Differential Effects of Morphine on Corneal-Responsive Neurons in Rostral Versus Caudal Regions of Spinal Trigeminal Nucleus in the Rat

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Meng, Ian D., James W. Hu, and David A. Bereiter. Differential effects of morphine on corneal-responsive neurons in rostral versus caudal regions of spinal trigeminal nucleus in the rat. J. Neurophysiol. 79: 2593–2602. 1998. The initial processing of corneal sensory input in the rat occurs in two distinct regions of the spinal trigeminal nucleus, at the subnucleus interpolaris/caudalis transition (Vi/Vc) and in laminae I–II at the subnucleus caudalis/spinal cord transition (Vc/C1). Extracellular recording was used to compare the effects of morphine on the evoked activity of corneal-responsive neurons located in these two regions. Neurons also were characterized by cutaneous receptive field properties and parabrachial area (PBA) projection status. Electrical corneal stimulation-evoked activity of most (10/13) neurons at the Vi/Vc transition region was increased [166 ± 16% (mean ± SE) of control, P < 0.025] after systemic morphine and reduced after naloxone. None of the Vi/Vc corneal units were inhibited by morphine. By contrast, all corneal neurons recorded at the Vc/C1 transition region displayed a naloxone-reversible decrease (55 ± 10% of control, P < 0.001) in evoked activity after morphine. None of 13 Vi/Vc corneal units and 7 of 8 Vc/C1 corneal units tested projected to the PBA. To determine if the Vc/C1 transition acted as a relay for the effect of intravenous morphine on corneal stimulation-evoked activity of Vi/Vc units, morphine was applied topically to the dorsal brain stem surface overlying the Vi/Vc transition region. Local microinjection of morphine at the Vc/C1 transition increased the evoked activity of 4 Vi/Vc neurons, inhibited that of 2 neurons, and did not affect the remaining 12 corneal neurons tested. In conclusion, the distinctive effects of morphine on Vi/Vc and Vc/C1 neurons support the hypothesis that these two neuronal groups contribute to different aspects of corneal sensory processing such as pain sensation, autonomic reflex responses, and recruitment of descending controls.

INTRODUCTION

The analgesic properties of morphine in behavioral studies are well established. Consistent with these properties, systemic or intrathecal morphine inhibits the responses of dorsal horn lamina I spinohalamic projection neurons to noxious mechanical and thermal stimulation (Craig and Serrano 1994; Hylden and Wilcox 1986) and intrathecal morphine selectively reduces electrically evoked C-fiber activity of superficial and deep dorsal horn neurons (Dickenson and Sullivan 1986). However, additional studies have demonstrated excitatory as well as inhibitory effects of systemic (Mokha 1993) or iontophoretically applied (Jones et al. 1990; Magnuson and Dickenson 1991; Woolf and Fitzgerald 1981) opiate agonists on laminae I–II neurons with unknown projections.
Vi/Vc neurons, a secondary aim was to determine, in a separate set of experiments, if drugs applied topically to the dorsal brain stem surface at the Vc/C1 transition region altered the activity Vi/Vc corneal units. Preliminary results have been presented in abstract form (Meng et al. 1996).  

**METHODS**

**Animals and surgery**

Experiments were conducted using 39 male Sprague-Dawley rats (300–460 g, Harlan) anesthetized initially with pentobarbital sodium (65 mg/kg ip) before surgery. The left femoral artery and jugular vein were catheterized, and after tracheostomy, animals were artificially respired with oxygen-enriched room air. Body temperature was maintained at 38°C with a heating blanket and thermal probe. Arterial blood gases were monitored periodically and respiratory volume was adjusted to maintain normal pH. Animals were mounted in a stereotaxic frame and the dorsal brain stem was exposed. After completion of all surgery, rats were paralyzed with gallamine triethiodide (20 mg/kg iv), and anesthesia was maintained with supplemental doses of α-chloralose (100 mg/kg iv) as needed. Experiments were terminated if blood pressure was not maintained >70 mmHg.

