Synaptic Inputs to Trigeminal Primary Afferent Neurons Cause Firing and Modulate Intrinsic Oscillatory Activity

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Verdier, Dorly, James P. Lund, and Arlette Kolta. Synaptic inputs to trigeminal primary afferent neurons cause firing and modulate intrinsic oscillatory activity. J Neurophysiol 92: 2444–2455, 2004; 10.1152/jn.00279.2004. In this paper, we investigated the influence of synapses on the cell bodies of trigeminal muscle spindle afferents that lie in the trigeminal mesencephalic nucleus (NVmes), using intracellular recordings in brain stem slices of young rats. Three types of synaptic responses could be evoked by electrical stimulation of the adjacent supratrigeminal, motor, and main sensory nuclei and the intertrigeminal area: monophasic depolarizing postsynaptic potentials (PSPs), diphasic PSPs, and all or none action potentials without underlying excitatory PSPs (EPSPs). Many PSPs and spikes were abolished by bath-application of 6,7-dinitroquinoxaline (DNQX) alone or combined with t,1,2-aminooxyphosphonovatric acid (APV), suggesting that they are mediated by non-N-methyl-D-aspartate (NMDA) and NMDA glutamatergic receptors, while some action potentials were sensitive to bicuculline, indicating involvement of GABA receptors. A number of cells showed spontaneous membrane potential oscillations, and stimulation of synaptic inputs increased the amplitude of the oscillations for several cycles, which often triggered repetitive firing. Furthermore, the oscillatory rhythm was reset by the stimulation. Our results show that synaptic inputs to muscle primary afferent neurons in NVmes from neighboring areas are mainly excitatory and that they cause firing. In addition, the inputs synchronize intrinsic oscillations, which may lead to sustained, synchronous firing in a subpopulation of afferents. This may be of importance during rapid biting and during the mastication of very hard or tough foods.

INTRODUCTION

It is now well established that the cell bodies of primary afferent neurons located within the dorsal root and trigeminal ganglia (DRG and TG, respectively) express receptors for a variety of neurotransmitters and neuroactive substances (Huettner 1990; Kilpatrick et al. 1989; Sahara et al. 1997) and that a number of putative neurotransmitters applied in vitro cause changes in membrane properties (Cook et al. 1997; Gallagher et al. 1978; Takeda et al. 2002). Although these data suggest that incoming sensory information could be modulated by the local release of transmitters, it is known that there are very few synaptic inputs within the ganglia (Yamamoto and Kondo 1989) and that the cell bodies of DRG neurons are insulated from the extracellular environment by satellite cells (Lieberman 1976).

Modulation of sensory transmission by synaptic inputs to primary afferent somata is more likely to occur in a particular subset of primary afferent neurons that have their cell bodies within the CNS, in the trigeminal mesencephalic nucleus (NVmes). These neurons are contacted by axo-somatic synaptic boutons (Liem et al. 1991, 1992; Zhang et al. 1997) that contain a variety of putative neurotransmitters (see Lazarov 2002 for review). They are depolarized by exogenous application of GABA, ATP, glutamate, and nitric oxide (Hayar et al. 1997; Khakh et al. 1997; Pelkey and Marshall 1998; Pose et al. 2003; Verdier et al. 2003). Like DRG and TG neurons, adult NVmes neurons have no dendrites in most species. Their single axon gives rise to a peripheral branch that innervates either the spindles of jaw closing muscles (Alvarado-Mallart et al. 1975; Capra et al. 1985; Shigenaga et al. 1988a), or pressoreceptors of the periodontal ligaments (Byers et al. 1986; Jerge 1963; Shigenaga et al. 1988c), and to a central branch that descends to the upper cervical cord (Shigenaga et al. 1988b). NVmes receives inputs from several structures in the forebrain, midbrain (Copray et al. 1990; Krettek and Price 1978; Nagy et al. 1986), and lower brain stem (Bae et al. 1997; Buissere-Delmas et al. 1997; Rokx et al. 1988; Ter Horst et al. 1991).

Like DRG cells (Amir et al. 1999), NVmes neurons are endowed with a set of conductances that produce high-frequency subthreshold membrane oscillations and repetitive discharge at slightly depolarized potentials (Pedroarena et al. 1999; Wu et al. 2001), and it has been shown that application of the ionotropic glutamate receptor agonists AMPA, kainate, and N-methyl-D-aspartate (NMDA) can elicit subthreshold oscillations and trigger action potentials in NVmes neurons (Pelkey and Marshall 1998). Some NVmes neurons are electrically coupled to one or two neighbors (Baker and Llinas 1971; Hinrichsen 1970), and this could help to spread and synchronize repetitive firing. It was suggested many years ago that synapses may control the degree of coupling because they are often found next to gap junctions (Bennett 1972).

We have previously described the effect of stimulation of local interneurons on action potential propagation along the central axons of NVmes neurons (Verdier et al. 2003). In this paper, we will describe the effects of synaptic inputs on the electrical properties of the soma, including their ability to trigger action potentials and modulate intrinsic oscillatory behavior.

