Responses of Medullary Dorsal Horn Neurons to Corneal Stimulation by CO2 Pulses in the Rat

HARUMITSU HIRATA,1 JAMES W. HU,2 AND DAVID A. BEREITER1,3
Departments of 1Neuroscience and 2Surgery, Brown University/Rhode Island Hospital, Providence, Rhode Island 02903; and 3Faculty of Dentistry, University of Toronto, Toronto, Ontario, Canada

Hirata, Harumitsu, James W. Hu, and David A. Bereiter. Responses of medullary dorsal horn neurons to corneal stimulation by CO2 pulses in the rat. J. Neurophysiol., 82: 2092–2107, 1999. Corneal-responsive neurons were recorded extracellularly in two regions of the spinal trigeminal nucleus, subnucleus interpolaris/caudalis (Vi/Vc) and subnucleus caudalis/upper cervical cord (Vc/C1) transition regions, from methohexital-anesthetized male rats. Thirty-nine Vi/Vc and 26 Vc/C1 neurons that responded to mechanical and electrical stimulation of the cornea were examined for convergent cutaneous receptive fields, responses to natural stimulation of the corneal surface by CO2 pulses (0, 30, 60, 80, and 95%), effects of morphine, and projections to the contralateral thalamus. Forty-six percent of mechanically sensitive Vi/Vc neurons and 58% of Vc/C1 neurons were excited by CO2 stimulation. The evoked activity of most cells occurred at 60% CO2 after a delay of 7–22 s. At the Vi/Vc transition three response patterns were seen. Type I cells (n = 11) displayed an increase in activity with increasing CO2 concentration. Type II cells (n = 7) displayed a biphasic response, an initial inhibition followed by excitation in which the magnitude of the excitatory phase was dependent on CO2 concentration. A third category of Vi/Vc cells (type III, n = 3) responded to CO2 pulses only after morphine administration (>1.0 mg/kg). At the Vc/C1 transition, all CO2-responsive cells (n = 15) displayed an increase in firing rates with greater CO2 concentration, similar to the pattern of type I Vi/Vc cells. Comparisons of the effects of CO2 pulses on Vi/Vc type I units, Vi/Vc type II units, and Vc/C1 corneal units revealed no significant differences in threshold intensity, stimulus encoding, or latency to sustained firing. Morphine (0.5–3.5 mg/kg iv) enhanced the CO2-evoked activity of 50% of Vi/Vc neurons tested, whereas all Vc/C1 cells were inhibited in a dose-dependent, naloxone-reversible manner. Stimulation of the contralateral posterior thalamic nucleus antidromically activated 37% of Vc/C1 corneal units; however, no effective sites were found within the ventral posteromedial thalamic nucleus or nucleus submedius. None of the Vi/Vc corneal units tested were antidromically activated from sites within these thalamic regions. Corneal-responsive neurons in the Vi/Vc and Vc/C1 regions likely serve different functions in ocular nociception, a conclusion reflected more by the difference in sensitivity to analgesic drugs and efferent projection targets than by the CO2 stimulus intensity encoding functions. Collectively, the properties of Vc/C1 corneal neurons were consistent with a role in the sensory-discriminative aspects of ocular pain due to chemical irritation. The unique and heterogeneous properties of Vi/Vc corneal neurons suggested involvement in more specialized ocular functions such as reflex control of tear formation or eye blinks or recruitment of antinociceptive control pathways.

INTRODUCTION

The cornea is a densely innervated organ (Rozsa and Beuerman 1982), supplied exclusively by small myelinated and unmyelinated sensory fibers that lack specialized endings (Beuerman et al. 1983; Maclver and Tanelier 1993; Rozsa and Beuerman 1982; Zander and Weddell 1951), that has long been associated with pain sensation. In addition, direct activation of corneal nerve terminals evokes protective reflexes such as eye blinks (Evinger et al. 1993), tear formation (Stern et al. 1998), endocrine and cardiovascular responses (Bereiter et al. 1996), as well as causing pain (Beuerman and Taneliac 1979; Kenhalo 1960; Lele and Weddell 1959). Although the general classes of primary afferent fibers that supply the cornea are well-established (see Belmonte and Gallar 1996), far less is known regarding the properties of the second-order brain stem neurons that receive input from corneal nociceptors. If the pathways for autonomic, motor and sensory responses to corneal stimuli are activated from a common population of nociceptors, then the initial relay in the trigeminal spinal nucleus (Vsp) onto second-order trigeminal neurons must play a critical role in mediating the various aspects of corneal nociception. As proposed for the spinal dorsal horn ( Lair and Cervero 1991; Price and Dubner 1977), the contribution of second-order neurons to the various aspects of nociception can be inferred on the basis of adequate knowledge of critical features such as neuron location, stimulus encoding properties, response to analgesics, and efferent projections to higher brain centers associated with established functions. The cornea afferent system provides a useful model to test this hypothesis for trigeminal nociception.

The sensory innervation of the corneal epithelium is supplied by the ophthalmic division of the trigeminal nerve (Marfurt et al. 1989; Zander and Weddell 1951). Axonal tract-tracing studies indicate that the central branches of corneal nerves terminate in two regions of the Vsp: at the subnucleus interpolaris/subnucleus caudalis (Vi/Vc) transition and in laminae I–II at the subnucleus caudalis/upper cervical cord (Vc/C1) (Marfurt 1981; Marfurt and Del Toro 1987; Panneton and Burton 1981). Also, c-fos gene expression, a marker for intense neural activation, is produced mainly at the Vi/Vc and Vc/C1 transition regions after corneal stimulation, a finding consistent with the conclusion that neurons in these regions receive the majority of direct input from cornea nociceptors (Bereiter 1997; Bereiter et al. 1996; Lu et al. 1993; Meng and Bereiter 1996; Strassman and Vos 1993). However, the basis for a dual representation of the cornea at the Vi/Vc and Vc/C1 transition regions and its importance in mediating the various aspects of corneal nociception remains uncertain. Recently, we determined that the general properties of corneal-responsive neurons at the Vi/Vc and Vc/C1 transition regions displayed substantial differences (Meng et al. 1997, 1998). For example, all Vc/C1 corneal units received convergent cutaneous input.
that could be classified as nociceptive (i.e., wide dynamic range or nociceptive specific) and the responses to electrical test stimulation of the cornea were inhibited by morphine. By contrast, many Vi/Vc units had receptive fields (RFs) restricted to the corneal surface, and morphine enhanced the responses to corneal electrical test stimuli. Although these results suggested that Vi/Vc and Vc/C1 units process corneal input differently, it was important to determine the encoding properties across a range of stimulus intensities using a well-defined, naturally occurring test stimulus. In the present study, pulses of CO2 gas of varying concentrations were applied to the cornea, and the responses of Vi/Vc and Vc/C1 units were recorded before and after morphine. Corneal nociceptors respond reliably to CO2 pulses at concentrations similar to that which evoke pain sensation in humans (Chen et al. 1995). Because the stimulus-response function of corneal nociceptors to graded concentrations of CO2 pulses has been described, comparison to the pattern for second-order neurons would address the issue of corneal sensory information transfer at multiple levels of the trigeminal neural axis. Also, corneal-responsive neurons were tested for projections to the contralateral thalamus, because it was predicted that Vi/Vc and Vc/C1 corneal units that contribute to the sensory-discriminative aspects of corneal nociception should encode CO2 stimulus intensity and project to sensory regions of the thalamus.

