Direct Comparison of Heat-Evoked Activity of Nociceptive Neurons in the Dorsal Horn With the Hindpaw Withdrawal Reflex in the Rat

MICHAEL M. MORGAN

Department of Psychology, Washington State University, Vancouver, Washington 98686

Morgan, Michael M. Direct comparison of heat-evoked activity of nociceptive neurons in the dorsal horn with the hindpaw withdrawal reflex in the rat. J. Neurophysiol. 79: 174–180, 1998. Although the sensory coding of nociceptive neurons in the dorsal horn has been studied extensively, surprisingly little is known about how these neurons contribute to nociceptive reflexes. The objective of the present study was to examine the characteristics of dorsal horn neurons capable of initiating hindpaw withdrawal. To this end, neural and reflex activity were measured simultaneously in response to noxious radiant heat applied to the hindpaw in lightly anesthetized rats. Subsets of both multireceptive (MR; 52/95) and nociceptive-specific (NS; 19/46) neurons showed a consistent burst of activity that preceded the reflex. However, when compared with NS neurons, MR neurons as a group were: more likely to be active before the reflex (55 vs. 41%); more active before the reflex (31 vs. 23 Hz); and active earlier (2.8 vs. 2.3 s before the reflex). Subsets of MR neurons were active before the reflex regardless of receptive field size or location in the dorsal horn. In contrast, NS neurons with small receptive fields or those located outside of superficial laminae were rarely active before the reflex and thus unlikely to be part of the reflex circuit. These results suggest that current classification schemes, in particular MR and NS categories, cannot be used as the sole criterion to predict involvement in nociceptive reflexes. However, simultaneous measurement of neural and reflex activity provides an opportunity to determine the characteristics of nociceptive neurons involved in withdrawal reflexes.

INTRODUCTION

In 1960, Wall described the sensory coding of stimulus intensity by nociceptive neurons in the dorsal horn of the spinal cord. Since then, dorsal horn nociceptive neurons have been studied extensively in an attempt to provide a better understanding of nociceptive processing (Willis and Coigeshall 1991). Although these studies have provided much information on the sensory coding and pharmacology of nociceptive neurons in the dorsal horn, surprisingly little is known about neural function. For example, although it is clear that nociceptive withdrawal reflexes are polysynaptic, the interneurons that relay input from primary afferent nociceptors, which terminate in the dorsal horn (Light and Perl 1979; Sugiuara et al. 1986), to motoneurons have not been identified.

Given that nociceptive reflexes may require as few as three serially linked neurons (Jasmin et al. 1997), determining the characteristics of dorsal horn neurons involved in these reflexes would seem to be a manageable problem. However, previous attempts to determine which dorsal horn neurons are part of the circuit for nociceptive reflexes are inconclusive because neural activity is either not compared with withdrawal reflexes or comparisons are carried out in separate experiments; e.g., examining the effects of the same noxious stimulus in different groups of animals (Ali et al. 1994; Mitchell and Hellon 1977; Schouenborg et al. 1995) or in the same animal at different times (Cahusac et al. 1990, 1995; Carstens and Douglass 1995; Nishioka et al. 1995). Although comparing neural and reflex activity in separate experiments is better than making no comparison at all, methodological differences (e.g., depth of anesthetic, immobilization, and surgical preparation) confound comparison of data collected in the two situations.

Such methodological problems can be overcome by measuring the activity of a dorsal horn neuron and motor nerve or muscle simultaneously (Falinower et al. 1994; Schouenborg and Dickenson 1985; Schouenborg and Sjolund 1983). Although such studies indicate that a class of dorsal horn neuron known as multireceptive (MR) is in principle parts of the circuitry for withdrawal reflexes, these studies are limited in that activity in a motor nerve is undefined and only correlations with the specific muscle(s) measured will be evident. For example, Falinower and colleagues (1994) showed that a distant noxious stimulus inhibited the C-fiber-evoked activity of MR neurons concomitant with inhibition of activity from the biceps femoris muscle. However, other studies show that a distant noxious stimulus can inhibit the activity of some hindpaw muscles while simultaneously enhancing the activity of others (Kalliomaki et al. 1992; Morgan and Whitney 1996).