METHODS

Preparation of slices

Crystals of the carbocyanine dye 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate DiI [DiIC18 (3); Molecular
Probes, Eugene, OR) were injected into the masseter muscles of Sprague-Dawley rat pups (1–2 days old; Charles River, Montreal, Quebec, Canada) under hypothermic anesthesia and allowed to diffuse for 9–28 days. The animals were anesthetized by methoxyflurane (Metofane, Janssen Pharmaceuticals, North York, Ontario, Canada) or isoflurane United States Pharmacopeia (Abbott Laboratories, Saint-Laurent, Quebec, Canada) inhalation and decapitated, and the brain stem was removed. It was put into ice-cold sucrose artificial cerebrospinal fluid (ACSF; composition in mM: 225 sucrose, 5 KCl, 1.25 KH₂PO₄, 4 MgSO₄, 0.2 CaCl₂, 20 NaHCO₃, and 10 d-glucose and saturated with 95% O₂-5% CO₂), embedded in agar (Agahajian and Rasmussen 1989), and cut into 400-μm transverse slices using a Vibratome (Electron Microscopy Study). The slices were transferred to an interface-type chamber saturated with a humidified mixture of 95% O₂-5% CO₂. They were perfused for 20 min with sucrose ACSF and then for 20 min with a mixture (50%-50%) of sucrose ACSF and normal ACSF (in mM: 125 NaCl, 5 KCl, 1.25 KH₂PO₄, 1.3 MgSO₄, 2.4 CaCl₂, 26 NaHCO₃, and 25 d-glucose and saturated with 95% O₂-5% CO₂) and finally, with normal ACSF alone, at a rate of 1 ml/min, at 29–31°C. In some experiments, the CaCl₂ was removed from the normal ACSF, and the MgSO₄ was increased to 3.8 mM. All procedures for dye injections and slice preparation conformed with National Ethics Committee Guidelines and were approved by an Institutional Ethics Committee.

Electrophysiology

The slices were viewed at low magnification (50×) under an epifluorescence microscope (Nikon), and the pool of Dil-labeled cell bodies of masseteric spindle afferents in NVMes was targeted for recording with glass microelectrodes (80–200 MΩ) filled with neurobiotine 2% dissolved in potassium acetate (1 and 3 M). Data were recorded through a bridge circuit with an Axoclamp 2B amplifier (Axon Instruments, Foster City, CA) or a BVC-700 amplifier (Corning) and sampled at 20 kHz. Data were stored on disk and analyzed using pClamp 6–8 software (Axon Instruments). Synaptic responses and antidromic action potentials were evoked by electrically stimulating regions of the slices through bipolar electrodes made of twisted nichrome wire (25 μm diam), insulated except at the tip. The intensity (0.01–7 mA) and duration (0.05–0.3 ms) of stimulus were varied to obtain optimal effects. We defined the action potentials as “antidromic” because the stimulating electrode was placed in regions through which traveled the central axon, or branches of the peripheral, central and stem axons. However, it is possible that some of the action potentials were generated by stimulation of the stem or peripheral axon.

Drug application

6,7-dinitroquininaloxine (DNQX; 10 μM; RBI), d,l-2-amino-5-phosphonovaleric acid (APV; 75 μM; Sigma), picrotoxine (PTX; 50 μM), and bicuculline methiodide (BIC; 10 μM; Sigma) were bath-applied using a syringe pump to block amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate, N-methyl-d-aspartate (NMDA), and GABAₐ receptor-mediated currents, respectively.

Labeling

Depolarizing current pulses (1.0–1.5 nA, 1 Hz, 500-ms duration) were passed through the recording electrode for 20–30 min to eject neurobiotine into some of the NVMes neurons. At the end of the experiment, the slices were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (PB). They were then treated with 0.5% H₂O₂ for 1 h, washed three times (10 min) in PB, incubated overnight in an avidin-biotin complex (ABC kit, Vector Laboratories), and reacted with 3,3’-diaminobenzidine tetrahydrochloride (DAB; 0.05%) and H₂O₂ (0.03%) in PB buffer. After dehydration, they were mounted with Entelan and examined under a light microscope.

Analysis

Only data from neurons with resting membrane potential (RMP) equivalent or more negative than −50 mV and which had overshooting action potentials were analyzed. The input resistance of NVMes cells was measured with square current step injections (0.2 nA, 500–650 ms). Response onset latencies and rise and decay times were measured. The height of action potentials was measured from RMP, and their latency was measured from the beginning of the stimulus artifact to spike onset. The amplitude of the afterhyperpolarization (AHP) was measured from RMP, and its duration was measured from the point at which the spike downstroke crossed the spike threshold to the return to RMP. Synaptic response latencies were measured on individual traces from the beginning of the stimulus artifact to the foot of the rising phase of the postsynaptic potential (PSP). The amplitude of the subthreshold PSPs was measured from the baseline to the peak of the average of all responses. In oscillating neurons, positive and negative peaks of membrane potential oscillations and of action potentials were detected automatically by custom-made software, and instantaneous frequencies were calculated. Stimulus triggered averaging was carried out on data from several trials. Quantitative variables were expressed as mean ± SE. Differences between means were compared with Student’s t-test (SigmaStat software, SPSS, Chicago, IL) and were considered to be significant if the probability of a type error was <0.05.