**METHODS**

**Animals and surgery**

Male rats (270–446 g, Sprague-Dawley, Harlan) were anesthetized initially with pentobarbital sodium (70 mg/kg ip) before surgery. The left femoral artery (blood pressure monitor) and jugular vein (anesthesia and drug infusions) were catheterized, and after tracheostomy, animals were artificially respired with oxygen-enriched room air. Anesthesia was maintained by a continuous infusion of methohexital sodium (∼35 mg·kg⁻¹·h⁻¹) and later switched to a mixture of methohexital sodium (26–40 mg·kg⁻¹·h⁻¹) and the paralytic agent, gallamine triethiodide (14–32 mg·kg⁻¹·h⁻¹), after completion of all surgical procedures and just before the electrophysiological recording session. The animal was placed in a stereotaxic frame, and a portion of the occipital bone and C1 vertebrae were removed to expose the dorsal surface of the medulla. The brain stem surface was bathed in warm mineral oil. A dental drill was used to remove a small portion of bone on the right side of the skull for placement of the array of antidiromic stimulating electrodes. Expired end-tidal CO2 was monitored continuously and kept at 3.5–4.5% by adjusting volume or rate of the respirator. Mean arterial pressure (MAP) remained above 100 mmHg throughout all experiments. Body temperature was maintained at 38°C with a heating blanket and thermal probe.

**Extracellular unit recordings**

Extracellular unit recordings were made using tungsten electrodes (9 MΩ, FHC, Brunswick, ME) as described previously (Meng et al. 1997). Neurons recorded at the Vi/Vc transition were approached at an angle of 28° off vertical and 45° off midline. Neurons recorded in laminae I–II at the Vc/C1 transition were approached at an angle of 43° off vertical, 60° off midline and were found just before exiting the lateral dorsal horn, 300–500 μm after surface penetration. Mechanical (von Frey filaments) and electrical (0.1–1 ms duration, maximum of 1.0 mA, 0.2 Hz) stimulation of the cornea was used to search for responsive neurons. Mechanical stimuli were applied from a bipolar electrode (2 mm separation, FHC) mounted on the ear bar and placed lightly on the cornea. Well-discriminated unit activity was amplified, displayed on a digital oscilloscope to monitor spike shape and amplitude, and passed through a window discriminator. Discriminated neural spikes, MAP, and a marker for CO2 stimulus pulses were acquired and displayed on-line with an Apple computer (PowerMac 7100) through a Lab-NB interface board using LabVIEW software (National Instruments). Also, these data were digitized (NeuroData) and stored on VCR tape as a backup and for further off-line analyses.

In each animal preparation a single neuron was isolated, and several general response properties were determined initially: A- or C-fiber type corneal input (electrical stimuli), mechanical thresholds for corneal input (von Frey filaments), presence of a convergent cutaneous RF, and tests for antidromic activation from the contralateral thalamus. Responses to electrical stimulation of the cornea occurring at latencies of >30 ms were assumed to indicate C-fiber input (Hu 1990; Meng et al. 1997). The ipsilateral face was explored for possible cutaneous input to corneal-responsive neurons, by first applying innocuous mechanical stimulation and then noxious pinch and deep pressure. Corneal-responsive neurons with a convergent cutaneous RF were classified as low-threshold mechanoreceptive (LTM), wide dynamic range (WDR), or nociceptive specific (NS) units (Hu 1990; Hu et al. 1981). LTM units responded to hair movement or light touch and did not increase in discharge rate with more intense stimuli. WDR units were sensitive to both nonnoxious and noxious mechanical stimuli and displayed an increase in firing rate with increasing stimulus intensity. NS units were activated only by noxious pinch applied to the cutaneous RF. Neurons with no apparent cutaneous receptive field were classified as cornea only (CO) units.

**Antidromic stimulation**

An array of two or four (1 or 2 mm tip separation) concentric bipolar stimulating electrodes (Rhodes Medical Instruments, SNE-100 or SNE-300) was used to stimulate medial (nucleus submedius, SM) or lateral (ventral posteromedial nucleus, VPM; and posterior nuclear group, PO) regions of the thalamus. The stereotaxic coordinates (AP from bregma, ML from midline, and DV from dorsal brain surface) were (in mm): −3, 0.5, −7 mm for SM; −3.5, 2.5, −6 mm for VPM; −4.6, 3, −6 mm for PO as adapted from the atlas of Paxinos and Watson (1986). Antidromic electrodes were lowered slowly while passing current (center negative; 0.1 ms, 20–800 μA, 1 or 8 Hz). Antidromic activation was defined by evoked activity at a constant latency (<0.1 ms jitter), ability to follow high-frequency stimulation (>200 Hz, 20 ms train duration), and collision with spikes evoked orthodromically by corneal stimulation (see Lipski 1981). The minimum intensity for antidromic activation was obtained by varying the electrode depth, and all putative antidromic spikes were evoked by current intensities of ∼500 μA.

