally at an angle of 30° to the vertical through the posterior portion of the cerebellum into the tract of the mesencephalic nucleus just dorsal and medial to the trigeminal motor nucleus (P 0.0–1.0, L 1.1–2.0, depth 5–6.5 mm). Jaw displacements of 2.5 mm then were produced via an electromagnetic vibrator attached to the jaw at the diastema and used as the search stimulus for stretch-sensitive afferents. Intraxonal recordings made from afferents in this region of the brain stem whose firing frequency increased during both jaw opening (muscle stretch) and gentle propping of jaw muscles and that failed to respond when pressure was applied to the teeth were tentatively characterized as jaw-elevator muscle spindles. The intracellular response of each of these stretch-sensitive afferents then was recorded on tape during 10 ramp and hold and 10 sinusoidal stretches (0.6 Hz) for off-line analysis.

Intracellular HRP labeling

Microelectrodes used for HRP labeling were fabricated from 1.0 mm OD borosilicate glass and initially filled with Tris HCl buffer (pH 8.6). After the electrode tips had filled, 20–30% HRP (Sigma VI) was placed into the electrode and allowed to diffuse into the tips for 24 h. These electrodes were bevelled just before recording to impedances of 80–120 MΩ.

In cases where stable intra-axonal penetrations with resting membrane potentials more negative than −40 mV were maintained from well-characterized spindle afferents, depolarizing current pulses (40 ms, 100 Hz, 0.5–5 nA) were applied through the microelectrode for 6–18 min, resulting in total injection times of 14–43 nA minutes.

Current injections were stopped every 30 s and discontinued if the membrane potential became more positive than −30 mV. Two to 3 h after the injection of HRP, heparin (500 units iv) was administered, followed after 15 min by an overdose of pentobarbital sodium. The animals then were perfused through the ascending aorta with 700 ml of 0.9% saline solution (38°C) containing 500 units of heparin and 1 ml of 2% xylocaine. This was followed by infusion of two liters of 1.25% glutaraldehyde and 1% paraformaldehyde in a phosphate buffer (pH 7.4) during 30 min. Finally, 1 l of 10% phosphate-buffered sucrose solution (pH 7.4) was infused for 30 min. The brain then was removed and stored overnight at 4°C in a 10% phosphate-buffered sucrose solution (pH 7.4). Sagittal sections (100 μm thickness) were cut on a vibratome and processed for the demonstration of HRP according to the method of Metz et al. (1989). These sections then were mounted on chrom-alum slides, air-dried, and cover-slipped.

Intracellular biotinamide labeling

Electrodes for biotinamide labeling were made from 1.0 mm OD borosilicate glass and filled with 3% biotinamide (Neurobiotin, Vector Laboratories) dissolved in 0.25 M KCl and 0.5 M Tris-HCl buffer (pH 7.6). Before use, these electrodes were bevelled to impedances of 60–80 MΩ. After physiological characterization of a stable intracellular jaw-muscle spindle afferent impalement, biotinamide was injected into the axon using DC currents ranging from 0.025% nickel ammonium sulphate, 0.02% DAB, and 0.00018 ± 0.00024% H₂O₂. After these procedures the sections were washed in PBS, air-dried, dehydrated, defatted in xylene, mounted onto slides coated with chrom-alum, and cover-slipped.
Physiological data analysis

The intracellular afferent responses of each well-stained neuron and the jaw displacement signal were replayed from tape and digitized off-line (80 kHz; Cambridge Electronic Design, 1401plus). Instantaneous and peak frequencies were computed with spike analysis software (Spike2, Cambridge Electronic Design). Dynamic indices were calculated from the averaged response of 10 ramp stretches by taking the difference between the maximum instantaneous frequency during the ramp stretch and 0.5 s later (Crowe and Matthews 1964).

Morphological analysis

Photomicrographs and camera lucida drawings of the intracellularly stained neurons were made before counterstaining the unstained tissue. Axonal morphology was reconstructed using a software assisted three-dimensional computer reconstruction system (Capowski and Sedivec 1981). The locations of swellings on fine axon collaterals, comparable with structures demonstrated in previous studies to contain synapses (Luo et al. 1995a,b), were examined at \( \times 1,000 \) magnification. After reconstruction and photography, the coverslips were removed from the slides, and the tissue was counterstained with either cresyl violet or neutral red to accurately determine the location of the intracellularly stained axons and boutons in relation to cell bodies of various brain stem nuclei.

The morphology of boutons stained with HRP was analyzed using a manual image analysis computer (Zeiss Videoplan). Boutons were observed at \( \times 1,000 \) magnification, and their outlines traced to determine bouton perimeter and area. Digital images of boutons stained with biotinamide initially were produced and stored on computer disk using a computer camera (Electrim, EDC 1000U) attached to an Olympus (BH-2) microscope. Bouton perimeter and area then were measured from these stored images using image measurement software (Jandel, SigmaScan). A shape factor also was calculated for these boutons using the formula

\[
\text{shapefactor} = \frac{4\pi\text{Area}}{\text{Perimeter}^2}
\]

Parametric and nonparametric statistical tests (SYSTAT) were used to determine differences in the central tendency of bouton area, perimeter, and shape within and outside the trigeminal motor nucleus.

RESULTS

HRP staining

Seventy-one afferents were intracellularly impaled, physiologically characterized and injected with HRP in 37 rats. Following histochemical procedures for the visualization of HRP, 31 afferents in 16 rats were considered to be well stained because their processes could be followed peripherally into the motor root of the trigeminal nerve, rostrally into the tract of the mesencephalic trigeminal nerve and caudally into the tract of Probst.

Biotinamide staining

Sixty-one afferents, which initially were identified as jaw-muscle spindle afferents on the basis of their afferent firing behavior, were impaled intracellularly, physiologically characterized, and injected with biotinamide in 23 rats. After histochemical processing for the visualization of biotinamide, 41 afferents in 17 rats were judged to be well stained because their processes could be visualized in the motor root of the trigeminal nerve, the tract of mesencephalic trigeminal nucleus (Vme) and the tract of Probst.

Physiological characteristics

All of the afferent neurons labeled in this study responded with an increased firing when the jaw-elevator muscles were palpated but failed to respond when pressure was applied to the teeth and gingiva. Two basic types of afferent response could be distinguished. One type was modulated strongly during stretching of the jaw-closing muscles. This kind of response exhibited a high dynamic sensitivity and high peak frequency during stretching of the jaw-elevator muscles and either a large reduction or a silencing of the afferent response during the release phase of muscle stretch (Fig. 1, A and B). The second type of afferent response was modulated only weakly during stretching of the jaw-elevator muscles. This variety of response showed little dynamic sensitivity, a low peak frequency during muscle stretch, and responded continuously during all phases of ramp and hold and sinusoidal stretching (Fig. 1, C and D).