**Recording technique**

As described previously (Meng et al. 1997) extracellular recordings were made using tungsten electrodes (9 MΩ, FHC, Brunswick, ME). Neurons recorded from the Vi/Vc transition region were approached at an angle of 28° off vertical and 45° off midline. Neurons in laminae I–II of Vc/C1 were approached at an angle of 43° off vertical and 60° off midline and were recorded just before exiting the dorsal horn, 350–500 µm after surface penetration. A bipolar stimulating electrode (2-mm separation, FHC) was mounted on the ear bar and placed lightly on the cornea. Both mechanical (von Frey filaments) and electrical (0.1–1 ms duration, maximum of 1.0 mA, 0.2 Hz) stimulation of the cornea were used as search stimuli. Corneal-responsive neurons were examined for cutaneous input, first using innocuous mechanical stimulation and then noxious pinch and deep pressure. Because not all receptive fields were continuous with the cornea, the entire facial region, especially the nose and underneath the eyelids, was explored. Corneal-responsive neurons with a convergent cutaneous receptive field were classified as low-threshold mechanoreceptive (LTM) units, wide dynamic range (WDR) units, nociceptive specific (NS) units, or deep nociceptive (D) units (Hu 1990). LTM units responded to hair movement and light touch and showed no increase in discharge with more intense stimuli. WDR units were sensitive to both nonnoxious and noxious stimuli and showed an increase in discharge as the intensity of the stimulation increased. NS units were activated only by noxious stimuli applied to the cutaneous receptive field. D units were activated only by deep pressure and did not respond to noxious pinch of the overlying skin. Neurons with no apparent cutaneous receptive field were classified as cornea only (CO) units.

Previously (Meng et al. 1997), we reported that all Vc/C1 corneal units but less than one-half of those at the Vi/Vc transition responded to noxious thermal stimulation of the cornea. Therefore, Vi/Vc units were further categorized by their responsiveness to heating (52°C) of the cornea using a contact thermode (LTS 3, Thermal Devices, Golden Valley, MN) with a stimulating area of 21 mm². No special precautions were taken to protect the surrounding pericocular tissue from thermal stimulation applied to the cornea. Also, the presence of diffuse noxious inhibitory controls (DNIC) was tested to determine if its occurrence correlated with the response to morphine. DNIC was assessed using electrical stimulation of the cornea (1.5–2 times threshold, 0.5–1 Hz) as the test stimulus and placement of the distal 2–4 cm of the rat’s tail in 35°C water for 1 min as the conditioning stimulus. The corneal test stimulus was presented for 15–30 s before, during, and within 1 min after the removal of the conditioning stimulus. A modulatory effect was considered significant if the evoked activity during the conditioning stimulus was <70% of control.

Antidromic activation was assessed from a stimulating electrode placed in the contralateral PBA (P: 0; L: 1.6–1.9 referred to the interaural midpoint with a 3.5° rostral inclination; V: 5.7–6.1 below the cerebral cortex). Antidromically activated neurons displayed a constant latency, followed high-frequency stimulation (200–300 Hz), and showed collision with naturally evoked orthodromic spikes (Lipski 1981).

**Test stimulation**

Electrical stimulation of the cornea was chosen as a test stimulus because it produced consistent, reproducible activation of trigeminal brain stem neurons without damaging the cornea (Meng et al. 1997). Receptive field test stimulation consisted of single pulses of 0.1–5 mA, 0.01–20 ms duration, and delivered at 1 Hz. Electrical stimulation of the cornea or cutaneous receptive field was presented at 1.2–1.5 times threshold relative to the threshold for A- and/or C-fiber primary afferents as estimated by latency of activation. If both A- and C-fiber inputs were present, an appropriate stimulus intensity was used to activate each fiber type. Late latency firing consistent with C-fiber input was defined as activity evoked at a latency of >30 ms (Hu 1990; Meng et al. 1997), which corresponded to a conduction velocity of ∼1 m/s.

**Intravenous drug administration**

After 15 trials of control stimulation (1.1–1.5 times threshold for A- and C-fiber input), morphine was given and electrical stimulation of the cornea was repeated. For Vi/Vc neurons, successively higher doses of morphine sulfate (0.1, 0.5, 1.0 mg/kg iv) were administered at 30-min intervals. Test stimulation (15 trials) was repeated 10 min after each drug injection. The total cumulative dose of morphine given during the 60-min interval was 1.6 mg/kg. For Vc/C1 neurons, after 15 trials of control test stimulation, a single dose of morphine sulfate (1.5 mg/kg iv) was administered and test stimulation was repeated after 10 min. Generally, only a single dose of morphine was used due to the difficulty in holding lamina II Vc/C1 neurons for the 3–4 h necessary to fully characterize each cell. However, in some cases, a second dose of morphine sulfate (1.5 mg/kg iv) was given and the test stimulation repeated. For all neurons, test stimulation was reapplied 10 min after naloxone (0.2 mg/kg iv). Only one neuron was examined per animal. The data were analyzed statistically by analysis of variance (ANOVA) corrected for repeated measures and individual treatment comparisons used the Newman-Keuls test after ANOVA (Winer 1971) and presented as mean percentage (±SE) of the control value. Statistical differences were considered significant at P < 0.05.