These problems can be overcome by simultaneously recording the activity of a dorsal horn neuron and a nociceptive reflex. Carstens and Campbell (1992) used this technique to examine descending modulation from the periaqueductal gray and lateral reticular formation and found a subset of MR neurons that were not inhibited despite inhibition of the hindpaw withdrawal reflex. This finding suggests that inhibition of a subset of MR neurons is sufficient to inhibit the hindpaw withdrawal reflex.

The objective of the present study was to examine systematically the relationship between nociceptive neurons in the dorsal horn and initiation of the hindpaw withdrawal reflex evoked by a noxious stimulus applied to the hindpaw. Neurons were analyzed on the basis of latency of evoked activity, cutaneous input, size of the receptive field, spinal location, and firing rate. This study differs from previous research examining the sensory coding of dorsal horn neurons by simultaneously measuring the activity of MR or nociceptive specific (NS) neurons and the hindpaw withdrawal reflex in lightly anesthetized rats.
METHODS

Surgery

Male Sprague-Dawley rats (275–325 g; Bantin and Kingman, Hayward, CA) were anesthetized with halothane, and a catheter was implanted in the trachea through which halothane could be administered continuously (0.3 l/min). A laminectomy was performed at L₄ and L₅. The dura mater was retracted, and the spinal cord was covered with a gelatin sponge (Gelfoam, Upjohn, Kalama-zoo, MI) soaked in saline. The rat was placed in a stereotaxic frame with vertebral segments T₁ and L₁ firmly clamped.

After surgery, the concentration of halothane was reduced from 2 to 1%, and the rat allowed to rest for 3 h. Immediately before recording, the halothane concentration was reduced further to as low as 0.6% so that nociceptive reflexes could be elicited by nox-ious stimuli but spontaneous or prolonged movements were not present. Rats were allowed to breathe spontaneously throughout the experiment. Body temperature was maintained by a 37°C water blanket beneath the rat.

Single unit recording

A stainless steel recording electrode (Frederick Haer, Brunswick, ME) was lowered into the spinal cord immediately to the left of the midline. The electrode was advanced in 4-μm steps by a hydraulic microdrive while the left hindpaw was rubbed and pinched until the activity of a single neuron could be isolated from background activity.

Only neurons that responded to noxious pinch of the hindpaw were studied. These neurons were classified as either MR (Wall 1960) or NS (Christensen and Perl 1970; Mendell 1966) depending on whether the neuron also responded to gentle stroking of the hindpaw with a cotton swab. The boundaries of the excitatory receptive fields to noxious pinch and innocuous touch were mapped. Neurons were classified as having either a small (single toe), medium (greater than 1 toe but less than the entire side of the paw), large (1 side of the hindpaw), or very large (the entire hindpaw) receptive field to noxious pinch.

Once the neuron had been classified as a MR or NS neuron and the receptive field mapped, the hindpaw was taped to an immovable block so that the pinch receptive field was exposed. The response of the neuron to brief noxious radiant heat (~5-mm diam) focused on the pinch receptive field was determined. A thermistor probe adjacent to the skin allowed feedback control of surface skin temperature. Each trial consisted of increasing the temperature of the skin from an intertrial level of 35±5°C during 10 s. The toes of the hindpaw were taped to the immovable block so that the heat stimulus could be applied to the same region of skin on each trial while also allowing the hindpaw reflex to be assessed by measuring movement of the ankle joint with a mechanical transducer.