General morphology of jaw-muscle spindle afferents

All of the axons labeled in this study entered the brain stem ventrally in the motor root of the trigeminal nerve and coursed dorsomedially toward the trigeminal motor nucleus (Vmo). Either within or slightly dorsal to the trigeminal motor nucleus these axons bifurcated with one branch coursing rostrally into the tract of the mesencephalic trigeminal nucleus and the other turning caudally to enter the tract of Probst. In most cases, the rostral process could be traced to a HRP- or biotinamide-stained cell body located in the mesencephalic trigeminal nucleus (Vme). All of these intracellularly stained somata exhibited a pseudounipolar morphology (Figs. 3, 5, 11, A and B, and 13) except one, which possessed a small dendrite (Fig. 11 C). In the caudal direction, each afferent possessed a prominent process that could be followed in the tract of Probst (Probst 1899; Corbin 1942) to the region between the facial motor nucleus and the inferior olivary nucleus (Fig. 13). All well-stained afferents possessed axon collaterals that emanated from the main axon into Vmo and the region dorsal to Vmo. These axons also possessed collaterals that emerged from the tract of Probst at the level of the facial motor nucleus and coursed ventrolaterally into the reticular formation. In addition to these major projections, a few afferents had collaterals with en passant and terminal swellings that coursed into the caudal part of Vme. A few intracellularly stained afferents also had axon collaterals that could be followed lateral and dorsolateral to Vmo. At more caudal levels, some afferents possessed axon collaterals that emerged from the tract of Probst at the level of the facial motor nucleus and coursed laterally through the reticular formation into the dorsomedial portion of the spinal trigeminal nucleus.

Morphological characteristics of dynamically sensitive jaw-muscle spindle afferents

The afferent response of a strongly modulated afferent (R161) labeled with HRP is shown in Fig. 2. This afferent was tested 15 min after the infusion of suxamethonium (22.8 mg/kg ip) and showed a high dynamic sensitivity (dynamic...
located within Vmo (Figs. 3 and 14A). Several of the axon collaterals that coursed into Vmo traversed the nucleus and could be followed into the region lateral to Vmo. This dynamically sensitive afferent also possessed collaterals that coursed into the region dorsal to Vmo, the reticular formation at the level of the facial motor nucleus, and the superior cerebellar peduncle. The peak frequencies of dynamically sensitive afferents labeled using the biotinamide protocol ranged from 290 to 320 impulses/s and exhibited dynamic indices between 152 and 178.5 impulses/s with a mean of 168.2 impulses/s (Table 1). The basic morphology of dynamically sensitive jaw-muscle spindle afferents stained with HRP. All dynamically sensitive, biotinamide-stained afferents possessed a process in the motor root of the trigeminal nerve, a process that extended rostrally into the tract of Vme and ended in a pseudounipolar-shaped soma and a thinner process that projected caudally in the tract of Probst. The greatest number of boutons and axon collaterals of dynamically sensitive afferents were located in the trigeminal motor nucleus (Table 2). More caudally, dynamically sensitive jaw-muscle spindle afferents possessed a process in the tract of Probst that extended beyond the caudal extent of the facial motor nucleus. Additional axon collaterals emerged from this process and coursed ventrolaterally through the reticular formation to reach the dorsomedial portion of the spinal trigeminal nucleus. No projections to the cerebellum were observed emanating from dynamically sensitive biotinamide-stained afferents.

The largest number of axon collaterals and boutons of dynamically sensitive afferents stained in this study either with HRP or biotinamide were located within the confines of the trigeminal motor nucleus. These boutons, however, only were found occasionally in close association to trigeminal motoneuron somata or proximal dendrites. All strongly modulated afferents also possessed axon collaterals and boutons throughout the region dorsal to the trigeminal motor nucleus and collaterals, which emerged from the tract of Probst and coursed into the reticular formation. In a few instances, dynamically sensitive afferents had axon collaterals with boutons that were overlying or adjacent to the somata of caudal Vme neurons. One dynamically sensitive afferent labeled with HRP possessed a collateral which could be traced into the superior cerebellar peduncle.

**Morphological characteristics of nondynamically sensitive jaw-muscle spindle afferents**

The behavior of a weakly modulated afferent is shown in Fig. 4. The response of this neuron (R170) increased during palpation of the region overlying the posterior portion of the masseter muscle but failed to respond to probing of the teeth and gingiva. This afferent showed a low dynamic sensitivity (dynamic index = 10.0 impulses/s) and low peak frequency (62.9 impulses/s) during stretching of the jaw closing muscles and showed only a modest reduction in firing during the release phase of muscle stretch. The pseudounipolar cell body of this afferent was located in the rostral part of the mesencephalic trigeminal nucleus. Figures 5 and 14C show the location of en passant and terminal boutons from this afferent in relation to the Nissl-stained boundaries of the
FIG. 2. Afferent response of a primary-like jaw-muscle spindle afferent (R161). A: intra-axonal response during ramp and hold displacement of jaw (top); bottom: jaw displacement signal. B: instantaneous frequency of afferent during ramp and hold jaw displacement. C: instantaneous frequency of afferent during sinusoidal displacement of jaw. Arrow, direction of jaw opening. Time bar in A is 0.1 s, in B and C, it is 1 s.

trigeminal motor nucleus. As is apparent in these figures, the axon collaterals and boutons of R170 were distributed preferentially to the region overlying the trigeminal motor nucleus. Additional but smaller projections to Vmo, Vme, and the reticular formation were present.

A more restricted distribution of axon collaterals and boutons in the region dorsal to the trigeminal motor nucleus can be seen in the afferent illustrated in Fig. 6. This neuron (R186) responded to palpation of the ipsilateral temporalis muscle and failed to respond to palpation of the teeth and gingiva. This afferent showed little dynamic sensitivity (dynamic index = 4.5 impulses/s), a low peak frequency (78.4 impulses/s), and failed to silence during the release phase of muscle stretch (Fig. 7). The pseudounipolar cell body of this weakly modulated afferent was located within a cluster of Vme neurons in the rostral portion of the mesencephalic trigeminal nucleus. Note that the axon collaterals and boutons of this afferent, which are dorsal to the trigeminal motor nucleus, are located primarily above the middle portion of Vmo (Figs. 6 and 14D) in contrast to the more caudal distribution of boutons overlying Vmo in R170 (Figs. 5 and 14C). Within Vmo itself, the distribution of axon collaterals was sparser than in the region dorsal to Vmo. A few axon collaterals were also found in Vme, and one collateral projected into the ipsilateral superior cerebellar peduncle. Axon collaterals emerging from the tract of Probst that coursed into the reticular formation also were present at the level of the facial motor nucleus.