**Microinjection of drugs**

In a separate set of experiments, drugs were applied topically to the dorsal surface of the brain stem overlying the Vc/C1 transition region while recording from Vi/Vc corneal units. A guide cannula (26 gauge) was positioned so that the injection cannula (30 gauge) lightly touched the surface of the dorsal horn at the Vc/C1 transition region ipsilateral to the corneal stimulus. Electrical test stimulation of the cornea (1.1–1.5 times threshold for A- and/or C-fiber input) was presented before and at 0.5, 1, 2, 3, 5,
TABLE 1. Summary of the effect of morphine given intravenously on corneal electrical stimulation-evoked activity in neurons located at the Vi/Vc and Vc/C1 transition regions

<table>
<thead>
<tr>
<th>Class</th>
<th>Vi/Vc Neurons</th>
<th>Vc/C1 Neurons</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inhibition</td>
<td>Facilitation</td>
</tr>
<tr>
<td>LTM</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>WDR</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>NS</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>CO</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>0</td>
<td>10</td>
</tr>
</tbody>
</table>

Corneal test stimulation consisted of 15 trials (1.1–1.5 times threshold, 1 Hz) applied before and 10 min after drug injection. Inhibition or facilitation of evoked activity was considered significant if the average postdrug response deviated by >30% from the predrug average. Vi/Vc, subnucleus interpolaris/caudalis transition; Vc/C1, laminae I–II at the subnucleus caudalis/spinal cord transition; LTM, low-threshold mechanoreceptive; WDR, wide dynamic range; NS, nociceptive specific; CO, cornea only.

Effect of morphine on Vi/Vc corneal units

The neurons included in this study represent a subpopulation of cells, with similar properties, of those characterized in a previous study (Meng et al. 1997). The effects of intravenous morphine on electrical stimulation-evoked activity were examined for 13 corneal-responsive neurons at the Vi/Vc transition region (Table 1). All Vi/Vc neurons were located at the ventral pole of the transition region between caudal Vi and rostral Vc (Fig. 1), and none of the 13 neurons could be antidromically activated from the contralateral PBA. Each Vi/Vc unit was classified according to its cutaneous receptive field properties prior to corneal test stimulation: LTM (n = 4), WDR (1), NS (1), and CO (7). Note

![Diagram](http://jn.physiology.org/)
that the anatomic distribution within the Vi/Vc transition region was similar for units of different classes. Eleven neurons received only A-fiber latency (<30 ms) input and 2 received both A- and C-fiber latency input. Six of the 13 neurons responded to noxious thermal stimulation of the cornea, and only 2 of 12 neurons tested showed DNIC.

As summarized in Fig. 2, the average corneal stimulation-evoked activity for neurons at the Vi/Vc transition region was increased significantly after each of three cumulative doses of morphine ($P < 0.025$) with the greatest effect ($+146.2 \pm 16.2\%$ of control) occurring after the highest dose. Naloxone reduced the morphine-induced facilitation to a mean of $118.3 \pm 13.6\%$ of control. There was no overt change in spontaneous activity after morphine or naloxone administration. Neither the ability to respond to noxious thermal stimulation of the cornea nor the presence of a convergent cutaneous receptive field was predictive of the magnitude of the enhanced response after morphine.