**Corneal stimulation by carbon dioxide**

Different concentrations of CO2 were obtained by mixing the outflow from tanks containing 100% CO2 and air through a proportional gas mixer (MX18; BOC, Warwick, RI) after the method of Chen et al. (1995). A constant flow of mixed gas was humidified and, after activation of an electronic switch, diverted to the left cornea through a short length of polyethylene tubing (∼2 mm ID). The separation between the tip of the tubing and the corneal surface was ∼5 mm. To estimate the force (in mg) caused by the flow of air, the tubing was positioned 5 mm above and perpendicular to the surface of a precision balance, and air pulses of varying flow rates were applied. At 260 ml/min the force displacement was ∼1 mg, and at 515 ml/min the force was 4 mg, values below or near the detection threshold for humans (Belmonte et al. 1999). The dead space from the electronic switch to the tip of tubing was 1.6–1.7 ml and created a time delay of ∼1 s. The timing and duration of CO2 pulses were computer controlled by the LabVIEW program (designed originally by D. Budai, University California, San Francisco and modified by H. Hirata,
Rhode Island Hospital). Concentrations of CO₂ were 0, 30, 60, 80, and 95% as monitored from the bleeder valve output by an infrared detector (CapStar 100, CWE). An ascending series of CO₂ pulses were used in most cases, and the duration of each pulse was 30 or 40 s presented at an interstimulus interval (ISI) of 4 min. The flow rate varied from 200 to 600 ml/min and was reduced to a minimum, depending on the cell’s sensitivity to 0% CO₂. The flow of air alone (0% CO₂) onto the cornea often produced transient initial (<5 s) discharges in neurons, but this activity was rarely proportional to CO₂ concentration and was readily distinguishable from the late discharges (>10 s delay, Fig. 2). Special care was taken to keep the cornea moist throughout surgery and the recording period with normal 0.9% saline. The integrity of the corneal surface was confirmed by a small rise in MAP that occurred after high concentrations (>80%) of CO₂ in each animal preparation.

Experimental design and drug administration

An initial control (no drug) series of CO₂ test stimuli consisted of pulses (0, 30, 60, 80, and 95%) of 30 or 40 s in duration with 4 min between each pulse. Morphine sulfate (MS; Marsam Pharmaceutical) was given (0.5–3.5 mg/kg iv) over 2–3 min, followed after 10 min by another series of CO₂ pulses. A single dose of morphine (1.5 or 3.0 mg/kg iv) was used for most Vc/C1 corneal units, because we determined previously (Meng et al. 1998) that doses lower than 1.0 mg/kg, had no effect on activity evoked by electrical stimulation of the cornea. For most Vi/Vc corneal units, morphine was given in cumulative doses of 0.5, 1.0, and 2.0 mg/kg up to a maximum of 3.5 mg/kg. After CO₂ testing in the presence of morphine, naloxone hydrochloride (RBI) was given (0.2 or 0.4 mg/kg iv) and after 10 min a final series of CO₂ pulses was presented. The response properties of a single neuron were studied in each experiment.

Data analysis

Neural recording data were acquired and displayed by LabVIEW as peristimulus time histograms (PSTHs) of spikes per 1-s bins. Together with signals for MAP and CO₂ stimulus onset and offset, each data set was exported to a spreadsheet and analyzed off-line. Spontaneous activity (see Table 1) was determined by averaging the spike counts during the 2-min epoch preceding the first CO₂ test series. Because the background activity of most units fluctuated during the 4-min period between CO₂ pulses, estimation of response magnitude (RCO₂) required that the background activity before each CO₂ pulse be accounted for statistically. The RCO₂ was defined as the spike count per bin (1 s) that exceeded the mean plus 2 times the standard deviation (SD) for the 2-min epoch of background activity sampled immediately preceding each CO₂ test pulse. The latency for RCO₂ was defined as the earliest time after stimulus onset for which three consecutive 1-s bins displayed a spike count that exceeded the mean ± 2 SD of background activity (i.e., the RCO₂ value). Similar methods have been used by us (Hirata and Aston-Jones 1994) and others (Neugebauer et al. 1993) to determine response magnitude and latency in central neurons with substantial background activity. Similarly, the onset of the inhibitory period observed in type II Vi/Vc neurons after CO₂ stimuli was defined as the minimum time at which the discharge rate fell below the mean ± 2 SD of the background activity for at least three consecutive 1-s bins (see Fig. 8). The average RCO₂ values for each CO₂ concentration under multiple experimental conditions (i.e., no drug, morphine, morphine plus naloxone) were analyzed statistically by ANOVA, repeated for repeated measures (Winer 1971), and individual comparisons were made with the Newman-Keuls test. Post hoc analyses revealed distinct classes of CO₂-responsive Vi/Vc corneal units. At the Vi/Vc transition, CO₂-responsive corneal units of different types (I, II, or III) were analyzed separately. At the Vc/C1 transition, all CO₂-responsive corneal units displayed a similar stimulus-response function and were considered as a single category.

<table>
<thead>
<tr>
<th>Table 1. Summary of general properties for Vi/Vc and Vc/C1 corneal units</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Spontaneous Activity, spikes/s</strong></td>
</tr>
<tr>
<td>-----------------------------</td>
</tr>
<tr>
<td>Vi/Vc (n = 39)</td>
</tr>
<tr>
<td>1.5 ± 0.5</td>
</tr>
<tr>
<td>&lt;0.5 (26)</td>
</tr>
<tr>
<td>&gt;0.5 (13)</td>
</tr>
<tr>
<td>Vi/C1 (n = 26)</td>
</tr>
<tr>
<td>5.2 ± 1.0†</td>
</tr>
<tr>
<td>&lt;0.5 (3)</td>
</tr>
<tr>
<td>&gt;0.5 (23)</td>
</tr>
</tbody>
</table>

Values are means ± SD. Numbers in parentheses indicate sample size. Vi/Vc, subnucleus interpolaris/caudalis; Vc/C1, subnucleus caudalis/upper cervical cord; LTM, low-threshold mechanoreceptive; WDR, wide dynamic range; NS, nociceptive specific; RF, receptive field. * Average spontaneous activity (mean ± SD, spikes per s) recorded for 2 min before CO₂ stimulation. † P < 0.01 versus Vi/Vc corneal units, mean ± SD.

Histology

At the end of the experiment, the animal was deeply anesthetized with an overdose of methohexitol sodium (60 mg/kg iv) and perfused transcardially with 10% Formalin containing potassium ferrocyanide. Blocks of medulla and thalamus were frozen, sectioned at 40 or 80 μm, respectively, and stained with cresyl violet. The recording sites in Vi/Vc and Vc/C1 regions of medulla were reconstructed from the tissues containing DC lesions. Antidromic stimulation sites in contralateral thalamus were reconstructed from brain sections containing the Prussian blue reaction product and drawn on a standard series of outlines adapted from the atlas of Paxinos and Watson (1986).