The afferent illustrated in Fig. 8 provides an even more extreme example of a preferential distribution of axon collaterals and boutons dorsal to Vmo. This neuron (R160) exhibited an increased firing during jaw opening (Fig. 9) and
when the posterior portion of the masseter muscle was palpated but failed to respond when the teeth and gingiva were probed. The firing of this afferent did not silence during the release phase of muscle stretch. Even though the response of this afferent was recorded 20 min after the infusion of suxamethonium (28.6 mg/kg ip), the afferent showed very little dynamic sensitivity (dynamic index = 17.8 impulses/s) and followed all phases of sinusoidal jaw movement. The HRP-stained, pseudounipolar cell body of R160 was located in the rostral part of the mesencephalic trigeminal nucleus. As seen in Fig. 14E, the majority of this afferent’s en passant and terminal boutons were located in a region dorsal to the

![Diagram](54x310 to 372x729)

**FIG. 3.** Computer-assisted reconstruction of primary-like jaw elevator muscle spindle afferent R161 in sagittal plane. Dotted line, outline of trigeminal motor nucleus. Dorsal is toward page top, rostral toward right. Tr<sub>p</sub>, tract of Probst; Tr<sub>vme</sub>, tract of mesencephalic trigeminal nucleus; Cb, cerebellar collateral; PP, peripheral process; S, soma. Scale bar: 200 μm.

**TABLE 1. Bouton measurements**

<table>
<thead>
<tr>
<th>Animal</th>
<th>Labeling</th>
<th>n</th>
<th>Dynamic Index</th>
<th>Perimeter, μm</th>
<th>Area, μm</th>
</tr>
</thead>
<tbody>
<tr>
<td>R161</td>
<td>HRP</td>
<td>162</td>
<td>61.0</td>
<td>11.5 ± 16.2</td>
<td>8.3 ± 4.7 (0.9–23.1)</td>
</tr>
<tr>
<td>PAR13r</td>
<td>Biotin</td>
<td>264</td>
<td>152.0</td>
<td>9.0 ± 2.4</td>
<td>5.1 ± 2.4 (1.0–12.0)</td>
</tr>
<tr>
<td>PAR14r</td>
<td>Biotin</td>
<td>294</td>
<td>174.0</td>
<td>8.5 ± 2.2</td>
<td>4.6 ± 2.2 (0.9–13.5)</td>
</tr>
<tr>
<td>PAR8r</td>
<td>Biotin</td>
<td>160</td>
<td>178.5</td>
<td>12.0 ± 2.2</td>
<td>5.6 ± 2.3 (1.5–15.9)</td>
</tr>
<tr>
<td>R170</td>
<td>HRP</td>
<td>513</td>
<td>10.0</td>
<td>11.7 ± 1.1</td>
<td>8.2 ± 5.5 (1.2–51.4)</td>
</tr>
<tr>
<td>R186</td>
<td>HRP</td>
<td>627</td>
<td>4.5</td>
<td>12.0 ± 4.5</td>
<td>6.0 ± 2.2 (0.2–37.6)</td>
</tr>
<tr>
<td>R160</td>
<td>HRP</td>
<td>451</td>
<td>17.8</td>
<td>11.7 ± 4.1</td>
<td>8.2 ± 5.5 (1.2–51.4)</td>
</tr>
<tr>
<td>PAR19r</td>
<td>Biotin</td>
<td>116</td>
<td>19.0</td>
<td>12.0 ± 4.5</td>
<td>6.0 ± 2.2 (0.2–37.6)</td>
</tr>
<tr>
<td>PAR20r</td>
<td>Biotin</td>
<td>456</td>
<td>8.4</td>
<td>11.7 ± 4.1</td>
<td>8.2 ± 5.5 (1.2–51.4)</td>
</tr>
<tr>
<td>PAR21r</td>
<td>Biotin</td>
<td>155</td>
<td>4.0</td>
<td>12.0 ± 4.5</td>
<td>6.0 ± 2.2 (0.2–37.6)</td>
</tr>
<tr>
<td>R174</td>
<td>HRP</td>
<td>636</td>
<td>N/A</td>
<td>11.8 ± 4.3</td>
<td>7.9 ± 5.7 (0.6–53.5)</td>
</tr>
</tbody>
</table>

Area and perimeter values are means ± SD with range in parentheses. HRP, horseradish peroxidase; Biotin, biotinamide; N/A, not applicable.
TABLE 2.  Distribution of boutons in the region of the trigeminal motor nucleus

<table>
<thead>
<tr>
<th>Animal</th>
<th>Labeling</th>
<th>Number of Boutons Inside Vmo</th>
<th>Number of Boutons Outside Vmo</th>
<th>Total Boutons</th>
<th>Percentage Inside Vmo</th>
</tr>
</thead>
<tbody>
<tr>
<td>R161</td>
<td>HRP</td>
<td>123</td>
<td>39</td>
<td>162</td>
<td>76</td>
</tr>
<tr>
<td>PAR121</td>
<td>Biotin.</td>
<td>405</td>
<td>225</td>
<td>630</td>
<td>64</td>
</tr>
<tr>
<td>PAR13r</td>
<td>Biotin.</td>
<td>198</td>
<td>160</td>
<td>358</td>
<td>55</td>
</tr>
<tr>
<td>PAR14l</td>
<td>Biotin.</td>
<td>326</td>
<td>129</td>
<td>455</td>
<td>72</td>
</tr>
<tr>
<td>PAR8r</td>
<td>Biotin.</td>
<td>269</td>
<td>131</td>
<td>400</td>
<td>67</td>
</tr>
<tr>
<td>Subtotal</td>
<td></td>
<td>1,321</td>
<td>684</td>
<td>2,005</td>
<td>mean = 67</td>
</tr>
<tr>
<td>R170</td>
<td>HRP</td>
<td>197</td>
<td>316</td>
<td>513</td>
<td>38</td>
</tr>
<tr>
<td>R186</td>
<td>HRP</td>
<td>123</td>
<td>504</td>
<td>627</td>
<td>20</td>
</tr>
<tr>
<td>R160</td>
<td>HRP</td>
<td>83</td>
<td>368</td>
<td>451</td>
<td>18</td>
</tr>
<tr>
<td>PAR19r</td>
<td>Biotin.</td>
<td>93</td>
<td>203</td>
<td>296</td>
<td>31</td>
</tr>
<tr>
<td>PAR20r</td>
<td>Biotin.</td>
<td>257</td>
<td>302</td>
<td>559</td>
<td>46</td>
</tr>
<tr>
<td>PAR21r</td>
<td>Biotin.</td>
<td>141</td>
<td>241</td>
<td>382</td>
<td>37</td>
</tr>
<tr>
<td>Subtotal</td>
<td></td>
<td>894</td>
<td>1,934</td>
<td>2,828</td>
<td>mean = 32</td>
</tr>
<tr>
<td>R174</td>
<td>HRP</td>
<td>306</td>
<td>330</td>
<td>636</td>
<td>48</td>
</tr>
</tbody>
</table>