Results

Anatomy

Two hundred seventy-eight neurons recorded from NVMes fulfilled the inclusion criteria. Eighty-one were successfully filled with neurobiotine, and all had the typical unipolar morphology of primary sensory neurons with only one process emerging from a round or ovoid cell body (Fig. 1A). Twelve were seen to be coupled to another cell following a single injection of neurobiotine: six to another unipolar neuron (Fig. 1B), while six others appeared to contact a multipolar neuron with extensive dendritic arbor. The junction between the NVMes neuron and the multipolar neuron was somato-somatic in three cases (Fig. 1C) and somato-dendritic in the three remaining cases (Fig. 1D).

Electrophysiological properties

The neurons had a RMP of −56 ± 0.2 mV and an input resistance of 28 ± 1.0 MΩ. Firing threshold was −46 ± 0.4 mV, and action potentials had an amplitude of 71 ± 1.0 mV, with a half-width (HW) of 0.54 ± 0.01 ms. The spike was followed by a pronounced but short-lasting AHP (9.5 ± 0.2 mV, 6.3 ± 0.4 ms). Almost all the recorded cells (99%) showed an off-spike at the end of hyperpolarizing pulses (Fig. 2A), while 95% of them exhibited a prominent sag with strong hyperpolarization (Fig. 2B, arrow). Accommodation of firing during depolarizing pulses, as shown in Fig. 2B, occurred in 64% of the cells, one-half of them discharging only one to three action potentials, even with long-duration pulses (100–600 ms). The I-V curve shown in Fig. 2C is typical of the majority (64%) of NVMes neurons and reveals an inward rectification during hyperpolarization and an outward rectification during depolarization. Ninety-nine neurons (36%)
showed subthreshold membrane oscillations spontaneously (Fig. 2D) or during injection of a small depolarizing current. The mean frequency of this oscillatory activity was 104 ± 4 Hz (range, 53–150 Hz), and peak-to-peak amplitude was 3.0 ± 0.2 mV. The oscillatory activity led to spontaneous discharge in 11 cells (11% of oscillating cells, Fig. 2D), and about 70% of the oscillating cells could be made to fire repetitively by membrane depolarization (Fig. 2E, top). The amplitude of the oscillations increased with depolarization until strong repetitive firing was elicited (Fig. 2E, 1st and 2nd). However, the frequency did not change markedly with the level of depolarization. Membrane hyperpolarization suppressed the oscillations in all cells (Fig. 2E, bottom).

All the electrophysiological properties mentioned above were found in 20 cells recorded in a calcium-free medium to prevent synaptic transmission. The electrophysiological properties of the cells that were coupled, whether to another afferent or to a multipolar neuron, were not different from those of the uncoupled neurons. The coupled cells had an input resistance of 27 ± 2 MΩ, a short-duration action potential (HW: 0.56 ± 0.03 ms), an off-spike after hyperpolarization, inward rectification during membrane hyperpolarization, and accommodation of firing with depolarizing pulses. Membrane potential oscillations and burst firing with depolarization were observed in four cases.

**Responses to stimulation**

A total of 421 sites were stimulated within four areas adjacent to NVmes while recording from the 278 neurons. The four stimulated regions were the supratrigeminal area (SupV), intertrigeminal area (IntV), motor trigeminal nucleus (NVmt), and the dorsal and medial part of the principal trigeminal sensory nucleus (NVsmp). The recording (R) and stimulating (S) positions are indicated on the schematic drawing of the slice in Fig. 3A.

**Action potentials caused by direct stimulation of the axon**

Very-short-latency (0.7 ± 0.02 ms; range, 0.4–1.1 ms) action potentials such as the one shown in Fig. 3B were evoked from all sites and were the most common response (n = 134; see Table 1). The amplitude and duration (69 ± 1 mV and HW: 0.51 ± 0.01 ms, respectively) of these spikes and of the AHP that followed them (9.3 ± 0.3 mV, 7.3 ± 0.4 ms) were similar to those of spikes occurring spontaneously or evoked by injection of depolarizing current, and all could follow high-frequency stimulation (100–250 Hz). Most (69%) disappeared with strong membrane hyperpolarization, but no underlying excitatory PSPs (EPSPs) were uncovered (Fig. 3B, bottom). Because of these characteristics, we assumed that these short-latency action potentials were evoked by direct stimulation of the axon of the investigated neuron. This was confirmed in 20 cases that were studied in calcium-free medium. Figure 3C shows an example of an antidromic action potential at RMP (left top) recorded under these conditions. The characteristics of these 20 antidromic spikes were similar to those described above (amplitude: 74 ± 2 mV; HW: 0.52 ± 0.03 ms; AHP amplitude: 9.0 ± 0.8 mV; AHP duration: 6.4 ± 0.5 ms). Their onset latency (0.6 ± 0.03 ms) ranged from 0.4 to 0.9 ms, and all could follow high-frequency (150–250 Hz) stimulation without alteration (Fig. 3C, right). The action potential remained on strong membrane hyperpolarization in nine cells and disappeared in the remaining cells (Fig. 3C, left bottom). However, some short-latency action potentials appeared to be synaptically mediated because they were blocked by bath-application of antagonists of AMPA/kainate or GABA_A receptors (Synaptic action potentials). On the basis of the observation that all antidromic spikes evoked in calcium-free ACSF occurred within 1.0 ms from stimulation, we chose to regard short-latency action potentials recorded in calcium-containing ACSF as being mediated synaptically only if they had a latency >1.5 ms and/or were blocked by receptor antagonists (n = 14). None of the others will be discussed further in this paper.