RESULTS

General properties

These results derive from 39 Vi/Vc and 26 Vc/C1 neurons that responded to mechanical and electrical stimulation of the cornea and subsequently were tested for sensitivity to CO₂ pulses. As shown in Table 1, most Vi/Vc corneal units had low levels of spontaneous activity (<0.5 spikes/s), whereas Vc/C1 corneal units had comparatively higher levels (>0.5 spikes/s; Vi/Vc vs. Vc/C1, P < 0.01) before CO₂ testing. Cornea RFs were determined by lightly rubbing the cornea with a von Frey filament. At the Vi/Vc, 4 of 28 neurons that were classified as CO units had a corneal RF that included only a portion of the cornea. However, for all Vi/Vc units that had a convergent cutaneous RF (n = 11), and for all 26 Vc/C1 units, the corneal RF covered the entire surface. The average von Frey threshold for Vi/Vc corneal units (111.9 ± 30.1 mg) was numerically lower than Vc/C1 units (139.0 ± 41.9 mg); however, this difference was not significant statistically (P > 0.05, Mann-Whitney U test). Based on the latency to electrically evoked corneal activity 87.1% (27 of 31) of Vi/Vc neurons were classified as receiving A-fiber input and four cells as receiving A- plus C-fiber volleys. Similarly, 86.7% (13 of 15) of Vc/C1 units received A-fiber only input from the cornea. The recording sites for Vi/Vc corneal units were located near the ventral edge of the Vsp within 0.5 mm of the obeb, and Vc/C1 corneal units were recorded within superficial laminae.
4 mm caudal to the obex (Fig. 1) consistent with previous reports (Meng et al. 1997). The recording locations of CO₂-responsive neurons (Fig. 1, left side of each outline) and nonresponsive corneal neurons (right side) were similar.

**Cutaneous RF properties**

**Vi/Vc.** As shown in Table 1, 72% (28 of 39) Vi/Vc corneal units were classified as CO, and of those that had a convergent cutaneous RF, 7 of 11 were classified as LTM. One LTM unit had a complex cutaneous RF that consisted of an excitatory field on upper eyelid and a low-threshold inhibitory RF on the nose. Two Vi/Vc units (multi-RF, Table 1) were excited by corneal stimulation but also had a low-threshold inhibitory cutaneous RF surrounding the cornea. Nine of 11 Vi/Vc corneal units had a cutaneous RF restricted to the ophthalmic division, one included ophthalmic and maxillary divisions, and one multi-RF Vi/Vc cell had a RF that included all three trigeminal divisions. The cutaneous RF of 9 of 11 Vi/Vc units was contiguous with the corneal surface; however, one unit had an NS field on the pinna, and another cell had a WDR-like field on the nose. One Vc/C1 neuron had a complex RF that included excitatory fields on cornea plus skin (ophthalmic and maxillary divisions) and a high-threshold inhibitory field on the nose. For 23 of 26 Vc/C1 corneal units, the convergent cutaneous RF was restricted to the ophthalmic division. Although the cutaneous RF area was not quantified, there did not appear to be overt differences in either RF size or location on the facial skin for Vi/Vc and Vc/C1 corneal units.

**Responses to CO₂ pulses applied to the cornea Vi/Vc.** Forty-six percent (18 of 39) Vi/Vc neurons were excited by the initial series of CO₂ pulses applied to the cornea. Two main patterns of response to graded concentrations of CO₂ were seen for Vi/Vc neurons. Type I neurons were encountered most frequently (n = 11) and displayed a progressive increase in discharge rate to increasing concentrations of CO₂ as shown by the example in Fig. 2A. Consistent evoked discharges to CO₂ pulses began after a delay (late responses) in all 11 cells (range, 7–22 s after 95% CO₂) and often outlasted the CO₂ stimulus, especially at higher CO₂ concentrations (80–95% CO₂). Note in Fig. 2A that a transient (<5-s delay) variable increase in firing was seen at stimulus onset (early RCO₂) that was not dependent on CO₂ concentration. A positive relationship between the early RCO₂ (where RCO₂ =
spike count (mean background + 2 SD) and CO₂ concentration was seen in only 3 of 11 type I Vi/Vc neurons as defined by a significant late RCO₂. By contrast, the late RCO₂ was proportional to CO₂ concentration (Fig. 2B) and the average late RCO₂ for all type I Vi/Vc neurons to 80% CO₂ pulses was significantly greater than to 60% CO₂ pulses (P < 0.01, ANOVA, see Fig. 6A). The derived population response for type I Vi/Vc units was described by a positively accelerating power function with an exponent of 1.81 (r² = 0.934, n = 11). In initial experiments CO₂ pulses of 30 s duration were used; however, as seen by the PSTHs in Fig. 3, the late increase in firing pattern of type I cells often appeared after a 15- to 20-s delay and continued beyond the end of the stimulus. To address the concern that a consistent maximum stimulus-response function to graded concentrations of CO₂ pulses of 30 s duration may have been obscured by a variable and incomplete “ON response,” the CO₂ pulse duration was increased to 40 s in subsequent experiments. As seen in Fig. 3B, the variability of the late RCO₂ to 30-s pulses at high CO₂ intensities (80 and 95%) was reduced by using 40-s pulses. The threshold concentration for the late RCO₂ (defined as >20% increase in RCO₂, sustained for 3 consecutive bins, above the RCO₂ to 0% CO₂) was 30% CO₂ in five units, 60% in four units, and 80% in two type I Vi/Vc units. The average latency of the late RCO₂ decreased significantly with an increase in CO₂ concentrations (60–95%) for type I Vi/Vc corneal units (see Fig. 6B). Eight of 11 type I units were classified as CO, 1 as WDR, 1 as LTM, and 1 as a multi-RF cell.