Although morphine-induced facilitation of Vi/Vc corneal units was a consistent finding (10/13 cells increased by $>30\%$ of premorphine controls), the nature of the facilitation was not uniform. Because only 2 of 11 neurons showed C-fiber latency firing before morphine administration, early and late latency firing were pooled for this analysis. In six neurons (2 LTM, 1 NS, and 3 CO), the increase in evoked activity after morphine was seen as an unmasking of late latency firing (Figs. 3 and 4). Two of these six neurons responded to noxious thermal stimulation of the cornea. The example in Fig. 3 shows unmasking of late latency firing for a CO unit that was unresponsive to thermal stimulation. Although this neuron did not respond to noxious thermal stimulation and received only A-fiber input from the cornea, the presence of DNIC was seen (Fig. 3B). Before morphine, electrical stimulation of the cornea produced responses at 10–20 ms, whereas after morphine the appearance of a second peak of firing occurred at a latency of 25–35 ms, and naloxone partially reversed the morphine-enhanced firing. Morphine caused a dose-dependent increase in the response to corneal stimulation (Fig. 3D). The second example of unmasking of late latency firing by intravenous morphine is shown in Fig. 4, a Vi/Vc unit with no cutaneous receptive field, yet was excited by noxious thermal stimulation of the cornea (Fig. 4B). Control test stimulation of the cornea evoked only A-fiber latency input and morphine produced an unmasking of late latency firing at 35–40 ms (Fig. 4C). Unlike the neuron shown in Fig. 3, naloxone completely reversed the increase in firing produced by morphine as summarized in Fig. 4D.

Four additional Vi/Vc neurons (2 CO, 1 LTM, 1 WDR) displayed a naloxone-reversible increase in evoked activity at A-fiber latency after morphine without an increase in late latency firing. Two of these four neurons responded to noxious thermal stimulation of the cornea. The activity of two thermally responsive neurons (1 LTM, 1 CO) was unaffected by morphine or naloxone, and in one CO neuron that did not respond to noxious thermal stimulation, naloxone decreased the response to corneal stimulation, whereas morphine had no effect. No Vi/Vc corneal neurons were inhibited by systemic morphine (Table 1).

Effect of morphine on Vc/C1 corneal units

At the Vc/C1 transition region, the effect of systemic morphine was tested on the electrical stimulation-evoked
MORPHINE AND CORNEAL-RESPONSIVE NEURONS

control and A-fiber latency input was 99 ± 3% of control. Morphine reduced the activity of the two Vc/C1 neurons that received only A-fiber latency input to <70% of control. The responses of three neurons were assessed after a second dose of morphine (1.5 mg/kg iv, cumulative dose of 3.0 mg/kg). The overall (A plus C fiber) evoked activity decreased to 62% after the initial dose and to 44% of control after the second dose of morphine. Three neurons showed an overshoot in C-fiber latency activity after naloxone that was >150% of control. Figure 5 presents an example of the effect of morphine on corneal stimulation-evoked responses tested at A-fiber (Fig. 5B) and C-fiber (Fig. 5C) intensities of a neuron classified as NS. Morphine produced a naloxone-reversible decrease in A-fiber and C-fiber latency responses to 58% and 8% of control, respectively (Fig. 5D).

Effects of drugs applied to the dorsal surface of brain stem overlying Vc/C1 on Vi/Vc corneal units

Because considerable evidence suggests that neurons in the Vc/C1 region may influence the activity of cells in more rostral regions of the Vsp, a second series of experiments was performed to test the hypothesis that excitatory (e.g., glutamate) or analgesic drugs (e.g., morphine, lidocaine) applied directly to the Vc/C1 transition alters the activity of Vi/Cc corneal units. The effects of successive topical

drug application to the Vc/C1 region was assessed by stimulating the Vc/C1 region with a single dose of morphine (1.5 mg/kg iv) and then stimulating the cornea with a dose of naloxone (0.2 mg/kg iv) to determine if an effect on A-fiber latency activity persisted.

FIG. 4. Effect of intravenous morphine on a thermally responsive Vi/Vc corneal unit with no cutaneous receptive field. A: recording site. B: response to noxious thermal stimulation before morphine administration. Binwidth = 0.5 s. C: top: control response to electrical stimulation of the cornea; middle: unmasking of C-fiber input after the highest dose of morphine (1.0 mg/kg); bottom: response after naloxone (0.2 mg/kg). Binwidth = 1 ms. D: summary of the effect of morphine on the total number of spikes evoked by electrical stimulation of the cornea. Treatments: control = (premorphine), morphine given as cumulative doses of 0.1, 0.5, and 1.0 mg/kg iv and +Nx = naloxone (0.2 mg/kg iv).

activity of eight neurons (5 WDR and 3 NS), seven of which projected to the contralateral PBA. The average latency for antidromic activation was 4.5 ± 2.2 (SD) ms with a range of 2.4–8.6 ms and a median of 3.7 ms. All Vc/C1 neurons were located superficially, in laminae I–II, at the transition region between Vc and the upper cervical spinal cord (Fig. 1). Six neurons received A- and C-fiber latency input from either the cornea or the cutaneous receptive field, and two neurons received only A-fiber latency input from both the cornea and the cutaneous receptive field. Five of eight neurons were inhibited by DNIC.