Procedure

The experimental protocol consisted of heating the hindpaw at 3-min intervals and measuring both the evoked activity of a MR or NS neuron in the dorsal horn and the latency for hindpaw withdrawal from the stimulus. Each neuron was tested on at least three trials, and one to five neurons were studied in each rat. After testing, an electrolytic lesion was made at the recording site. If more than one neuron was studied in a rat, a lesion was made at the site of the first and last neuron examined. The rat then was given a lethal injection of pentobarbital (100 mg/kg ip) and perfused intracardially with saline followed by formalin (10%). The lumbar enlargement of the spinal cord was removed, sectioned (50 μm), and stained with cresyl violet so as to localize the recording site.

Data analysis

Surface skin temperature, hindpaw movement, and unit activity were digitized and analyzed on-line by computer (DataWave, Thornton, CO). Four measurements were made from each trial: latency for onset of the hindpaw reflex; latency for the heat-evoked burst of neural activity (the beginning of the burst was defined as 2 spikes occurring within 200 ms followed by ≥1 spike every 500 ms until the reflex occurred); the number of heat evoked spikes preceding the reflex; and the total number of spikes in the 12.5 s before and 12.5 s after onset of the reflex. Mean neuronal firing rate preceding the reflex was calculated by dividing the number of spikes preceding the reflex by the time between the beginning of the burst and onset of the reflex. Parametric data, such as neural and reflex latencies, were analyzed using Student’s t-test for independent samples. Data that were not normally distributed, such as neural activity, were analyzed using the Mann-Whitney U test. Proportions of neurons exhibiting specific characteristics were analyzed using χ². Statistical significance was defined as a probability of <5%.

RESULTS

The present data were derived from 141 neurons recorded from the 68 rats in which a hindpaw withdrawal reflex could be evoked. Ninety-five of these neurons were characterized as MR and 46 as NS. Hindpaw heat reliably (i.e., on ≥3 consecutive trials) evoked a burst of activity that began before onset of the hindpaw reflex in 52 of the 95 MR neurons (55%) and 19 of the 46 NS neurons (41%). Two neurons were spontaneously active and are not included in data analysis.

The locations of 140 of the 141 neurons studied are plotted in Fig. 1. Most MR and NS neurons (61 and 62%, respec-

FIG. 1. Location of multireceptive (MR) and nociceptive-specific (NS) neurons distinguished by whether noxious heat consistently evoked a burst of activity before or after the occurrence of the hindpaw withdrawal reflex (Paxinos and Watson 1986). Both MR and NS neurons tended to be located in superficial laminae and lamina V. NS neurons located in superficial laminae were more likely to be active before the reflex than NS neurons located in other regions.
Nociceptive-specific neurons (NS) were located in lamina I, II, or V (defined as any recording site located in or touching the border of these regions). For statistical purposes, neurons were assigned to one of three location categories: superficial laminae, lamina V, or regions outside of these areas (i.e., laminae III, IV, or in the ventral horn). This analysis revealed that the location of a MR neuron was not related to whether it was active before the reflex (Fig. 1). In contrast, NS neurons in superficial laminae were much more likely to be active before the hindpaw reflex than NS neurons located in lamina V or outside these regions ($\chi^2 = 9.31, P < 0.05$). Ten of the 13 NS neurons (77%) located in laminae I and II were active before the reflex, compared with only 5 of 15 NS neurons (33%) located in lamina V (Fig. 1).

Both MR and NS neurons had receptive fields to noxious stimuli that ranged from small to very large. Receptive fields that were greater than one toe but less than the entire hindpaw (i.e., medium and large receptive fields) were the most common, occurring in 82% of MR neurons and 76% of NS neurons (Table 1). Comparison of neurons that were and were not active before initiation of the hindpaw reflex revealed that of the eight NS neurons with a small receptive field, none were active before onset of the hindpaw withdrawal reflex. In contrast, a subset of MR neurons were active before onset of the reflex regardless of the size of the receptive field.