Type II Vi/Vc corneal units (n = 7) displayed a biphasic response to CO₂ pulses; an initial inhibition of activity followed by excitation, and in some cases followed by a second inhibitory period (Fig. 8A). The initial inhibitory period for all type II units appeared rapidly (<5-s delay) after stimulus onset at threshold intensity of 30% CO₂ for six neurons and at 60% in one unit, but was not seen with 0% CO₂. For all type II units the early inhibitory response to CO₂ pulses occurred at a lower CO₂ concentration than the well-defined excitatory phase. For most type II units the excitatory phase (late RCO₂) was characterized as a progressive increase in firing rate at CO₂ concentrations of >60%; however, two type II units displayed a saturated-like response at 95% CO₂. The derived population response for type II Vi/Vc units was described by a positively accelerating power function with an exponent of 1.23 (r² = 0.961, n = 5). The magnitude of the late RCO₂ to 95% CO₂ pulses was greater (P < 0.01) than after 60% CO₂ pulses. The latency for onset of the excitatory phase of type II units decreased with higher concentrations of CO₂ as occurred for type I units (see Fig. 6B). Note that the excitatory phase of type II cells often terminated abruptly (Fig. 8A), whereas the OFF response for type I units was more prolonged (see Fig. 3). The total duration of the inhibitory phase did not appear related to stimulus intensity; however, this was difficult to quantify because the end of the inhibitory period was not well-defined in most type II units. Interestingly, the onset of the inhibitory phase of type II units preceded the rise in MAP and further, the excitatory phase of type II units was not necessary to observe an increase in MAP to CO₂ pulses (see 60% pulse, Fig. 8). Five
of seven type II units were classified as CO, and two units had an LTM-like RF on the facial skin surrounding the cornea.

Vs/C1. Fifty-eight percent (15 of 26) of Vs/C1 corneal units responded to CO₂ pulses. Compared with Vi/Vc corneal units, the stimulus-response patterns of Vs/C1 units to CO₂ pulses (30–95%) were homogeneous. All Vs/C1 units displayed a progressive increase in discharge rate with increasing CO₂ intensity. A majority of Vs/C1 units (10 of 15) showed a progressive increase in firing rate throughout the entire range of CO₂ concentrations (Fig. 4A, left column) and the remainder displayed an apparent saturation to 95% CO₂ pulses (Fig. 4B, right column). Two subclasses of Vs/C1 units with linear and saturated response patterns also were apparent if a less conservative method of subtracting only the mean background (versus mean ± 2 SD for RCO₂) was used to calculate the RCO₂. This suggested that the level of spontaneous activity before CO₂ testing did not influence the classification (bottom panels of Fig. 4, △). As shown in Fig. 5A, Vs/C1 units often responded to CO₂ pulses (30 or 40 s duration) with a brief transient (<5 s delay) increase in firing rate that was not related to CO₂ concentration and, followed by a delay (range of latency, 8–29 s at 95% CO₂), a late RCO₂ that was proportional to CO₂ concentration (P < 0.01, ANOVA). The late RCO₂ often persisted for several seconds after stimulus offset, similar to the response seen for type I Vi/Vc units (Fig. 3). Comparison of 30- and 40-s CO₂ pulses for the average early and late RCO₂ values (calculated as spikes per stimulus) shown in Fig. 5B were not different statistically (P > 0.05, ANOVA). The average late RCO₂ stimulus-response function for all Vs/C1 units shown in Fig. 6A revealed a progressive increase with greater CO₂ intensity similar to that seen for Vi/Vc units. The derived population response for all Vs/C1 corneal units was described by a positively accelerating power function with an exponent of 1.41 (r = 0.889, n = 15). The latency for late RCO₂ onset also decreased significantly with increasing CO₂ intensity similar to that of Vi/Vc units (Fig. 6B). The threshold CO₂ concentration was 60% for 7 of 15 Vs/C1 units, 80% for 4 units, and 95% for 4 units. Although the results suggested that Vs/C1 units required higher minimum CO₂ concentration than Vi/Vc units for activation, this was not significant (P > 0.05, χ² analysis). Thirteen of 15 Vs/C1 corneal units were classified as WDR on the basis of the properties of the convergent cutaneous RF, and two cells were classified as NS neurons.

Repeated presentation of the series of CO₂ pulses was tested on several Vi/Vc and Vs/C1 corneal units before morphine. Each of five Vs/C1 units became desensitized (defined as >20% decrease to the combined response to 60, 80, and 95%)
to repeated CO₂ stimulation, whereas three of seven Vi/Vc cells showed a decrease, one was enhanced, and three units were not influenced. Although repeated CO₂ stimulation had variable effects on the responsiveness of Vi/Vc neurons to subsequent CO₂ testing, a majority of Vi/Vc units (7 of 11) displayed an increase in background activity with repeated CO₂ testing as seen for a type II Vi/Vc unit in Fig. 8. Repeated presentation of the CO₂ pulse series did not enhance the background activity of Vc/C1 units.

Effects of intravenous morphine

Vi/Vc. Four of six type I Vi/Vc units showed an increase in spontaneous activity (>50% change) after morphine (0.5–3.5 mg/kg iv), and two units were not affected. Of the type I units tested for CO₂ responses before morphine, after morphine, and after naloxone, two units were markedly enhanced by morphine (Fig. 8A), one unit was inhibited and two were not affected. Both the excitatory and inhibitory effects of morphine on type I Vi/Vc units were reversed by naloxone. Morphine did not affect the early transient responses (<5-s delay) to CO₂ pulses in type I units. There was no apparent difference in the recording location for Vi/Vc corneal units that were excited, inhibited, or unaffected by morphine.

Six type II Vi/Vc units were tested for CO₂ responses before and after morphine. Morphine (cumulative dose of 0.5–3.5 mg/kg iv) caused an increase in background activity of one unit, decrease in four units, and no effect in one neuron. By contrast, morphine enhanced (>50% above premorphine value) the late RCO₂ after high concentrations of CO₂ (80 or 95%) in three units (see example in Fig. 8) and decreased the late RCO₂ in two units. Morphine did not affect the duration of the inhibitory phase of type II units evoked by 30–95% CO₂ pulses, although the example of Fig. 8 suggested that it may have been reduced in some cases.