Morphine (1.5 mg/kg iv) produced a naloxone-reversible decrease in the composite A- plus C-fiber latency activity of Vc/C1 neurons (55 ± 10% of control, P < 0.001). All Vc/C1 neurons showed a decrease in evoked activity after morphine, including the one neuron that could not be antidromically activated from the PBA (Table 1). Neurons classified as WDR or NS displayed a similar magnitude of inhibition after morphine. There was no significant difference in the amount of A- versus C-fiber inhibition, although numerically C-fiber latency input was decreased to 38 ± 15% of control (P < 0.001) and A-fiber latency input was reduced to 64 ± 8% of control (P < 0.001, Fig. 2). After naloxone C-fiber latency input increased to 130 ± 19% of control and A-fiber latency input was 99 ± 3% of control. Morphine reduced the activity of the two Vc/C1 neurons that received only A-fiber latency input to <70% of control. The responses of three neurons were assessed after a second dose of morphine (1.5 mg/kg iv, cumulative dose of 3.0 mg/kg). The overall (A plus C fiber) evoked activity decreased to 62% after the initial dose and to 44% of control after the second dose of morphine. Three neurons showed an overshoot in C-fiber latency activity after naloxone that was >150% of control. Figure 5 presents an example of the effect of morphine on corneal stimulation-evoked responses tested at A-fiber (Fig. 5B) and C-fiber (Fig. 5C) intensities of a neuron classified as NS. Morphine produced a naloxone-reversible decrease in A-fiber and C-fiber latency responses to 58% and 8% of control, respectively (Fig. 5D).

FIG. 5. Effect of morphine on a Vc/C1 corneal unit that projected to the contralateral PBA and had a WDR cutaneous receptive field. A: recording site in laminae I–II. B: top: control response to electrical stimulation at A-fiber intensity; middle: response after morphine; bottom: response after naloxone. C: top: control response to electrical stimulation at C-fiber intensity. A-fiber responses were truncated for clarity; middle: response after morphine; bottom: response after naloxone. D: summary of total A- and C-fiber latency evoked activity before (control) and after morphine (1.5 mg/kg) and after naloxone (+Nx, 0.2 mg/kg). Binwidth = 1 ms.
TABLE 2. Summary of the effect of glutamate and morphine microinjections onto the dorsal surface of the Vc/C1 region on corneal electrical stimulation-evoked activity in neurons located at the Vi/Vc transition region

<table>
<thead>
<tr>
<th>Class</th>
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<th>No change</th>
<th>Morphine Inhibition</th>
<th>Facilitation</th>
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<td>0</td>
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</tr>
<tr>
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<td>0</td>
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</tr>
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<td>Total</td>
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<td>1</td>
<td>13</td>
<td>2</td>
<td>4</td>
<td>12</td>
</tr>
</tbody>
</table>

Microinjections: glutamate = 20–40 nmol, 100–200 nl; morphine = 2.3–4.6 nmol, 100–200 nl. Corneal test stimulation (15 trials, 1.1–1.5 times threshold, 1 Hz) applied before and at 0.5, 1, 2, 3, 5, 10, 15, and 20 min after drug microinjection. Inhibition or facilitation of evoked activity was considered significant if the average postdrug response deviated by >30% from the predrug average.