A comparison of the firing characteristics of MR and NS neurons that were active before the hindpaw reflex is presented in Table 2. Although the total number of heat-evoked spikes in the 25 s surrounding the hindpaw reflex did not differ between MR and NS neurons, there was a difference in the temporal distribution of these spikes. The heat-evoked burst of activity tended to produce more spikes before the reflex and to occur earlier for MR compared with NS neurons. The firing rate before the reflex for MR neurons was significantly greater than for NS neurons (Table 2). Moreover, there was a tendency for the mean onset time of the burst of activity in MR neurons to occur earlier than in NS neurons relative to the onset of the hindpaw reflex, but this 0.5-s difference did not reach statistical significance (Table 2).

The mean latency for onset of the heat-evoked burst of activity and the hindpaw reflex was very consistent from trial to trial when either MR or NS neurons were tested (Table 3). Although the mean number of spikes that preceded the reflex was consistent across these same three trials, some individual neurons were quite variable from trial to trial. In fact, 21% of the MR neurons and 37% of the NS neurons had standard deviations that were >50% of their individual means. This magnitude of variability occurred in only 3 of 71 neurons (4%) when latency of burst onset was assessed and in only 1 of 71 experiments when reflex latency was assessed.

An example of the relationship between the heat-evoked activity of a MR neuron and the associated hindpaw withdrawal reflex is shown in Fig. 2. A burst of activity and a reflex withdrawal of the hindpaw is evident on every trial. Neural activity precedes the reflex and firing rate increases as the stimulus temperature rises. An example of the heat-evoked activity of a NS neuron is shown in Fig. 3. This neuron is typical of NS neurons in that the number of spikes preceding the reflex was typically less than occurred with MR neurons and much of the evoked activity of NS neurons occurred subsequent to initiation of the hindpaw reflex.

**DISCUSSION**

The present data demonstrate that two common assays of nociception, recording the activity of dorsal horn neurons and measuring the latency for a nociceptive reflex, can be measured simultaneously (see also Carstens and Campbell 1992). This approach is an improvement over previous studies investigating the role of dorsal horn neurons in nocicep-

### Table 1. Percentage of multireceptive and nociceptive-specific neurons with different-sized receptive fields

<table>
<thead>
<tr>
<th>Receptive Field Size</th>
<th>Small</th>
<th>Medium</th>
<th>Large</th>
<th>Very Large</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Multireceptive neurons</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active before reflex</td>
<td>12</td>
<td>65</td>
<td>21</td>
<td>2</td>
</tr>
<tr>
<td>Active after reflex</td>
<td>16</td>
<td>60</td>
<td>16</td>
<td>7</td>
</tr>
<tr>
<td><strong>Nociceptive-specific neurons</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active before reflex</td>
<td>0</td>
<td>74</td>
<td>21</td>
<td>5</td>
</tr>
<tr>
<td>Active after reflex</td>
<td>31</td>
<td>46</td>
<td>15</td>
<td>8</td>
</tr>
</tbody>
</table>

$n = 52$ multireceptive neurons active before reflex and 43 after reflex; $n = 19$ nociceptive-specific neurons active before reflex and 26 after reflex.

### Table 2. Firing characteristics of MR and NS neurons

<table>
<thead>
<tr>
<th></th>
<th>MR Mean ± SE</th>
<th>NS Mean ± SE</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean latency from burst to reflex, s</td>
<td>2.8 ± 0.2</td>
<td>2.3 ± 0.3</td>
<td>$t = 1.32$</td>
</tr>
<tr>
<td>Median number of heat-evoked spikes</td>
<td>270 (32–1343)</td>
<td>284 (70–777)</td>
<td>$z = 0.27$</td>
</tr>
<tr>
<td>Median number of spikes preceding reflex</td>
<td>88 (11–915)</td>
<td>144 (12–262)</td>
<td>$z = 2.08^*$</td>
</tr>
<tr>
<td>Median firing rate preceding reflex, Hz</td>
<td>31 (9–132)</td>
<td>23 (12–56)</td>
<td>$z = 2.40^*$</td>
</tr>
</tbody>
</table>

Data in parentheses represent range. MR, multireceptive neuron; NS, nociceptive-specific neuron. * $P < 0.05$.