**Synaptic responses**

Eighty-eight postsynaptic responses were recorded from 76 cells. Thirty-five of the 76 neurons with synaptic responses were successfully filled with neurobiotine. These neurons were...
distributed over the rostrocaudal and dorsoventral extent of NVmes, except in the long anterior column of NVmes, which was not investigated. Eight were coupled to other neurons: four to primary afferents and four to multipolar neurons. We separated the responses into four groups: monophasic depolarizing PSPs, all or none synaptic action potentials, biphasic PSPs, and enhancement of oscillations. Table 1 summarizes the distribution of the responses according to stimulus type.

Monophasic PSPs

Twenty-eight monophasic depolarizing PSPs were obtained from the four sites (Table 1). However, in one-half of these \((n = 14)\), it was necessary to hyperpolarize the cell to about \(-76 \text{ mV}\) to detect the PSP. In the example shown in Fig. 4A, a stimulus of 400 \(\mu\text{A}\) produced no response at RMP, but a slight increase in stimulus intensity (410 \(\mu\text{A}\)) elicited an action potential. Membrane hyperpolarization disclosed a subthreshold PSP (Fig. 4A, bottom), while depolarization led to repetitive poststimulus action potentials (Fig. 4A, top). The subthreshold PSPs occurred at a very short latency (0.9 \(\pm\) 0.1 ms), had a short duration (8.3 \(\pm\) 0.9 ms), and rapid rise and decay times (0.6 \(\pm\) 0.1 and 5.1 \(\pm\) 0.7 ms, respectively). The spikes recorded at RMP followed high-frequency stimulation (sometimes \(\pm 250 \text{ Hz}\)), but in each case, subthreshold responses could be distinguished. In three cases, bath-application of DNQX (\(n = 3\); Fig. 4B) eliminated the spikes and the subthreshold PSPs without changing the membrane potential. The synaptic nature of these responses is also supported by the finding that six cells showing this type of response were filled with neurobiotine, and all were seen to be single uncoupled unipolar neurons.

Another type of monophasic depolarizing PSP with an average amplitude of 2.4 \(\pm\) 0.4 mV was obtained at RMP in 14 cells. These occurred at a considerably longer latency (5.9 \(\pm\) 0.5 ms) and had slower kinetics (51 \(\pm\) 10 ms duration; 5.6 \(\pm\) 1.1 ms rise time; and 32 \(\pm\) 7 ms decay time) than the preceding type of PSP. These differences in latency and duration were statistically significant (\(P < 0.05\)). Voltage dependency was assessed by hyperpolarizing and depolarizing the cell with current injection 40–80 ms prior to electrical stimulation. Hyperpolarization increased PSP amplitude in all cases (Fig. 5, A and B, bottom), while the PSP triggered an action potential in eight of these cells when they were depolarized (Fig. 5A, left top). In the remaining cases (\(n = 6\)), depolarizing the cells up to \(-45 \text{ mV}\) eliminated the PSP (Fig. 5B, top), but the response did not reverse with greater depolarization (\(\pm 5 \text{ mV}\)). Five of the long-latency monophasic PSPs were tested with high-frequency stimulation, and only one could follow 100 Hz; the others failed at 60 Hz or less. Bath application of DNQX alone eliminated the depolarizing PSP in two cells (Fig. 5C) and decreased it in three other cases (Fig. 5D, 2nd trace). The addition of APV eliminated the remaining component in two of these (Fig. 5D, bottom). Seven cells with this kind of response were filled with neurobiotine: four were single unipolar neurons, and three others were coupled to a multipolar neuron.

Synaptic action potentials

Responses recorded from 15 neurons following electrical stimulation of the four nuclei were classified as synaptic action potentials (Table 1) rather than antidromic responses because of their latency (\(> 1.5 \text{ ms}\)) and suppression by antagonists of synaptic transmitters. Like the example in Fig. 4A, these action potentials appeared fully formed at RMP as stimulus intensity was increased (Fig. 6A, left top), but unlike Fig. 4A, no PSP could be disclosed by hyperpolarization (Fig. 6A, right). Their
mean onset latency was 3.2 ± 0.5 ms (range, 1.0–6.6 ms). This is significantly longer than that of the antidromic potentials (0.6 ± 0.03 ms, \( P < 0.001 \)). Their amplitude and duration were 69 ± 3 mV and 0.66 ± 0.06 ms, respectively. AHP amplitude and duration were 10 ± 1 mV and 6.1 ± 0.7 ms, respectively. Membrane hyperpolarization eliminated the synaptic spike in seven cells (Fig. 6A, right bottom), but had no effect in the remaining eight cases. Three synaptic action potentials evoked by stimulation of SupV were blocked by bath-application of DNQX (Fig. 6B), and two others were blocked by BIC (Fig. 6C). The three blocked by DNQX were impaired by membrane hyperpolarization, while the ones blocked by BIC were not. Three of the pharmacologically tested spikes had a short onset latency (1.0–1.2 ms) and could follow high-frequency stimulation (\( \approx 200 \) Hz). The latency of the remaining spikes (\( n = 12 \)) ranged from 1.5 to 6.6 ms (4.6 ± 0.6 ms), and they could follow repetitive stimulation up to frequencies of between 50 and 130 Hz (e.g., Fig. 6A, right bottom). Six cells with all or none synaptic action potentials were filled with neurobiotine: four were single unipolar neurons, and two were coupled to another unipolar neuron.