Vmo, trigeminal motor nucleus. For other abbreviations, see Table 1.

The most rostral portion of Vmo. Some axon collaterals also projected to Vmo, Vme, the dorsomedial portion of the principal sensory trigeminal nucleus (Vpdm), and the reticular formation.

Nondynamically sensitive jaw-muscle spindle afferents labeled with biotinamide had peak frequencies between 52 and 105 impulses/s and dynamic indices that ranged from 4 to 19 impulses/s with a mean of 10.5 impulses/s (Table 1). The general morphology of nondynamically sensitive afferents stained with biotinamide corresponded to the morphology of nondynamically sensitive afferents labeled with HRP. All of the nondynamic biotinamide-stained afferents had a single process that could be followed laterally to exit the brain stem in the motor root of the trigeminal nerve. These afferents also possessed single processes that coursed rostrally in the tract of the mesencephalic nucleus of the trigeminal nerve and terminated in a pseudounipolar cell body. A thinner process bifurcated at the level of the trigeminal motor nucleus and traveled caudally in the tract of Probst. Axon collaterals from this caudally directed process coursed ventrolaterally through the reticular formation to reach the dorsomedial portion of the spinal trigeminal nucleus. The largest number of axon collaterals from these neurons were located in the region dorsal to the trigeminal motor nucleus. No axon collaterals of biotinamide-stained nondynamically sensitive afferents were found in the cerebellar peduncle.

The most distinctive morphological feature of nondynamically sensitive afferents, stained either with HRP or biotinamide, was that the distribution of axon collaterals and boutons were densest in the region dorsal to the trigeminal motor nucleus. The local distribution and density of this projection within the region dorsal to Vmo, however, varied among nondynamically sensitive afferents. In some neurons (Figs. 5 and 14C), axon collaterals and boutons tended to be located dorsal to the middle and caudal portions of Vmo, whereas in other instances, the distribution was located more rostrally (Figs. 8 and 14E). Although swellings on axon collaterals in the area dorsal to Vmo were generally not closely associated with Nissl-stained neurons, a case in which they were is shown in Fig. 10A. Although the largest number of collaterals and boutons of nondynamically sensitive afferents were located dorsal to Vmo, all of these afferents also possessed some axon collaterals and boutons within Vmo. In most instances, these terminal and en passant swellings were not closely associated with trigeminal motoneuron somata. Figure 10 (E and F), however, shows examples in which boutons were adjacent to or overlying Nissl-stained trigeminal motoneurons. Several nondynamically sensitive afferents also possessed axon collaterals with boutons closely opposed to or overlying other Vme neurons (Fig. 11F). More caudally at the level of the facial motor nucleus, axon collaterals emerged from the tract of Probst of all nondynamically sensitive afferents and coursed ventrolaterally into the reticular formation and dorsomedial portion of the spinal trigeminal nucleus. An additional projection into the ipsilateral cerebellar peduncle was observed in two nondynamically sensitive jaw-muscle spindle afferents.

Morphological characteristics of an unclassified jaw-muscle spindle afferent

The response of an HRP-stained afferent that was modulated phasically in a different manner is shown in Fig. 12. This afferent (R174) responded with an increase in firing during the initial portion of the jaw opening phase followed by a silencing when the jaw was held open. During jaw closing, the afferent responded and continued to discharge when the jaw was held closed. Manual manipulation of the mandible revealed that this afferent also could be activated by anteroposterior movement of the mandible and palpation in the region of the posterior temporalis muscle. This afferent failed to respond, however, to palpation of the teeth or gingiva. Afferent R174 exhibited a well-stained central axon...
FIG. 4. Afferent response of secondary-like jaw-muscle spindle afferent. A: intra-axonal response generated during ramp displacement of jaw. B: instantaneous frequency of afferent during ramp and hold displacement of jaw. C: instantaneous frequency of afferent during sinusoidal displacement of jaw. Arrow, direction of jaw opening. Time bar in A is 0.1 s, in B and C, time bars are 1 s.

located in the tract of Vme that could be traced to a pseudounipolar cell body located in the caudal portion of the mesencephalic trigeminal nucleus underlying the anterior portion of the cerebellum. This cell body was juxtaposed to a cluster of several other unlabeled Vme somata. The largest number of axon collaterals of afferent R174 were located within Vmo (Fig. 13). A smaller number of axon collaterals emanated from the tract of Vme into the region dorsal to Vmo with a few collaterals reaching the dorsomedial portion of the trigeminal principal sensory nucleus. More caudally, additional collaterals emanated from the tract of Probst at the level of the facial motor nucleus and coursed ventrolaterally into the reticular formation with a small number of collaterals reaching the dorsomedial portion of the spinal trigeminal nucleus.

Morphology and distribution of boutons

The morphology of 2,389 HRP-stained en passant and terminal swellings on axon collaterals presumed to be synaptic boutons were examined in detail for five afferents in five animals (Table 1). The total number of boutons located on these HRP-stained afferents ranged from 162 to 636 (Table 2). One hundred sixty-two boutons were found on the HRP-stained, dynamically sensitive afferent. The average number of boutons on nondynamically sensitive afferents stained with HRP was 530. The perimeter of these boutons ranged from 2.0 to 41.4 \( \mu m \). The mean perimeter of boutons on the dynamically sensitive afferent was 11.5 \( \mu m \), whereas that for the nondynamically sensitive afferents was 11.9 \( \mu m \). The average area of the boutons on dynamically sensitive afferents was 8.3 \( \mu m^2 \), whereas that for the boutons on nondynamically sensitive afferents was 8.7 \( \mu m^2 \). In three neurons, the presence of a few very large boutons, which ranged from 16 \times 12 to 30 \times 10 \( \mu m \), were observed. These "giant" boutons were located in the region dorsal to Vmo and within the caudal portion of Vme.