Twenty-one of 39 Vi/Vc corneal units did not respond to the initial series of CO₂ pulses before morphine. However, after morphine a third category (type III, n = 3) of Vi/Vc neuron was uncovered that displayed a vigorous response to CO₂ in naloxone-reversible manner. Eleven cells that initially were unresponsive to CO₂ pulses were retested after morphine, and 3 of these 11 units displayed an increase in late RCO₂ as shown in Fig. 7B. The dose of morphine required for uncovering CO₂-evoked activity was >1 mg/kg in each case. The magnitude of the late RCO₂ after 95% CO₂ was enhanced significantly after morphine (P < 0.025, ANOVA, vs. premorphine or postnaloxone). The ability of naloxone to return type III
units to a state of low CO₂ responsiveness was not due to a
general loss of unit amplitude or activity, because rubbing the
cornea evoked a prompt and significant increase in firing rate
(R, in Fig. 7B). Type III Vi/Vc units displayed low levels of
background activity (<0.2 spikes/s) and morphine caused only
small additional increases. Two of three type III Vi/Vc units
were classified as CO₂, and one unit had an LTM-like RF on the
upper and lower eyelids. All three type III units received
A-fiber only input from the cornea. It was possible that the
frequency of occurrence of type III cells (3 of 11) was under-
estimated, because seven additional corneal units that did not
respond to the initial series of CO₂ pulses were not retested
after morphine. It was possible that the occurrence of CO₂-
unresponsive Vi/Vc neurons (21 of 39) may have been over-
estimated by desensitization caused by prior CO₂ testing of
cells that were not held long enough to be included in the data
analyses.

Vc/C1. Morphine inhibited the CO₂ responsiveness of all
Vc/C1 corneal units tested. A total of six Vc/C1 units were
tested before morphine, after morphine (1.5 or 3.0 mg/kg iv),
and after naloxone. As summarized in Fig. 9, the late RCO₂ of
Vc/C1 units, expressed as percent of control, was inhibited by
morphine and reversed, at least partially, by naloxone. Because
desensitization of Vc/C1 units was common, it was important
to compare the percentage decrease due to repeated presenta-
tion of CO₂ pulses alone (n = 5). Figure 9 indicates that repeat
presentation of CO₂ pulses alone (C1 vs. C2) caused numeri-
cally smaller reductions in the late RCO₂ than was seen after
1.5 or 3 mg/kg morphine. Morphine never enhanced the late
response of a Vc/C1 corneal unit and did not affect the early
transient response to CO₂ pulses. In contrast to type III Vi/Vc
corneal units, no Vc/C1 unit that was unresponsive to the initial
series of CO₂ pulses became responsive after morphine (n =
5). Several additional Vc/C1 units were examined after mor-
phine, but were lost before CO₂ testing was completed; how-
ever, background activity was generally reduced by morphine
(11 of 17 units) with no effect in six units.

Antidromic responses from contralateral thalamus
Vi/Vc. None of 18 Vi/Vc corneal units tested were activated
antidromically from sites in the contralateral nucleus subme-
dius (SM) or ventral posteromedial thalamic nucleus (VPM).
Three Vi/Vc neurons also were tested for activation from sites
in the caudal aspect of contralateral posterior thalamic nucleus
(POc), and none were driven antidromically (bipolar concen-
tric electrode, center negative, 0.2 ms pulse duration; up to 2.0 mA).

Vc/C1. None of 30 Vc/C1 corneal units tested were antidromically activated from sites within the SM or VPM. However, 7 of 19 Vc/C1 units were driven from POc. An example of a Vc/C1 unit antidromically activated from the POc is shown in Fig. 10. Two of seven Vc/C1 corneal units driven from sites in the contralateral POc were responsive to CO2 pulses, and five units were not. The average current required for antidromic activation was 329 μA (range, 84–540 μA; n = 7), and the latency to antidromic activation ranged from 2.8 to 8.0 ms. Calculated conduction velocities ranged from 1.8 to 5.4 m/s, suggesting that mainly small-diameter myelinated fibers mediated the projection from Vc/C1 neurons to the contralateral POc.

**DISCUSSION**

The present study used a chemical stimulus of CO2 pulses to determine the stimulus encoding properties of Vi/Vc and Vc/C1 corneal units. It was predicted that neurons that mediate the sensory-discriminative aspects of corneal pain should encode the concentration of CO2, be inhibited by systemic morphine, and project to the contralateral thalamus. The results indicated that Vc/C1 corneal units satisfied each of these criteria. Although Vi/Vc units also displayed a positive stimulus-response function to increasing concentrations of CO2, morphine often enhanced neural responsiveness to CO2, and Vi/Vc corneal units were not activated antidromically from stimulation sites in the contralateral thalamus. These results confirm and extend previous findings (Meng et al. 1997, 1998) that Vi/Vc and Vc/C1 neurons likely contribute to different aspects of corneal pain.

**Dual representation of the cornea at the Vi/Vc and Vc/C1 transition regions**

The caudal portion of the trigeminal spinal nucleus (Vsp), subnucleus caudalis (Vc), shares anatomic and physiological similarities with the spinal dorsal horn (Dubner and Bennett 1983; Sessle 1987). However, the Vsp also displays distinctive features suggesting that the initial integration of nociceptive input from craniofacial structures may be processed differently from that of other body tissues. One unique organizational

**FIG. 6.** Comparison of the average magnitude (A) and latency (B) of the late RCO2 for type I Vi/Vc, type II Vi/Vc, and Vc/C1 corneal units. Late RCO2 was calculated as the activity that exceeded the mean ± 2 SD of the background activity. * P < 0.05, ** P < 0.01 vs. response to 30% CO2 pulses.