### Biphasic PSPs

Biphasic PSPs were evoked by stimulation of SupV, IntV, and NVsnpr. Six of them consisted of a depolarizing potential that decayed in two phases (Fig. 7A, middle). The latency was 4.6 ± 0.5 ms, amplitude was 3.2 ± 0.6 mV, and rise time was 3.6 ± 1.1 ms. At hyperpolarized potentials, the responses became monophasic, and amplitude and duration increased (Fig. 7A, bottom), while an action potential was triggered in the depolarized state (Fig. 7A, top). The two neurons filled with neurobiotine were noncoupled unipolar cells.

Sustained depolarization was characteristic of this type of biphasic response, and after several stimuli (50–125 Hz), the cell remained depolarized \( \leq 150 \) ms. In the example shown in Fig. 7B, four stimuli at 50 Hz (Fig. 7B, top) caused prolonged depolarization, while stimulation at 80 Hz elicited an action potential during the poststimulus depolarization (Fig. 7B, bottom).

The remaining biphasic responses (\( n = 22 \)) began with a positive component followed by a negative one (Fig. 7C, middle). The amplitude of the positive peak ranged from 1.3 to 6.7 mV (4.3 ± 0.3 mV), with a rise time of 1.27 ± 0.35 ms. The negative peak was of lower amplitude (1.4 ± 0.2 mV). Most (\( n = 14 \)) began at an extremely short onset latency (1.0 ± 0.1 ms; range, 0.6–1.4 ms), but some (\( n = 7 \)) occurred at a much longer onset latency (7.8 ± 1.6 ms; range, 4.3–16.4 ms). Hyperpolarization abolished both components of the response in 11 of 16 cases tested, and only the negative component in 5 cases (Fig. 7C, bottom). At depolarized potentials, stimulation elicited an action potential in most cells (Fig. 7C, top). Eleven neurons with these biphasic PSPs were filled with neurobiotine: eight were single unipolar neurons, two were coupled to a unipolar neuron, and one was coupled to a multipolar cell.

### Table 1. Responses of NVmes neurons to stimulation of adjacent areas

<table>
<thead>
<tr>
<th>Stimulation Site</th>
<th>Latency of PSPs, ms</th>
<th>Monophasic PSPs</th>
<th>Biphasic PSPs</th>
<th>Enhancement of Oscillatory Behavior</th>
<th>Antidromic Spikes*</th>
<th>Short-latency Action Potentials†</th>
<th>No Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>SupV (( n = 214 ))</td>
<td>2.4 ± 0.5</td>
<td>0</td>
<td>23</td>
<td>15</td>
<td>17</td>
<td>97</td>
<td>38</td>
</tr>
<tr>
<td>IntV (( n = 113 ))</td>
<td>4.9 ± 1.0</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>0</td>
<td>3</td>
<td>18</td>
</tr>
<tr>
<td>NVmt (( n = 49 ))</td>
<td>2.1 ± 0.6</td>
<td>6</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>16</td>
<td>24</td>
</tr>
<tr>
<td>NVsnpr (( n = 45 ))</td>
<td>4.1 ± 0.9</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>34</td>
</tr>
<tr>
<td>Total (( n = 421 ))</td>
<td>28</td>
<td>15</td>
<td>28</td>
<td>17</td>
<td>20</td>
<td>134</td>
<td>179</td>
</tr>
</tbody>
</table>

Values are mean ± SE. *Recorded in calcium-free ACSF. †Not considered in this study due to uncertainty about their antidromic or synaptic nature. NVmes, trigeminal mesencephalic nucleus; PSP, postsynaptic potential.
Effects on oscillatory activity

Electrical stimulation of SupV triggered oscillatory activity in one neuron. In 16 additional neurons, electrical stimulation of SupV (n = 14) or the adjacent regions of NVsnpr (n = 1) and NVmt (n = 1) modified existing oscillations. Stimulation of inputs to these neurons during periods of quiescence evoked a few (Fig. 8A) or several (Fig. 8A1) oscillatory cycles. When stimulation occurred during spontaneous oscillations (Fig. 8B), it increased the amplitude of both the positive and negative component of the waveform (peak-to-peak amplitude: 5.1 ± 0.4 mV vs. spontaneous 2.7 ± 0.2 mV, paired t-test, P < 0.05, n = 16; Fig. 8B) without changing the mean membrane potential. However, there was no difference in the frequency of the oscillations before and after stimulation (94 ± 5 Hz vs. spontaneous 95 ± 6 Hz, paired t-test, P = 0.2, n = 16).

When the neurons were hyperpolarized, the spontaneous and evoked oscillations disappeared, and stimulation caused simple depolarization of PSPs (Fig. 8C, bottom) in 8 of 12 tested cells. When the neurons were depolarized, stimulation triggered repetitive firing in all cells (Fig. 8C, top). The PSPs revealed by hyperpolarization occurred at a latency of 5.6 ± 0.7 ms.

Stimulation reset the spontaneous rhythm. In Fig. 8D, the stimulus coincided with the hyperpolarizing phase of the oscillation in trace 1 and the peak of depolarization in trace 2 (dotted lines 1 and 2). Nevertheless, the positive peak of the first poststimulus cycle occurred at the same latency in the two trials, and the two rhythms remained in synchrony for the next few cycles (dotted lines 3 and 4). However, synchronicity was lost with time (dotted lines 5 and 6). These observations were confirmed by averaging the responses around the time of stimulation (Fig. 8D, bottom). The first few cycles following stimulation remained visible, but averaging eliminated spontaneous oscillations because these were not phase-linked to the stimuli.