The morphology of 1,445 biotinamide-stained en passant and terminal boutons was measured from six afferents in an
additional six animals. Every axonal swelling in the trigeminal motor nucleus and surrounding regions was examined and its position recorded. Due to the thickness of the tissue sections, however, the perimeters of some boutons could not be resolved exactly enough to measure accurately. The total number of boutons that could be measured precisely ranged from 116 to 456. The average number of boutons measured on dynamically sensitive, biotinamide-stained afferents was 239, whereas the average number of boutons measured on nondynamically sensitive afferents stained with biotinamide was 242. The perimeter measurements of the total population of biotinamide-stained boutons ranged from 1.9 to 19.3 μm. The mean perimeter of the dynamically sensitive afferents was 9.1 μm, whereas that of the nondynamically sensitive afferents was 8.4 μm. The average area of the dynamically sensitive afferents was 5.1 μm² in contrast to 4.6 μm² for the nondynamically sensitive afferents. The shape factor for biotinamide-stained boutons ranged from 0.35 to 0.91. The mean shape factor for dynamically sensitive afferents was 0.75, whereas that for nondynamically sensitive afferents also was 0.75. No statistical difference was found between the shape factors of dynamically sensitive versus nondynamically sensitive afferent boutons (t-test; $P = 0.920$; Mann-Whitney $U$-test, $P = 1.0$).

The distribution of 5,469 boutons located in the region of the trigeminal motor nucleus was examined in five HRP-stained and seven biotinamide-stained afferents (Table 2). For dynamically sensitive afferents, an average of 66.8% of boutons located in the region of Vmo were distributed within the confines of the trigeminal motor nucleus. In contrast to this, an average of only 31.7% of boutons on nondynamically sensitive afferents were located within Vmo. When examined as a group, jaw-muscle spindle afferents with high dynamic sensitivity, as indicated by a large dynamic index, consistently distribute a greater percentage of their boutons within the Vmo as compared with surrounding regions.

To examine the distribution of boutons in more detail, the location of afferent boutons in relation to the trigeminal motor nucleus was plotted for five HRP-stained afferents (Fig. 14). Note that for both dynamically sensitive and nondynamically sensitive afferents, the majority of those boutons that are located outside Vmo are located dorsal to Vmo. Within the trigeminal motor nucleus, boutons were distributed to relatively restricted regions for both dynamically sensitive and nondynamically sensitive afferents. The size of boutons located inside the trigeminal motor nucleus was compared with boutons located in regions surrounding Vmo for individual HRP- or biotinamide-stained afferents (Table 3). The boutons of all six of the nondynamically sensitive afferents examined were significantly larger within Vmo than outside it. In only one of four cases ($PAR8r$) was a significant difference in bouton size inside and outside of Vmo found for dynamically sensitive afferents.

**DISCUSSION**

Two morphological features demonstrate that the intracellularly labeled cells presented in this study are first-order neurons. First of all, their peripheral processes were observed exiting the brain stem in the motor root of the trigeminal nerve. Second, the intracellularly labeled somata of these afferents exhibited the pseudounipolar morphology, which, within the brain stem, is unique to Vme neurons and corresponds to that reported in studies where retrograde neuroanatomic tracers have been injected into the muscles of mastication (Capra and Wax 1989; Gottlieb et al. 1984; Nomura and Mizuno 1985; Rokx et al. 1985).

**Physiological response of the afferents**

Several features evident in the physiological responses of the neurons stained in this study indicate that they are jaw-
muscle spindle afferents. One of these distinguishing features is that the afferents responded with increased firing during passive stretching of the jaw-elevator muscles. Additional support for this identification is given by the fact that all of the afferents had single receptive fields restricted to the region of the jaw-elevator muscles. These characteristics are comparable with those previously attributed to jaw-muscle spindle afferents (Appenteng et al. 1978; Inoue et al. 1981; Miyazaki and Luschei 1987). The possibility that the afferents labeled in this study are Vme periodontal afferents was excluded because they failed to respond to probing of the teeth and gingiva and were modulated when the jaw-elevator muscles were stretched. It is also important to consider the level of fusimotor activity during these experiments because fusimotor drive has the capability to alter the afferent response of muscle spindles. Because trigeminal fusimotor axons exit the brain stem in the motor root of the trigeminal nerve, it is impractical to deafferent jaw-elevator muscle spindles. Therefore fusimotor drive in the HRP-labeling experiments was reduced by deeply anesthetizing the animals with barbiturate anesthesia and administering chlorpromazine (Cody et al. 1972). The constant level of fusimotor drive obtained under these conditions is evident in the reproducible patterns of afferent discharge observed during repeated muscle stretches.

Two basic kinds of jaw-muscle spindle afferent response were observed in this study. One type was modulated phasically during stretching of the jaw-closing muscles. This modulation consisted of a marked dynamic sensitivity during muscle stretch and a cessation or substantial reduction when the stretch was released. Afferent response behavior like this is known to be produced by primary muscle spindle afferents (Boyd and Ward 1975; Cooper 1961). Examples of this type of afferent response that show a dramatic sensitivity during stretching of the jaw-closing muscles are illustrated in Figs. 1, A and B, and 2. Because these afferents were recorded after the infusion of suxamethonium, which contracts intrafusal bag fibers (Boyd 1985; Gladden 1976; Smith 1966; Taylor et al. 1993b), the large dynamic response of these afferents is most likely due to bag fiber contraction and is indicative of a primary spindle afferent ending (Taylor et al. 1995). Intracellular recordings during the infusion of suxamethonium were difficult to maintain, and therefore no attempt was made to further subdivide the spindle afferent response types using depolarizing drugs as some extracellular electrophysiological studies have done (Donga and Tay-
FIG. 7. Afferent response of spindle afferent R186. 
A: instantaneous frequency of afferent during ramp and hold displacement of jaw. 
B: instantaneous frequency of afferent during sinusoidal displacement of jaw. 
Arrow, jaw opening, time bars are 1 s.