Microinjections of glutamate then morphine onto the brain stem surface at the Vc/C1 transition region were assessed on corneal stimulation-evoked activity of 18 Vi/Vc units. These results are summarized in Table 2. Corneal stimulation-evoked activity in 5 of 18 neurons was altered by glutamate microinjections (>30% change), and the evoked activity in each of these 5 cells also was affected by morphine. Also one cell (a CO unit) showed enhanced evoked activity after morphine but was not affected by glutamate. Of the six cells affected by morphine, four showed an increase and two a decrease in evoked responses. Glutamate reduced the evoked activity in three of the four neurons that were enhanced after morphine. Two of these three neurons were classified as CO and one as NS; all three responded to noxious thermal stimulation and two showed DNIC. Facilitation of evoked activity by morphine was seen as an unmasking of late latency firing for two of the four neurons, and the other two cells showed C-fiber input before morphine that was enhanced after morphine. An example of morphine-induced unmasking of C-fiber activity is shown in Fig. 6. This neuron had no cutaneous receptive field, displayed only A-fiber input before morphine, did not show DNIC, and had a small response to thermal stimulation of the cornea before the microinjection of morphine. Microinjection of glutamate reduced the evoked responses to 13% of control by 30 s and returned to 69% of control by 5 min (Fig. 6B, trial 1). A second injection of glutamate, made 40 min after the first injection, inhibited corneal stimulation-evoked responses to 6% of control by 30 s (Fig. 6B, trial 2). Microinjection of morphine (4.6 nmol) 40 min after glutamate produced an increase in activity seen as an increase in late latency firing (Fig. 6C). Maximal facilitation of activity to 212% of control occurred 2 min after morphine and remained elevated at 141% of control after 25 min. In addition, the response to noxious thermal stimulation of the cornea was enhanced at 30 min after the injection of morphine (Fig. 6D).

Two neurons (1 CO, 1 D) responded to microinjection of morphine with a decrease in corneal stimulation-evoked responses, and both were excited by noxious thermal stimulation of the cornea and showed DNIC. In one case (Fig. 7), microin-
Injection of glutamate produced an increase and morphine a decrease in activity. Evoked responses were facilitated to 135% of control by 10 min after glutamate (32 nmol, Fig. 7B). Microinjection of morphine (3.7 nmol), 40 min after glutamate, reduced the activity to 12% of control by 5 min (Fig. 7C) and was reversed partially by naloxone. Five of the six neurons that were affected by morphine also were tested after naloxone (0.2 mg/kg iv) and in each case naloxone caused at least a partial reversal of the morphine effect as shown in the example of Fig. 7C (57% of premorphine control). Eight of 18 cells also were tested after topical application of lidocaine (160 nl, 2% solution) at the Vc/C1 transition at the end of the experiment. As shown in the example of Fig. 7D, lidocaine decreased the evoked responses at the Vi/Vc transition in two cells (compare with Fig. 7C, postnaloxone). However, evoked activity could be recovered by increasing the intensity of the corneal stimulus (Fig. 7E). In two other neurons in which morphine produced an increase in corneal stimulation-evoked responses, microinjection of lidocaine also caused an increase in activity. The fact that evoked activity could be enhanced by lidocaine application at the Vc/C1 transition or that lidocaine inhibition could be overcome by increasing stimulus strength suggested that the drug effects were not simply the result of diffusion to the Vi/Vc transition. Twelve neurons were unaffected by morphine microinjection into the Vi/Vc transition region (Table 2), and 5 of these 12 units responded to noxious thermal stimulation and 2 showed DNIC.

**DISCUSSION**

Although anatomic evidence suggested that the ventral Vi/Vc transition region represents a rostral extension of Vc laminae I–II (Phelan and Falls 1989; Shults 1992; Strassman and Vos 1993; Yoshida et al. 1991), the present physiological results indicated that intravenous morphine produced opposite effects on the evoked activity of corneal-responsive neurons located at the Vi/Vc transition region and laminae I–II of the Vc/C1 transition region. At the Vc/C1 transition region, all corneal-responsive neurons displayed a naloxone-reversible decrease in activity after intravenous morphine. By contrast, the activity of most corneal-responsive neurons at the Vi/Vc transition region was increased after morphine regardless of receptive field properties. These results also revealed for the first time that corneal stimulation-evoked activity among neurons at the Vi/Vc transition region could be altered by local microinjection of morphine or glutamate onto the dorsal brain stem surface overlying the Vc/C1 transition region.