The enhancement of spontaneous oscillations by the synaptic inputs augmented the probability of discharge, so that stimulation at RMP elicited single spikes or doublets intermingled with the oscillations (Fig. 8E, top, inset) in four cells or trains of 3–16 action potentials (Fig. 8E, bottom) in six cells, none of which discharged spontaneously at rest. When trains of
action potentials were evoked, the frequency of the action potentials was, in all cases, higher than the frequency of the underlying oscillations (143 / 11006 vs. 114 / 11006 Hz, respectively, paired t-test, $P < 0.05$).

Bath application of DNQX suppressed the poststimulus response from SupV ($n = 2$) and NVmt ($n = 1$) without affecting the spontaneous membrane oscillations (Fig. 8F). Eight of these cells were filled with neurobiotine, and all of them were found to be uncoupled unipolar neurons.

**DISCUSSION**

The results of this study show that synaptic inputs to the somata of some muscle spindle primary afferent neurons of NVmes can elicit action potentials and increase the amplitude of intrinsic membrane oscillations. However, quantitative electronmicroscopic analyses show that the somatic region of NVmes neurons receive few synaptic inputs (Hinrichsen and Larramendi 1970; Honma et al. 2001; Liem et al. 1991). In the rat, no more than five boutons per 100-μm cell perimeter were found (Liem et al. 1991), and <8% of the boutons were in contact with the cell membrane (Liem et al. 1992). This probably explains why we obtained responses from only one-quarter (27%) of the NVmes neurons that we sampled. Nevertheless, our data show that, when present, synaptic inputs have very strong effects, and this is due, in large part, to the distinctive properties of the target neurons.

**Intrinsic properties**

The recorded neurons had the distinctive electrophysiological properties already ascribed to NVmes: inward rectification on membrane hyperpolarization (Khakh and Henderson 1998; Tanaka et al. 2003), accommodation of firing during depolarizing pulses (Del Negro and Chandler 1997), and subthreshold membrane oscillations (Pedroarena et al. 1999; Wu et al. 2001). These provide a recognizable signature for NVmes primary afferents, but in addition, we confirmed that all 81 neurons filled with neurobiotine had the typical unipolar morphology of primary afferents.

**Coupling**

Approximately 14% of our filled neurons were coupled to other neurons, one-half of which were also primary afferents. It has been known for decades that some NVmes neurons are closely coupled to one or two other primary afferents (Hinrichsen 1970; Hinrichsen and Larramendi 1970) and that gap junctions allow action potentials to pass from one neuron to another (Baker and Llinas 1971; Hinrichsen 1970). In this paper, we provide the first evidence that a similar proportion are coupled to small multipolar neurons within NVmes, some of which have been shown to contain GABA (Lazarov and Chouchkov 1995).

**Synaptic inputs**

Stimulation of four cell groups at the caudal end of NVmes, SupV, IntV, NVmt, and NVsnpr, evoked postsynaptic activity in the muscle spindle afferents. Inputs from SupV seem to be the most widespread or effective (29% response rate), followed by NVmt (18%), NVsnpr (17%), and IntV (8%).

**Monophasic depolarizing potentials**

We showed that many monophasic depolarizing PSPs could be blocked by bath-application of DNQX alone or in combination with APV, suggesting that both non-NMDA and NMDA receptors were being activated. NVmes neurons express kainate, AMPA, and NMDA receptor subunits (Mineff et al. 1998; Petralia et al. 1994; Turman et al. 1999), and Pelkey and Marshall (1998) have shown that glutamate depolarizes NVmes neurons in vitro via NMDA and non-NMDA receptors. It is possible that some of these PSPs were generated by the recurrent axon collaterals of NVmes neurons described by Shigenaga et al. (1990).
The lack of dendrites may account for very rapid rise and decay of some of these potentials, but the persistent sodium current ($I_{NaP}$) found in NVmes neurons (Wu et al. 2001) may also contribute to this. In NVmes neurons, $I_{NaP}$ is activated around −69 mV and reaches its peak around −40 mV (Wu et al. 2001). It has been reported that $I_{NaP}$ amplifies AMPA-

FIG. 7. Characterization of biphasic PSPs. A: IntV elicited a depolarizing PSP with 2 decay phases (RMP). With hyperpolarization, it increased in amplitude and duration (bottom). Action potential was triggered during depolarization (top). B: example of sustained depolarization subsequent to the stimulation of IntV at 50 Hz (top). With an increase in the frequency (80 Hz), an action potential was elicited during sustained depolarization (bottom). C: example of a short-latency biphasic PSP (RMP) elicited by stimulation of IntV. Membrane hyperpolarization eliminates the negative component (bottom), while membrane depolarization allowed an action potential to be triggered (top).