lor 1995; Price and Dutia 1987; Taylor et al. 1992). Larger peak frequencies and dynamic indices were recorded from dynamically sensitive afferents encountered during the biotinamide staining experiments than during HRP labeling. A strong contributor to these differences was the presence of suxamethonium, which increases the dynamic sensitivity of primary muscle spindle afferents (Rack and Westbury 1966) and which was administered to all animals used for biotinamide-labeling but only a few employed for HRP labeling. When the afferent responses of dynamically sensitive, biotinamide-stained afferents were compared with dynamically sensitive, HRP-stained afferents, which also were exposed to suxamethonium, biotinamide-stained afferents exhibited larger peak frequencies and dynamic indices. A likely contributor to these differences is the presence of gallamine, which more readily blocks neurotransmission from dynamic fusimotor axons onto intrafusal bag fibers (Proske and Carr 1995; Yamamoto et al. 1994) and was not used in the biotinamide protocol. An additional consideration is that chlorpromazine, which was administered in the HRP-staining experiments, has been shown to reduce fusimotor activity and produce a more passive spindle (Cody et al. 1972; Henatsch and Ingvar 1956).

Figure 12 shows an afferent with a more modest dynamic response during the initial phase of jaw opening followed by a strong silencing. This type of response implies that the spindle was only stretched during the initial jaw-opening movement and then was unloaded. Afferents like this characteristically were activated more effectively by anteroposterior movement of the mandible. These afferents were interpreted as primary-like jaw-muscle spindle afferents because of the silencing of their afferent response. It is likely that the spindle unloading that creates this silencing is due to the peripheral muscle receptor being at an oblique angle to the direction of jaw opening and closing. Spindle afferents like this indicate that masticatory muscle spindle afferent feedback can exist during mandibular movements of various directions.

The second type of afferent response seen in this study was only slightly modulated during stretching of the jaw-closing muscles and exhibited tonic activity. This response type showed little dynamic sensitivity during muscle stretch as evidenced by a dynamic index, which ranged from 4.5 to 17.8 impulses/s in the reconstructed afferents illustrated here. Characteristically these afferents also discharged continuously during all phases of stretching of the jaw-closing muscles and essentially mimicked the displacement of the jaw. This type of afferent response previously has been demonstrated to originate from secondary muscle spindle afferents contacting nuclear chain fibers (Boyd and Ward 1975; Cooper 1959). We were also able to examine this afferent response type after the infusion of large doses of suxamethonium and even under these circumstances, the afferent response showed little dynamic sensitivity during stretching.
FIG. 8. Computer-assisted reconstruction in sagittal view of a masseter secondary-like jaw-elevator muscle spindle afferent (R160). Dotted line, location of trigeminal motor nucleus. Dorsal is toward page's top, rostral is toward page right. Scale bar: 500 μm.

of the jaw muscles and no evidence of silencing during the release of muscle stretch. The afferent responses of nondynamically sensitive afferents stained with biotinamide were comparable with those stained with HRP.

**Basic axonal morphology and projections**

The basic axonal trajectory of the afferents stained in this study is comparable with that described previously for unclassified jaw-muscle spindle afferents stained with HRP (Dessem and Taylor 1989; Lingenhöhöld and Friauf 1991; Luo et al. 1991; Shigenaga et al. 1988, 1990) and biotinamide (Luo and Dessem 1995; Luo et al. 1995a) but differs from that reported by Appenteng and co-workers (1985) using Lucifer yellow. Appenteng et al. (1985) contended that the jaw-muscle spindle afferent impulses first reach Vmo and then invade the spinal trigeminal subnucleus oralis followed by supratrigeminal region (Vsup) and finally Vme. In this study, we found no jaw-muscle spindle afferent axonal trajectories consistent with the projection of spindle afferent impulses from Vmo through the spinal trigeminal nuclei to the supratrigeminal region.

**Projection areas of primary-like jaw muscle spindle afferents**

The most distinguishing feature of primary-like jaw-muscle spindle afferents is that their strongest projection, based on the number of axon collaterals and axonal swellings, is to the trigeminal motor nucleus. Consistent with this finding is the previous report by Taylor et al. (1993a) that the largest extracellular field potential generated by primary jaw-muscle spindle afferents in cats is also within the trigeminal motor nucleus. Even though a large number of intracellularly stained primary-like muscle spindle afferent axon collaterals and boutons are located in Vmo, most of the en passant and terminal boutons of these afferents were not closely associated with Nissl-stained trigeminal motoneurons. There was typically, however, a small subpopulation of trigeminal motoneurons, whose soma and proximal dendrites were apposed closely by up to five labeled, primary-like jaw-muscle spindle afferent boutons (Fig. 10, C and D). Although some previous electrophysiological studies have implied that masticatory muscle spindle afferents contact trigeminal motoneuron distal dendrites (Appenteng et al. 1978; Chandler et al. 1980), others (Grimwood et al. 1992; Nozaki et al. 1985) report larger EPSPs with sharp rising phases that may be indicative of the proximal dendritic and somatic contacts found in this study.

In addition to a strong projection to Vmo, all primary-like jaw muscle spindle afferents possessed axon collaterals with en passant and terminal boutons, which were distributed throughout the region dorsal to the trigeminal motor nucleus including an area more medial than the supratrigeminal nucleus described by Lorente de Nó (1922). Because no contacts between primary-like jaw-muscle spindle afferents and Vsup neurons were observed in this study, it remains to be determined how many spindle afferent boutons contact the distal dendrites of trigeminal motoneurons that extend into...
this region (Mong et al. 1988) versus interneurons and therefore whether afferent information from primary-like jaw-muscle spindle afferents is relayed through Vsup.

Caudal to the trigeminal motor nucleus, primary-like jaw-muscle spindle afferents typically possessed five to eight axon collaterals that emerged from the tract of Probst and coursed ventrolaterally into the parvicellular reticular formation. In some instances, collaterals with en passant and terminal boutons were located in the dorsolateral part of the parvicellular reticular formation and the adjacent dorsomedial portion of the spinal trigeminal subnucleus oralis (Vo). Several large, multipolar neurons, in the region of Vo were closely apposed by intracellularly stained primary-like jaw muscle spindle afferent boutons. Falls et al. (1985) have described similar large, multipolar neurons in this region that project to orofacial regions of the cerebellum, suggesting that the contacts observed in this study may represent a disynaptic mossy fiber pathway for the transmission of trigeminal proprioceptive feedback to the cerebellum. Because neurons in Vo and the adjacent reticular formation also are known to project to the thalamus (Travers and Norgren 1983), the trigeminal motor nucleus (Luo and Dessem 1996b; Mogoseanu et al. 1993; Yoshida et al. 1994) and the cervical spinal cord (Dessem and Luo 1996), the possibility also exists that this projection conveys polysynaptic stretch reflexes or proprioceptive information to the thalamus, trigeminal motor nucleus and spinal cord.