**General properties of Vc/C1 and Vi/Vc corneal units**

As reported previously (Meng et al. 1997), Vi/Vc and Vc/C1 corneal units had different projection and receptive field properties. More than 80% of corneal-responsive neurons located in laminae I–II at the Vc/C1 transition region could be antidromically activated from the ipsilateral or contralateral PBA, whereas none of the corneal-responsive neurons at the Vi/Vc transition were activated. The response properties of Vc/C1 corneal units were similar to those of other projection neurons of the superficial spinal and medullary dorsal horn (Dado et al. 1994). All Vc/C1 neurons had a nociceptive cutaneous receptive field on the periorbital skin contiguous with the corneal surface and responded to
noxious thermal stimulation. At the Vi/Vc transition region, >50% of the corneal-responsive neurons had no cutaneous receptive field, many had low threshold cutaneous receptive fields, and <50% responded to noxious thermal stimulation. The convergent cutaneous receptive fields of Vi/Vc units often were discontinuous with the corneal surface such as on the tip of the nose or distant vibrissae. Thus our previous (Meng et al. 1997) and present physiological studies support the hypothesis that the Vi/Vc transition region that processes corneal input is a functionally distinct region and not simply a rostral extension of laminae I–II neurons from Vc.

**Morphine inhibition of Vc/C1 corneal units**

Intravenous morphine inhibited all Vc/C1-PBA projection neurons consistent with the notion that trigemino-parabrachial pathways may contribute to multiple aspects of nociception (Bernard and Besson 1990). Although the site of action for systemic morphine-induced inhibition of Vc/C1 neurons was not determined, direct actions at the spinal level as well as supraspinal activation of descending inhibitory controls were likely (Basbaum and Fields 1984; Yaksh and Malmberg 1994). A peripheral site of action was also possible because topical application of morphine reduced the pain associated with corneal injury in humans (Peyman et al. 1994). Previous studies indicated that topical spinal application of morphine in the rat (Hylden and Wilcox 1986) or intravenous morphine in cat (Craig and Serrano 1994) inhibited noxious mechanical and thermal stimulation-evoked activity of laminae I spinothalamic projection neurons, often with an overshoot in activity after naloxone. A significant number of Vc/C1-PBA projection neurons in the present study may send collaterals to thalamus because axonal trac-tracing results indicated that a majority of spinothalamic tract neurons also projected to the PBA in the rat (Hylden et al. 1989). Excitation of dorsal horn neurons in superficial laminae has been reported after by intravenous and iontophoretic application of morphine (Jones et al. 1990; Mokha 1993) as well as from in vitro preparations (Magnuson and Dickenson 1991). These studies did not determine the projection status of each neuron, a variable that may be critical in the interpretation of such results. When the effects of intravenous morphine on superficial dorsal horn neurons with projections to the thalamus were compared with those with unknown projection status, it was found that morphine reduced the noxious stimulation-evoked responses of all spinothalamic neurons but only one of five nonprojecting neurons (Craig and Serrano 1994).

**Morphine facilitation of Vi/Vc corneal units**

Intravenous morphine enhanced the responses to corneal input in most (10/13) Vi/Vc units. Although not expected, similar results have been noted previously for evoked activity of Vc corneal units by intravenous morphine (see Fig. 4) (Ayliffe and Hill 1979; Hill et al. 1984). The exact placement of recording sites in Vc was not reported but most were in rostral Vc near the level of the obex (R. Hill, personal communication). Consistent with these results, intracerebroventricular administration of morphine reduced the expression of the immediate early gene product, Fos, at the Vc/C1 transition produced by corneal stimulation, whereas the number of Fos-positive neurons at the Vi/Vc transition was not affected (Bereiter 1997). Interestingly, in many neurons, the increase in electrical corneal stimulation-evoked activity after morphine was manifested as an unmasking of late latency firing. Although the late latency firing may not be caused by direct C-fiber input, this observation may be related to our previous results where C-fiber latency input in Vi/Vc neurons decreased with increasing intensity of the electrical stimulus (see Meng et al. 1997) (Fig. 5). Furthermore, Vi/Vc corneal units typically had phasic responses to both mustard oil and noxious thermal stimulation and desensitized to repeated noxious thermal stimulation, properties not observed in Vc/C1 corneal units (Meng et al. 1997). Therefore, late latency firing of Vi/Vc neurons could be masked by input from inhibitory neurons that are activated by corneal stimulation and inhibited by morphine. It will be necessary to use intracellular recording techniques to determine the nature of an apparent inhibitory masking of evoked neural activity at the Vi/Vc transition region (see De Koninck and Henry 1991).