### Table 3. Neural and reflex responses across trials

<table>
<thead>
<tr>
<th></th>
<th>Trial 1 Mean ± SE</th>
<th>Trial 2 Mean ± SE</th>
<th>Trial 3 Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Burst onset latency, s</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MR</td>
<td>4.3 ± 0.2</td>
<td>4.0 ± 0.2</td>
<td>4.2 ± 0.2</td>
</tr>
<tr>
<td>NS</td>
<td>5.3 ± 0.4</td>
<td>4.7 ± 0.4</td>
<td>4.8 ± 0.5</td>
</tr>
<tr>
<td><strong>Reflex latency, s</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MR</td>
<td>6.7 ± 0.2</td>
<td>6.7 ± 0.3</td>
<td>7.0 ± 0.2</td>
</tr>
<tr>
<td>NS</td>
<td>7.6 ± 0.4</td>
<td>7.1 ± 0.4</td>
<td>7.1 ± 0.4</td>
</tr>
<tr>
<td><strong>Spikes before reflex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MR</td>
<td>118 ± 20</td>
<td>126 ± 20</td>
<td>123 ± 21</td>
</tr>
<tr>
<td>NS</td>
<td>59 ± 14</td>
<td>68 ± 16</td>
<td>66 ± 15</td>
</tr>
</tbody>
</table>

Values are means ± SE.
DORSAL HORN NEURONS AND THE HINDPAW REFLEX

177

Characteristics of dorsal horn neurons mediating the hindpaw reflex

A subset of nociceptive neurons in the dorsal horn must be part of the circuitry for nociceptive reflexes given that primary afferent nociceptors appear to terminate exclusively in the dorsal horn (Light and Perl 1979; Sugiura et al. 1986). Although it has been difficult to identify the dorsal horn neurons involved in nociceptive reflexes, the minimum expectation is that these neurons would have the following characteristics: reflex-related neurons should be activated by noxious stimuli; inhibition of neurons in the reflex circuit should inhibit the reflex; and these neurons should project to the ventral horn or to other neurons that project to the ventral horn.

The two known classes of neuron activated by noxious stimuli in the dorsal horn are MR and NS neurons (Christensen and Perl 1970; Mendell 1966; Wall 1960). Although designating neurons as MR or NS (or wide dynamic range, convergent, high-threshold, etc.) demonstrates that nociceptive information is processed in more than one way, knowledge of these categories has provided surprisingly little information about neural function. Given that a noxious stimulus causes many effects (e.g., sensory discriminative, affective, and reflex), these specific effects must be coded either by subclasses of MR and NS neurons (Chung et al. 1986; Dubner et al. 1989; Maixner et al. 1989; Surmeier et al. 1988) or patterns of activity in MR, NS, and low-threshold neurons (Craig and Bushnell 1994). The recent finding by Schouenborg and colleagues (1995) that the cutaneous receptive field for activation of specific hindlimb muscles corresponds with the receptive fields of MR neurons suggests that some MR neurons have a specific motor function. The only other likely function of such a neuron would be to code stimulus location. However, like many electrophysiology studies, this study (Schouenborg et al. 1995) is limited in scope because a search stimulus was used that favored examination of MR over NS neurons.

The second criterion states that inhibition of nociceptive neurons in the dorsal horn should inhibit nociceptive reflexes. Although correspondence on these two measures of nociception is often quite good, in certain circumstances, neural and reflex activity does not appear to coincide. Micro-injection of opioids into supraspinal sites at doses sufficient to inhibit nociceptive reflexes has been shown to facilitate and inhibit the evoked activity of MR neurons in the dorsal horn (Gebhart and Jones 1988). This finding suggests that neurons with seemingly identical characteristics (i.e., MR neurons) may be quite different. Second, stimulation of the periaqueductal gray has been shown to inhibit nociceptive reflexes without inhibiting the activity of MR neurons in the dorsal horn (Curstens and Campbell 1992). Finally, it is well known that application of a distant noxious stimulus inhibits the activity of nociceptive neurons throughout the dorsal horn (Gerhart et al. 1981; Le Bars et al. 1979; Ness and Gebhart 1991a,b; Morton et al. 1987; Tomlinson et al. 1983), but recent reports show that such a stimulus facilitates and inhibits different nociceptive reflexes (Morgan et
Nociceptive Specific