Convincing evidence for a projection of primary-like jaw muscle spindle afferents to the mesencephalic trigeminal nucleus and the cerebellum also was found in the present study. The close association between primary-like spindle afferent boutons and caudal Vme neurons that was observed implies that neuronal communication can occur directly between the rostral and caudal portions of the mesencephalic trigeminal nucleus. The occurrence of a primary-like jaw-muscle afferent axon collateral in the ipsilateral superior cerebellar peduncle suggests that muscle spindle feedback can reach the cerebellum without relay and may allow the gain of the jaw-stretch reflex to be regulated (Donga and Dessem 1993).

**Projection areas of secondary-like jaw-muscle spindle afferents**

The strongest projection of secondary-like jaw-muscle spindle afferents, as indicated by both axon collaterals and boutons, was in the region dorsal to the trigeminal motor nucleus. As was the case with primary-like spindle afferent boutons, most of the secondary-like spindle afferent boutons in this region do not appear to contact Nissl-stained neurons. In a few instances, however, intracellularly labeled, secondary-like boutons were observed in close association with neurons dorsal to Vmo. Previous studies have reported that some neurons in this region project to the contralateral trigeminal motor nucleus (Kamogawa et al. 1988; Mizuno et al. 1978; Rokx et al. 1986; Travers and Norgren 1983). Secondary jaw-muscle spindle afferent projections to this area therefore may be involved in polysynaptic jaw muscle stretch reflexes. Neurons in this region also project to the cerebellum (Somana et al. 1980), making this region a potential relay for secondary jaw-muscle spindle proprioceptive feedback to the cerebellum. Although it remains uncertain what percentage of secondary-like jaw-muscle spindle afferent boutons in this region contact interneurons and how many contact the distal dendrites of trigeminal motoneurons, at least some information transmitted via secondary-like jaw-muscle spindle afferents is relayed through interneurons dorsal to Vmo.
A smaller number of secondary-like jaw-muscle spindle afferent boutons were located within the trigeminal motor nucleus than in the region dorsal to Vmo. In a manner similar to the primary-like spindle afferent boutons, only a few secondary-like jaw-muscle spindle afferent boutons were associated closely with the cell bodies and proximal dendrites of trigeminal motoneurons. Even though the number of motoneurons receiving this input is small, multiple boutons were found overlying single motoneurons in some cases, implying that these inputs may be powerful. These inputs from secondary-like jaw-muscle spindle afferents to trigeminal motoneurons provide morphological corroboration of previous electrophysiological data (Appenteng et al. 1978; Taylor et al. 1993a).

In addition to projections at the level of the trigeminal motor nucleus, all secondary-like jaw-muscle spindle afferents had collaterals that emerged from the tract of Probst caudal to Vmo and coursed into the reticular formation and adjacent dorsomedial portion of the spinal trigeminal nucleus. As with primary-like afferents, boutons were observed in the reticular formation and spinal trigeminal nucleus.

Evidence also was found that some secondary-like jaw-muscle spindle afferents project directly to the cerebellum via processes in the ipsilateral superior cerebellar peduncle. Both primary and secondary jaw-muscle spindle afferent feedback, therefore, can be transmitted to the cerebellum without relay.

Secondary-like jaw-muscle spindle afferents whose somata were located rostrally in Vme also were seen to possess axon collaterals with boutons overlying more caudally located Vme neurons. Luo and Dessem (1996a) recently have reported that some Vme, jaw-muscle spindle afferent boutons contact the somata of other Vme, jaw-muscle spindle afferents, although these experiments did not differentiate between muscle spindle afferent types. In this study, we have been able to characterize this circuitry further by demonstrating the involvement of both primary and secondary jaw-muscle spindle afferents in the interaction of Vme neurons. An additional observation within the mesencephalic trigeminal nucleus is that one labeled secondary-like Vme neuron possessed a small dendrite (Fig. 11C). Some previous studies (Capra et al. 1984; Gottlieb et al. 1984; Nomura and Mizuno 1985; Shigenaga et al. 1988; Walberg 1984) have reported a subpopulation of Vme neurons with small dendrites after the injection of neuroanatomic tracers into the jaw-elevator muscles. This study demonstrates that in the rat, some of these multipolar Vme neurons are secondary jaw-elevator muscle spindle afferents.

Comparison with previous attempts to correlate the physiology and morphology of jaw-muscle spindle afferents

Previous attempts to correlate the physiology and morphology of jaw-muscle spindle afferents are limited. Tay-
lor and co-workers (1993a) used spike-triggered averaging to determine the location of unitary field potentials generated from single jaw-muscle spindle afferents in the cat. Due to volume conduction of the recorded potential and the large size of the recording electrode, however, this technique cannot distinguish the detailed morphology of individual afferents including axon collaterals and synaptic boutons. Taylor et al. (1993a), for instance, were unable to detect jaw-muscle spindle afferent projections within Vme or into the cerebellar peduncle nor were they able to discern the relationship between afferent terminations and individual trigeminal motoneurons. In a general sense, however, the results of Taylor et al. (1993a) are similar to those reported here in that Taylor and co-workers reported a large extracellular field potential generated by single secondary jaw-muscle spindle afferents in the region dorsal to the trigeminal motor nucleus and a large field within the trigeminal motor nucleus by primary jaw-muscle spindle afferents.