Several studies have reported facilitatory effects of morphine on neurons in the superficial laminae of the medullary and spinal dorsal horns (Jones et al. 1990; Magnuson and Dickenson 1991; Mokha 1993; Woolf and Fitzgerald 1981). It has been hypothesized that these cells are interneurons that inhibit projection neurons in superficial or deeper laminae (Dickenson 1994; Kemp et al. 1996; Magnuson and Dickenson 1991). Failure to observe antidromic invasion of Vi/Vc units from the lateral PBA suggested that some of these cells could be interneurons, which, on activation by corneal input, cause local inhibition of Vi/Vc projection neurons. However, little is known regarding the local circuitry of the Vi/Vc transition region which is necessary to ascribe functional implications (Phelan and Falls 1989).

**Vc/C1 modulation of Vi/Vc corneal units**

Considerable evidence has indicated modulatory effects from Vc on the activity of neurons in more rostral subnuclei of Vsp (Davis and Dostrovsky 1988; Greenwood and Sessle 1976; Scibetta and King 1969; Sessle and Greenwood 1974; Young and Perryman 1984). However, the majority of these studies were performed in the cat, and none examined specifically the relationship between caudal Vc and the ventral pole of the Vi/Vc transition region. Intravenous morphine may act at multiple brain sites to affect the activity of Vi/Vc neurons and central administration of morphine can facilitate dorsal horn activity, consistent with the enhanced response of Vi/Vc neurons (Bouhassira et al. 1988; Duggan et al. 1980). Because autoradiographic studies revealed significant opioid receptor agonist binding at the ventral pole of the Vi/Vc transition region and in the superficial laminae at the Vc/C1 transition (Xia and Haddad 1991), it was also possible that the Vc/C1 region acted as a relay for modulating the activity of Vi/Vc neural activity. This was tested by applying glutamate and morphine locally to the dorsal surface at the Vc/C1 transition while recording from Vi/Vc corneal neurons.

As summarized in Table 2, topical application of glutamate and morphine to the Vc/C1 transition altered the
evoked activity in ~30% of the Vi/Vc corneal units tested. All cells that were affected by glutamate also were affected by morphine. However, the different directional changes in evoked activity seen after glutamate and morphine suggested a complex relationship between corneal stimulation-evoked activity at the Vi/Vc and Vc/C1 transition regions. Morphine caused an unmasking of late latency firing in two Vi/Vc neurons after morphine microinjections into Vc/C1 similar to that seen in six neurons after systemic morphine. Thus morphine inhibition of Vc/C1 neurons in part could account for the enhanced corneal input to Vi/Vc cells observed after intravenous morphine. The exact mechanism of these modulatory effects is not known and could be mediated through a supraspinal loop originating from the Vc/C1 transition as well as by local projections (direct or via internurons) from the Vc/C1 to the Vi/Vc transition region. Microinjection of lidocaine produced a similar directional change in activity as morphine, indicating that the effect of the morphine likely was due to inhibition of Vc/C1 neurons.

The functional significance of morphine-induced modulation of Vi/Vc neurons by the Vc/C1 transition region is not known. Several lines of evidence indicate that the Vi/Vc transition region may contribute to the modulation and integration of trigeminal nociceptive input. The Vi/Vc transition region that processes corneal input also receives input from the inflamed temporomandibular joint region (Hathaway et al. 1995), meninges (Strassman et al. 1994), and nasal mucosa (Anton et al. 1991) as determined by c-fos immunocytochemistry. Cutaneous receptive fields of Vi/Vc corneal units often include regions that are discontinuous with the cornea such as the tip of the nose (Meng et al. 1997). Widespread convergent input and the facilitatory effects of morphine on Vi/Vc neurons are consistent with a population of cells whose function of which is to recruit opioid-dependent descending controls after nociceptive input (Bereiter 1997; Jasmin et al. 1994). As evidence, retrograde tracer studies have shown a large projection from the Vi/Vc transition region to nucleus submedius of the thalamus in the rat (Yoshida et al. 1991), and electrical stimulation of nucleus submedius has been shown to increase tail flick latency (Zhang et al. 1995) and to inhibit the jaw opening reflex (Tang et al. 1996) by activating the periaqueductal gray descending inhibitory system.

In conclusion, the inhibition of Vc/C1-PBA projection neurons by intravenous morphine is consistent with opioid-mediated reductions in the sensory-discriminative and autonomic aspects of trigeminal nociception. Morphine-induced facilitation of Vi/Vc neurons is consistent with a contribution to recruitment of supraspinal descending control pathways.

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