100 Hz

\[ \text{5 sec} \]

FIG. 3. Ratemeter record showing 3 consecutive trials in which the activity of a nociceptive specific neuron (top line; 100-ms bins), movement of the hindpaw measured with a mechanical transducer (middle line), and surface skin temperature (bottom line) were measured. Skin temperature increased from 35 to 53°C in 10 s on every trial regardless of when the reflex occurred. Although noxious heat evoked a consistent burst of activity in the neuron and a hindpaw withdrawal reflex, this NS neuron was not active before the reflex and, thus, cannot initiate this response. Neural activity that occurs after the reflex could contribute to the reflex or could be caused by hindpaw movement. Mean latency for the burst of activity in this neuron was 4.4 s (42.9°C), whereas the mean latency for the reflex was 3.4 s (41.1°C).

al. 1994; Morgan and Whitney 1996). Unfortunately, until recently, clear interpretation of these data was not possible because electrophysiological and behavioral data were collected in different experiments using different methodologies. However, using the methodological technique described here, I have found that inhibition of nociceptive neurons in the dorsal horn by a distant noxious stimulus inhibits the hindpaw withdrawal reflex and releases hindpaw extension (personal observation; for discussion, see Morgan 1996).

Satisfying the third criterion, identifying nociceptive neurons that project either directly or indirectly to the ventral horn, has been difficult. Neurons that project to supraspinal sites can be identified by antidromic activation from ascending fiber pathways or supraspinal termination sites. In contrast, dorsal horn neurons that project to the ventral horn have short axons or project indirectly via interneurons, making it difficult to identify putative reflex related neurons using antidromic activation. However, given that different populations of dorsal horn neurons appear to project to supraspinal and ventral horn sites (Jasmin et al. 1997), combining electrophysiological and anatomic techniques should allow identification of reflex related neurons.

It should be noted that identifying reflex related neurons is complicated by the fact that nonreflex related neurons may satisfy these criteria. In fact, given the correlational nature of these criteria, only neurons that are not part of the reflex circuit can be positively identified. For example, the present data demonstrate that many dorsal horn neurons cannot be involved in the reflex because they are not active before initiation of the reflex. Although this is obvious, the present study is the first to examine systematically the evoked activity of MR and NS and the hindpaw withdrawal reflex simultaneously. A number of studies have compared neural and reflex activity by applying the same noxious stimulus to different animals in electrophysiological and behavioral experiments (Ali et al. 1994; Mitchell and Hellon 1977; Schouenborg et al. 1995) or the same animal at different times (Cahusac et al. 1990, 1995; Carstens and Douglass 1995; Nishioka et al. 1995), but precise comparisons are precluded because of methodological differences in the two test situations. Carstens and Campbell (1992) simultaneously assessed the activity of a subset of MR neurons that were active before the hindpaw reflex but did not systematically examine the characteristics of dorsal horn neurons that were and were not active before the reflex.

The operating assumption of the present study is that some characteristic of dorsal horn neurons that are active before the reflex will distinguish them from other neurons. Nociceptive neurons in the dorsal horn have been classified along a number of dimensions: range of sensory coding (MR vs. NS) (Christensen and Perl 1970; Mendell 1966), spinal location (superficial vs. deep) (Rexed 1952), projection site (spinothalamic vs. nonspinothalamic) (Ferrington et al. 1986; Giesler et al. 1976), etc. The degree to which these are arbitrary or functional classifications are not known. The present study suggests that sensory coding, spinal location, or size of receptive field, in and of themselves, are not predictive of a role in initiating nociceptive reflexes. That is, a subset of MR and NS, superficial and deep, and small and large receptive field neurons were active before the reflex, and thus each of these types of neuron is capable of initiating the reflex.