FIG. 8. Synaptic effects on membrane oscillations. A and A1: examples of oscillatory potentials in 2 neurons that were triggered by stimulation of SupV (A, average of 18 traces; A1, average of 12 traces). B: stimulation of SupV (bottom) increased the amplitude of the spontaneous oscillations (top). C: middle: displays an oscillatory potential at RMP following stimulation of SupV. Membrane hyperpolarization reveals a monophasic depolarizing PSP (bottom) following stimulation, while membrane depolarization triggers action potentials discharge (top). D: 2 recordings from the same cell show spontaneous membrane oscillations that were clearly out of phase in the 2 cycles (dotted lines 5 and 6). Bottom: average of 12 traces. E: examples of doublet (top) and repetitive discharge (bottom) after stimulation of SupV in 2 different cells. Inset: oscillations at higher magnification. F: bath-application of DNQX abolishes the stimulus related oscillatory activity (right) but does not impair the spontaneous oscillations (left). The 1st and 3rd traces of each panel are single traces. The 2nd and 4th traces are averages of 8 traces.

2451 SYNAPTIC INPUTS TO PRIMARY SENSORY NEURONS

J Neurophysiol • VOL 92 • OCTOBER 2004 • www.jn.org
mediated EPSPs in pyramidal cortical neurons (Gonzalez-Burgos and Barrionuevo 2001), which may explain why action potentials were easily triggered at RMP (mean, $-57 \text{ mV}$). It was necessary to hyperpolarize these neurons, which inactivates $I_{\text{Nap}}$ channels, to see the underlying PSP.

A few depolarizing potentials were greatly impaired by membrane depolarization, suggesting that they depend on a current with an equilibrium potential positive to the RMP, but negative to the cationic currents mediating glutamatergic responses. They are likely mediated by GABA, which depolarizes primary afferent neurons of NVmes (Hayar et al. 1997; Verdier et al. 2003), TG, and DRG (Feltz and Rasminsky 1974; Gallagher et al. 1978; Puil and Spigelman 1988; Valeyev et al. 1999) through GABA$_A$ receptor activation. GABA depolarizes primary afferent neurons because the chloride reversal potential is above RMP. These neurons have a high intracellular concentration of Cl$^-$ because they lack the Cl$^-$ extruder KCC2 (Kanaka et al. 2001). Gallagher et al. (1978) showed that the reversal potential for iontophoretically induced GABA depolarization in cat spinal ganglia neurons was $-23.5 \pm 6.1 \text{ mV}$. In six of our tested neurons, the response disappeared at potentials ranging from $-45$ to $-40 \text{ mV}$, but we were unable to determine the reversal potential of these responses because the cells rectified and could not be depolarized above $-40 \text{ mV}$. Since these depolarizing potentials were modified by voltage changes imposed on the soma, they are likely to be caused by activation of the GABAergic synapses found on the cell body (Chen et al. 2001).

### Biphasic potentials

It is probable that the small number of depolarizing PSPs that showed two phases of decay resulted from the summation of two excitatory inputs to the same cell. However, most of the biphasic responses included a hyperpolarizing phase, and up to now, all tested neurotransmitters depolarize these neurons, including GABA, and they have no receptors for glycine (Lazarov 2002). Glycine, however, would have been depolarizing since it also acts through chloride channels. It is possible that a positive-negative PSP sequence could be the result of electrotonic coupling, with the depolarizing phase mirroring a spike in the adjacent coupled cell, while the hyperpolarizing phase reflects the AHP (Galarreta and Hestrin 1999). We did record positive-negative PSPs in three of seven pairs of coupled neurons, but also recorded similar potentials from cells that appeared to be uncoupled. It seems more likely that the late hyperpolarization is caused by a yet unidentified transmitter or by activation of an intrinsic voltage-dependent hyperpolarizing current triggered by the depolarization.

The four areas from which PSPs were evoked contain a mixed population of glutamatergic and GABAergic cells (Ginestal and Matute 1993; Kolta et al. 2000; Li et al. 1996; Turman and Chandler 1994a,b). Stimulation of SupV caused short latency, perhaps monosynaptic PSPs, in NVmes, which is consistent with anatomical evidence of a direct projection (Rokx et al. 1986). Although there are direct pathways from NVsnpr to NVmes (Buisset-Delmas et al. 1997), the onset latency suggests that most NVsnpr PSPs were at least disynaptic. The mean latency of IntV responses also suggests that the pathway is di- or polysynaptic.

Most responses elicited from NVmt were monophasic depolarizing and biphasic PSPs. The PSPs had a short mean onset latency, suggesting that most result from monosynaptic transmission. It is unlikely that they were caused by stimulation of trigeminal motoneurons, because these do not have axon collaterals (Shigenaga et al. 1988d). However, the nucleus contains glutamatergic, GABAergic, and glycineric interneuron subpopulations (Bourque and Kolta 2001; Ginestal and Matute 1993; Kolta et al. 2000).

### Synaptic action potentials

Several ($n = 14$) all or none action potentials that appear to be of synaptic origin were recorded. The absence of an underlying subthreshold PSP suggests that these spikes arise from inputs to the axon. We showed that some synaptic spikes could be blocked by bicuculline, suggesting that they rely on activation of GABA$_A$ receptors, while others were abolished by DNQX, indicating that they depend on the activation of non-NMDA receptors. The six that disappeared with membrane hyperpolarization were probably initiated in the initial segment of the axon, which is sometimes contacted by synaptic boutons (Honma et al. 2001). Other “synaptic” spikes were not impaired by membrane hyperpolarization, suggesting that they are generated far from the soma. Activation of GABA synapses on terminals of sensory neurons has been shown to cause primary afferent depolarization (PAD), which, if strong, can trigger action potentials that are conducted antidromically toward the soma and the periphery (Catteart et al. 1994; Dubuc et al. 1985; Gossard et al. 1991). Most synaptic spikes were caused by SupV stimulation, and there are GABAergic terminals on many NVmes boutons found in this nucleus (Bae et al. 1997). Synaptic action potentials could also arise from synapses onto NVmes terminals in NVmt, where about 25% of them are contacted by presynaptic P-type boutons (Luo and Dessem 1999; Luo and Li 1991).