Shigenaga and co-workers (1988, 1990) also attempted to compare the physiological responses of jaw-muscle spindle afferents with their axonal morphology using intracellular HRP staining in the cat. These authors were able to differentiate two morphological types of afferent. One type, which they designated as type I, showed its strongest projection to Vmo. The second type (type II) had the majority of its axon collaterals and boutons in the supratrigeminal region with much sparser projections to Vmo. The morphology of the type I afferents described by Shigenaga et al. (1988) is similar to the morphology of the primary-like jaw-muscle spindle afferents that we describe here. The type II axonal morphology reported by Shigenaga et al. (1988) resembles that of the secondary-like jaw-muscle spindle afferents reported here. Shigenaga et al. (1990), however, reported no relationship between the response of the spindle afferent and these morphological types and therefore concluded that there was no correlation between the type of spindle afferent response and its central morphology. This is completely the opposite from what we report in this study in which it was found that distinct morphological types of jaw-muscle spindle afferent corresponded to distinctly different afferent responses. The most likely explanation for this discrepancy is that Shigenaga et al. (1990) recorded the response of afferents during a step displacement of the jaw, which is inadequate to physiologically characterize muscle spindle afferents. In addition, Shigenaga et al. (1990) did not try to control the fusimotor drive to the spindles or to test any afferents in the presence of suxamethonium to see if the bag fibers could be activated. In a more recent study (Yabuta et al. 1996), a few afferents were recorded during ramp and hold displacement of the jaw before intracellular staining. These authors, however, provide no quantitative measure of the dynamic sensitivity of the afferents making their classification into traditional muscle spindle afferent types problematic.

Comparison with muscle spindle projections in the spinal cord

The central projection of jaw-muscle spindle afferents resembles the projection pattern of limb muscle spindle afferents in a number of ways. In the lumbosacral spinal cord, HRP-stained primary muscle spindle afferents show their strongest projection to the motor nuclei (Brown and Fyffe 1978; Burke et al. 1979; Honga et al. 1987; Ishizuka et al. 1979). Similarly, the primary-like jaw-muscle spin-
Jaw-muscle spindle afferents reported here have their strongest projection to the trigeminal motor nucleus. Primary jaw-muscle spindle afferents, however, appear to directly contact a smaller percentage of the motoneuron pool than hindlimb spindle afferents based on the number and distribution of boutons located within the motor nucleus (Honga et al. 1987; Ishizuka et al. 1979). An additional consideration, however, is that some jaw-muscle spindle afferent boutons dorsal to the anatomically defined trigeminal motor nucleus may be contacting the distal dendrites of trigeminal motoneurons. Presently, however, the relationship between masticatory muscle spindle afferents and motoneurons appears to resemble more closely the distribution of primary muscle spindle afferents in the cervical spinal cord; these afferents make monosynaptic contacts with only ~10% of neck motoneurons (Keirstead and Rose 1988a,b).

Hindlimb secondary muscle spindle afferents project mainly to interneurons located in laminae V, VI, VII (Fyffe 1979). The region dorsal to the trigeminal motor nucleus may be homologous because secondary-like jaw-muscle spindle afferents project predominately to this region. Some spinal secondary spindle afferents make monosynaptic contacts with motoneurons (Kirkwood and Sears 1974; Stauffer et al. 1976); the close association of secondary-like jaw-muscle spindle afferents reported here provides evidence for comparable monosynaptic connections in the trigeminal system.

**Bouton distribution and morphology**

Previous studies (Bae et al. 1996; Conradi et al. 1983; Luo and Li 1991; Luo et al. 1995a,b) provide ultrastructural evidence that swellings on HRP- and biotinamide-filled axon collaterals are synaptic boutons. In these studies, a few additional synapses usually are revealed during electron microscopic analysis, implying that the bouton counts presented in this study are likely to be an underestimate of the total number of jaw-muscle spindle afferent synapses.

The total number of boutons present on the spindle afferents stained with HRP in this study ranged from 162 to 636 with a mean of 478, whereas those labeled with biotinamide ranged from 296 to 559 with a mean of 408. By comparison, Shigenaga and co-workers (1990) reported a range of 245 to 2,182 HRP-stained boutons with a mean of 1,219 boutons on intra-axonally stained cat jaw-muscle spindle afferents. Although the sample sizes in both of these studies are small, these data imply that rat jaw-muscle spindle afferents possess fewer synaptic boutons than those of cats.

A small number of the swellings on primary- and secondary-like axon collaterals in Vme and the supratrigeminal region are very large (16 × 12 to 30 × 10 μm). In the cat, Shigenaga and co-workers (1990) also have reported a few jaw-muscle spindle afferent boutons that were >7 μm. These putative giant boutons appear to be comparable with those found on Ia fibers in Clarke’s column; those on Clarke’s...
column are \( \pm 20 \mu m \) in length and contain multiple release sites (Walmsley et al. 1987). Ultrastructural analysis of these Vme axonal structures is needed to confirm their synaptic nature and determine the number of release sites that they contain.

The distribution of boutons in the region surrounding the trigeminal motor nucleus differed for primary- and secondary-like jaw-muscle spindle afferents. Primary-like afferents had 48–76% (mean = 64%) of their boutons within the confines of Vmo in contrast to 18–46% (mean = 32%) for secondary-like afferents. The largest percentage of secondary-like jaw-muscle spindle afferent boutons were located in the supratrigeminal region dorsal to Vmo. These secondary-like afferent boutons were statistically larger within Vmo than those outside of the trigeminal motor nucleus whereas primary-like spindle afferent boutons showed no significant size differences inside and outside of Vmo. Further support for this difference in bouton size is provided by a previous study (Dessem and Taylor 1989), which reported that the boutons of unclassified rat jaw-muscle spindle afferents dorsal to the motor nucleus are smaller than those within the motor nucleus. Pierce and Mendell (1993) have shown that the volume of spinal cord Ia affrent boutons is correlated in a positive manner with active zone number and total active zone area. This observation, coupled with the fact that larger terminals have been shown to release greater amounts of transmitter and have larger postsynaptic effects (Kuno et al. 1971, 1973), make it conceivable that secondary jaw-muscle spindle afferent boutons within the motor nucleus may be more powerful than those in surrounding regions.

**Functional considerations**

These findings demonstrate that there are distinct morphological differences between jaw-muscle spindle afferents that transmit jaw muscle velocity and positional feedback to the brain stem. Primary jaw-muscle spindle afferents capable of providing velocity and dynamic muscle feedback project most strongly to the trigeminal motor nucleus. Secondary jaw-muscle spindle afferents project most heavily to interneuronal regions, suggesting that positional feedback from the jaw muscles arrives at trigeminal motoneurons primarily via relayed pathways. The results of this study also imply a partitioning of monosynaptic sensory feedback from jaw-muscle spindle afferents to small groups of trigeminal motoneurons similar to that described in the spinal cord (Stuart et al. 1988). Future studies will be needed to determine how this sensory partitioning relates to the compartmentalization of the jaw muscles and their differential contraction.

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Address for reprint requests: D. Dessem, Dept. OCBS, Room 4G-15, University of Maryland Dental School, 666 W. Baltimore St., Baltimore, MD 21201-1586.

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