Although MR neurons were more likely to be active before the reflex, to have more activity before the reflex, and to be active earlier than NS neurons, these responses cannot be used as evidence that MR, and not NS, neurons are part of the circuit for nociceptive reflexes. These responses merely may reflect the fact that MR neurons, by definition, have a wider response range. A causal role in initiating nociceptive reflexes cannot be assigned merely on the basis of a bigger response or better correlation. However, direct comparison of neural and reflex activity allows neurons that are unlikely to be involved in initiating the hindpaw reflex
to be identified. The present study shows that NS neurons located outside of superficial laminae or with small receptive fields rarely were active before the hindpaw reflex and thus unable to initiate the reflex.

Nonetheless, it is becoming increasingly clear that at least a subset of MR neurons are involved in nociceptive reflexes. MR neurons have receptive fields that match the receptive fields of specific muscles involved in hindpaw withdrawal (Schouenborg et al. 1995), show a burst of activity that correlates with the magnitude of reflex withdrawal (Carstens and Ansley 1993), and show an increase in activity after repeated noxious stimulation that closely matches enhancement of the discharge in a motor nerve to the same stimulus (Schouenborg and Sjölund 1983). The present study is consistent with this body of knowledge by demonstrating that a subset of MR neurons are consistently active before the hindpaw reflex.

Evaluation of methodology

The obvious advantage of simultaneously measuring neural and reflex activity is that it controls for the methodological confounds associated with comparing neural and reflex activity in separate experiments. Another advantage is that it provides an independent measurement of nociception in electrophysiological experiments. Although it is assumed that changes in neural activity reflect changes in pain or nociceptive-reflex sensitivity, in certain circumstances, this assumption cannot be true. For example, intracerebral microinjection of morphine inhibits nociceptive reflexes (Cheng et al. 1986; Jacquet and Lajtha 1973; Yaksh et al. 1976) but has been reported to facilitate, inhibit, and have no effect on the activity of dorsal horn neurons (Bennett and Mayer 1979; Clark et al. 1983; Dickenson and Le Bars 1983, 1987; Du et al. 1984; Gebhart et al. 1984; Llewelyn et al. 1986). Facilitation of dorsal horn neuronal activity after opioid microinjection can be explained in two ways: a subset of inhibitory interneurons in the dorsal horn are facilitated by descending mechanisms or facilitation is the result of repeated testing after an ineffective microinjection. When the only measure of the effectiveness of morphine administration is a change in the activity of a dorsal horn neuron, it is impossible to distinguish between these two explanations.

However, these advantages must be weighed against the technical difficulty of simultaneously recording neural and reflex activity. Strong vertebral clamps adjacent to the recording site allow stable neural recordings despite reflexive movement of the hindpaw. Moreover, restricting hindpaw movement as was done in the present experiment allows application of the stimulus to the same skin location across trials and controls for possible changes as a result of varying limb position (Schomburg 1997). Nonetheless, the number of spikes preceding the reflex was surprisingly variable across trials. This variability could be the result of slight changes in burst and reflex latency, slight variations in the site of stimulus application, and/or the effects of repeated testing. In contrast, both the onset latency for the heat-evoked burst of neural activity and the hindpaw reflex were relatively consistent across trials.

The technical assistance of B. Budra and scientific discussions with Drs. Mary Heinricher and Howard Fields are greatly appreciated.

M. M. Morgan was supported by National Institute of Drug Abuse training grant DA-05399.

Address for reprint requests: M. M. Morgan, Washington State University, 14204 NE Salmon Creek Ave., Vancouver, WA 98686.

Received 2 July 1997; accepted in final form 17 September 1997.

REFERENCES