We recently showed that GABA has a direct action on the central axons of NVmes spindle afferents through GABA$_A$ receptors and that these receptors can be activated from SupV and the adjacent region of NVsnpr (Verdier et al. 2003). However, activation of these axonal receptors does not cause firing of NVmes afferents; instead, it blocks the propagation of action potentials, perhaps through a local shunt (Verdier et al. 2003). Thus it is very unlikely that this type of synapse is responsible for the BIC- and DNQX-sensitive action potentials. These action potentials may also be reflections of spikes produced in a coupled cell, but the fact that their kinetics are as rapid as those of “normal” spikes argues against this.

### Effect of PSPs on intrinsic oscillations

Thirty-six percent of the recorded neurons showed spontaneous membrane potential oscillations or could be made to oscillate by the injection of a small depolarizing current. The mean frequency was just under 110 Hz. Action potentials were generated on the positive peaks of the waves in about 12% of the neurons at RMP, and the majority of oscillating neurons could be made to fire repetitively by injecting current. These results confirm earlier findings in NVmes (Pedroarena et al. 1999; Wu et al. 2001) and are similar to those of Amir et al. (1999) in the dorsal root ganglia. Amir et al. showed that about
35% of large DRG neurons (low-threshold mechanoreceptors) recorded from young rats in vitro showed oscillations of 88–195 Hz when depolarized. Although few fired at RMP, injections of current induced firing that was sometimes repetitive. Oscillations in DRG and NVmes are both blocked by TTX (Amir et al. 1999; Pedroarena et al. 1999; Wu et al. 2001), and Wu et al. (2001) believe that those seen in NVmes result from the interaction between intrinsic membrane properties, a 4-AP-sensitive noninactivating K+ current, and a persistent Na+ current (I_{NaP}). Their independence from synaptic inputs is confirmed by our observation that they persist in presence of DNQX and the reports of Pedroarena et al. (1999) and Wu et al. (2001) that they persist in calcium-free medium.

We are the first to show that synaptic inputs modify the spontaneous oscillations and that they are able to induce membrane oscillations in quiescent cells. The input resets the rhythm of spontaneous oscillations and increases their amplitude for several cycles. Although a single shock can lead to a prolonged, high-frequency burst of action potentials, membrane hyperpolarization revealed that the underlying depolarizing PSP could be of very short duration. DNQX blocked the enhancement of oscillations caused by stimulation of SupV and NVmt, which is in accordance with the finding of Pelkey and Marshall (1998), that bath applications of ionotropic glutamate receptor agonists cause oscillations and firing in NVmes neurons.

Functional implications of synaptic inputs to NVmes neurons

Because the PSPs reset the ongoing membrane oscillations, they will tend to synchronize the depolarizing waves and associated action potentials in all oscillating cells that share the same input. This will reinforce the effects of electrotonic coupling, which also synchronizes the firing of bonded NVmes afferent neurons (Baker and Llinás 1971; Hinrichsen and Larramendi 1970). Furthermore, the same multipolar neuron could be linked through gap junctions or chemical synapses to several NVmes cells, which could be a mechanism that synchronizes oscillations in distant groups of primary afferents.

It has been suggested that electrotonic coupling between primary afferents in NVmes of elasmobranchii has been retained during evolution because it amplifies an input from a subpopulation of receptors and increased synchronous firing in the jaw closing motoneurons that they activate (Hinrichsen 1970). This would be an advantage to predators during hunting. Bennett (1972) suggested that synaptic inputs to these neurons could decouple them, thus reducing the strength of the jaw closing reflex. However, we have shown that synaptic inputs to NVmes appear to have the opposite effect: they cause firing of the afferents and probably increase synchronicity. All four areas that produce PSPs in NVmes neurons receive monosynaptic inputs from them (Dessem and Taylor 1989; Luo et al. 1991; Luschei 1987; Miyazaki and Luschei 1987; Takata and Kawamura 1970). This mechanism could allow spindles afferent inputs to trigger synchronous bursts of firing in NVmes cell bodies under certain circumstances. The bursts may propagate throughout the central, stem, and peripheral axons and their branches, and in turn, trigger bursts of activity in jaw closing motoneurons. Such a mechanism could maximize the speed and force of biting, during both attack and defense, but may also be brought into play during the chewing of hard or tough foods.

However, many things need to be determined before we can assign any clear function to these processes. We have already shown that the axonal tree becomes compartmentalized during fictive mastication (Westberg et al. 2000) and that the propagation of action potentials along parts of the main axons can be blocked by GABAergic axo-axonic synapses controlled by neurons within SupV and NVsnpr (Verdier et al. 2003). The relationship between action potentials coming from the stem axon and intrinsic membrane oscillations and the ultimate effects of somatically generated spikes on NVmes targets must be investigated before functions can be assigned.

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