**FIG. 7.** Examples of the effects of intravenous morphine on the activity of a type I and type III Vi/Vc neuron. A: type I Vi/Vc cornea only unit displayed increased responses to initial series of CO2 pulses before morphine (top panels), enhanced responses after morphine (3 mg/kg, middle panel) and return toward premorphine response level after naloxone (0.2 mg/kg, bottom panel). Note that naloxone did not reverse the increase in spontaneous activity caused by morphine. B: type III Vi/Vc cornea only unit did not respond to the initial series of CO2 pulses before morphine (top panels), displayed intensity-related responses after morphine (1 mg/kg, middle panel) and no response after naloxone (0.2 mg/kg, bottom panel). Note that rub (R) of the cornea after naloxone caused a vigorous response indicating integrity of the recorded cell was not lost after naloxone.
feature of craniofacial input to the Vsp not seen at the spinal level is a multiple somatotopic representation within the Vsp. Multiple representation of the cornea is suggested by transganglionic tract-tracing studies in which the central projections of corneal sensory nerves are seen to terminate mainly at the ventrolateral pole of the Vi/Vc transition and in the lateral superficial laminae at the Vc/C1 transition region (Marfurt 1981; Marfurt and Del Toro 1987; Panneton and Burton 1981). Similarly, c-fos immuncytochemical studies reveal that nocxious thermal (Lu et al. 1993; Meng and Bereiter 1996), mechanical (Strassman and Vos 1993), or chemical (Bereiter 1997; Bereiter et al. 1996; Meng and Bereiter 1996) stimulation of the corneal surface produces a high-density of Fos-positive neurons in the Vi/Vc and Vc/C1 transition regions. The basis for a dual representation of the cornea in two spatially distinct portions of the Vsp remains uncertain and two general organizational schemes can be considered. First, it is possible that subpopulations of corneal primary afferent neurons may project preferentially to either the Vi/Vc or Vc/C1 transition region. Such a segregation of corneal afferents could be based on fiber type and/or modality. Partial support for fiber type segregation derives from studies in which the marker for unmyelinated fibers, isoleucin B4, was injected into the trigeminal ganglion to reveal a dense termination of C-fibers in the superficial laminae of Vc but not at the Vi/Vc transition region (Sugimoto et al. 1997a). By contrast, both substance P–like and calcitonin gene-related peptide (CGRP)-like immunoreactivity (Meng and Bereiter 1996; Strassman and Vos 1993; Sugimoto et al. 1997b), neuropeptide markers for small diameter fibers, overlap substantially with the location of Fos-positive neurons produced at the Vi/Vc and Vc/C1 transition regions by corneal stimulation. Also, we have determined previously (Meng et al. 1997), and confirmed in the present study, that the activity evoked at A-fiber or A- plus C-fiber latencies after electrical stimulation of the cornea occurred in a similar proportion for Vi/Vc and Vc/C1 units. Thus these results suggest that Vi/Vc and Vc/C1 corneal units receive a similar proportion of afferent input on the basis of fiber diameter. Alternatively, it is possible that corneal afferents distribute preferentially to the Vi/Vc or Vc/C1 transition regions based on the stimulus modality encoded by the primary afferent neuron. Recording studies, mainly from cat and rabbit, indicate that the majority of corneal afferent fibers can be classified as polymodal nociceptors (>70%), mecanoreceptive, or cold receptors in descending frequency of occurrence (see Belmonte and Gallar 1996). In the first systematic examination of corneal-responsive units within the caudal Vsp (Meng et al. 1997), we determined that all Vc/C1 corneal units responded to mustard oil stimulation of the cornea regardless if electrical stimuli evoked activity at A-fiber alone or A- plus C-fiber latencies. By contrast, Vi/Vc units typically responded to mustard oil only if electrical stimulation of the cornea evoked activity at C-fiber–like latency. A second line of evidence to suggest that Vi/Vc and Vc/C1 corneal units receive different forms of orofacial input concerns convergent cutaneous RFs. At the Vc/C1 transition, all corneal units received a convergent cutaneous RF that could be classified as nociceptive, i.e., WDR- or NS-like. However, at the Vi/Vc transition, >50% of corneal units had no convergent cutaneous RF, and of those that received cutaneous input, most were classified as LTM confirming previous findings (Meng et al. 1997). Also, the number of Fos-positive neurons produced at the Vi/Vc transition increased similarly over a broad range of thermal stimuli applied to the cornea, whereas at the Vc/C1 transition only nocuous thermal intensity levels enhanced c-fos expression (Meng and Bereiter 1996). Despite the limitations of c-fos expression as a marker for central pain pathways (see Bullitt 1990; Strassman and Vos 1993), these results were consistent with the notion that nocuous thermal input from corneal nociceptors was processed differently by neurons at the Vi/Vc and Vc/C1 transition regions. Although the available evidence suggested that Vi/Vc and Vc/C1 corneal units receive a different complement of sensory input, it was important to examine this possibility more closely by determining the stimulus-response function of corneal units to a naturally occurring chemical stimulus.

Encoding of corneal stimuli by Vi/Vc and Vc/C1 neurons and primary afferent fibers

It has been proposed that a unique feature of nociceptive processing compared with other sensory systems is a relative constancy of the stimulus-response function for nociceptive neurons across different levels of the nervous system (McHaffie et al. 1994). Comparison of the response properties of second-order corneal units at the Vi/Vc and Vc/C1 transition regions to those of corneal primary afferents permits inferences to be made regarding the transfer of sensory information at the initial sites of integration of presumptive corneal pain pathways. First, the corneal RF area for single primary afferent fibers from cat (Belmonte and Giraldez 1981; Gallar et al. 1993; Lele and Weddell 1959), rabbit (Tanelian and Beuerman 1984) and rat (Mark and Maurice 1977) comprise only a fraction of the corneal surface as determined by mechanical stimulation. Although some polymodal afferent fibers supply up to 70% of the corneal surface area (Belmonte et al. 1991; Gallar et al. 1993), the present study indicated that most brainstem units displayed whole corneal RFs, indicating considerable spatial convergence onto second-order trigeminal neurons at both the Vi/Vc and Vc/C1 transition regions. Second, the percentage of neurons responsive to mechanical and chemical stimulation of the cornea was similar for primary afferents and second-order neurons. In the cat, ~60% of mechanically sensitive corneal primary afferent fibers also responded to acetic acid (Belmonte et al. 1991; Gallar et al. 1993). Assuming that most pH-sensitive corneal fibers also respond to CO2, as was found in the rat skin in vitro preparation (Steen et al. 1992), the present finding that 46% of Vi/Vc and 58% of Vc/C1 corneal units were excited by the initial series of CO2 pulses agreed well with the percentages reported for primary afferents. This also suggested that select classes of CO2-sensitive neurons were not excluded from the present study due to microelectrode sampling bias. In fact, it was more likely that the percentage of CO2-responsive Vsp neurons was underestimated because repeated presentation of high concentrations of CO2 desensitized most Vc/C1 units and some Vi/Vc units. Also, some Vi/Vc corneal units may be tonically inhibited, because morphine caused an apparent disinhibition of the responses to CO2 pulses (type III units). It is not known whether corneal primary afferent fibers exist in the rat that respond to chemical irritants only and not to mechanical stimuli as reported for the rabbit (Tanelian 1991). At the Vi/Vc transition, two types of cells responded to the initial series of CO2 pulses. Type I Vi/Vc
units displayed a progressive increase in discharge rate with increasing CO₂ concentration and type II Vi/Vc units showed a reduced discharge rate at 30–60% CO₂ concentrations and a progressive increase in firing at higher concentrations. At the caudal Vc/C1 transition, most units displayed a progressive increase in firing rate with increasing CO₂ concentration.

Third, despite some units in each category that displayed a saturation-like response to 95% CO₂, it was unexpected to find similar CO₂ thresholds and an average derived population response for type I, type II, and Vc/C1 units that was described by a positively accelerating power function with an exponent of >1.0. This suggested that a majority of CO₂-responsive neurons at the Vi/Vc and Vc/C1 transition regions received similar chemosensory information from corneal primary afferents. In the cat Aδ and C-fiber corneal afferents responded to CO₂ pulses with an increasing firing rate to CO₂ concentration and an average power exponent of 1.12 (Chen et al. 1995). In the rabbit the stimulus-response pattern of corneal primary afferent fibers to a variety of irritant chemicals also was fit by an exponential function (Beuerman et al. 1992). It is not yet known whether corneal afferent fibers in the rat display a similar stimulus-response pattern to graded concentrations of CO₂ or acid. Most Vi/Vc and Vc/C1 corneal units responded to CO₂ pulses with long latencies (10–20 s), whereas Chen et al. (1995) found most primary afferent fibers in the cat responded within 5 s. The basis for this difference is not certain; however, the evoked pressor responses to CO₂ pulses also occurred after a 10- to 20-s delay (see Fig. 8). The integration of corneal sensory information by second-order neurons may require spatial and/or temporal summation. Alternatively, it is possible that CO₂-responsive nerve endings are situated deeper within the corneal epithelium of rat compared with cat. Small differences in the barrier thickness or the temperature of the gas delivered to the cornea could affect the diffusion of CO₂ and/or the enzymatic conversion to protons by carbonic anhydrase, which, in turn, activate nerve endings (Steen et al. 1992).

Fourth, desensitization of Vi/Vc and Vc/C1 units often was seen after repeated stimulation with pulses at high CO₂ concentrations (80–95%). This result differed from that seen after repeated noxious thermal stimulation of the cornea in which Vc/C1 units became sensitized and Vi/Vc units generally had reduced responses (Meng et al. 1997). Interestingly, in the cat the activity of corneal primary afferents also was enhanced by repeated noxious thermal stimulation (Belmonte and Giraldez 1981) and reduced by repeated CO₂ pulses (Chen et al. 1995). Carstens et al. (1998) have noted that repeated application of irritant chemicals such as nicotine or capsaicin to the corneal surface caused pronounced tachyphylaxis in rostral Vc neurons. Collectively, these results indicated that type I Vi/Vc and Vc/C1 corneal units displayed similar stimulus-response patterns to CO₂ pulses consistent with that expected to be transmitted from corneal primary afferent fibers, and sufficient to encode CO₂ concentration. Type II Vi/Vc units had more complex responses to CO₂ pulses that must derive from central processing.

Effects of morphine

Despite similar responses to the initial series of CO₂ pulses, Vi/Vc and Vc/C1 corneal units had markedly different responses to CO₂ after morphine. Morphine enhanced the response to CO₂ pulses of ~50% of type I and type II Vi/Vc corneal units, whereas all Vc/C1 units were inhibited. The enhancement of Vi/Vc units after morphine often appeared as an increase in spontaneous activity accompanied by an increase in the CO₂ stimulus-response pattern to the previous series of CO₂ pulses. A third category, so-called type III Vi/Vc corneal units, became “unmasked” by morphine in that they were initially unresponsive to CO₂ pulses and became responsive after morphine. However, it was possible that type III units represented cells that had become desensitized by prior corneal testing. The effects of morphine on Vi/Vc and Vc/C1 units were reversed by naloxone, indicating an opioid receptor-selective action. These results confirmed and extended previous findings in which morphine enhanced the activity of Vi/Vc units and inhibited that of Vc/C1 corneal units to electrical or noxious thermal stimulation of the corneal surface in a naloxone-reversible manner (Meng et al. 1998). Although less well-

FIG. 8. Example of CO₂ encoding by a type II Vi/Vc cornea only unit displaying a distinctive inhibition–excitation response pattern to graded concentrations of CO₂. Typically, the threshold for early inhibition (30% CO₂) was lower than for the excitatory phase (80% CO₂) for most type II neurons. A: responses to initial series of CO₂ pulses. B: repeated series of CO₂ pulses reduced mainly the excitatory phase (*) of the response pattern. C: morphine (0.5 mg/kg iv) given 10 min before start of CO₂ pulse series enhanced the evoked activity. Note that background activity increased after high CO₂ concentrations (80–95%) of initial pulse series, returned toward prestimulus values during the 2nd pulse series, and was enhanced by morphine before the 3rd pulse series.
described than the inhibitory effects, morphine or mu opioid receptor agonists have been reported to enhance the activity of nociceptive neurons in the spinal dorsal horn after systemic (Craig and Serrano 1994), intrathecal (Dickenson and Sullivan 1986), or iontophoretic (Jones et al. 1990) routes of administration. Among medullary dorsal horn neurons, morphine increased the activity of some cold-responsive (Mokha 1993) and nociceptive trigeminalthalamic projection neurons in the rat (Wang and Mokha 1996). Also, morphine did not attenuate c-fos gene expression produced by corneal stimulation at the Vi/Vc transition but did cause a significant reduction at the Vc/C1 transition (Bereiter 1997). In contrast to the progressive facilitative effects of increasing doses of intravenous morphine on Vi/Vc corneal units, the majority of studies in which low doses of opioids were shown to enhance neural activity also concluded that higher doses were inhibitory (however, see Craig and Hunsley 1991; Grudt and Williams 1994). For example, Craig and Serrano (1994) found that low doses of morphine (0.125 mg/kg iv) increased the activity in 9 of 13 lamina I neurons in cat spinal cord to noxious pinch or heat stimulation, whereas higher doses inhibited most neurons. In rat spinal dorsal horn, intrathecal doses of <5 µg morphine enhanced the evoked activity at C-fiber intensity, but higher doses were inhibitory (Dickenson and Sullivan 1986). Similarly, low doses of opioids enhanced the activity of some dorsal root ganglion cells in culture (Crain and Shen 1990); however, when tested on peripheral Aδ fibers, morphine caused only inhibition of spontaneous activity (Russell et al. 1987). Although lower doses of morphine than used here (0.5 mg/kg iv) may have enhanced the responses of caudal Vc/C1 corneal units to CO2 pulses, it seemed unlikely that a common mechanism could explain the enhanced activity of both Vi/Vc corneal units and spinothalamic tract lamina I nociceptive neurons. Unlike the effects on spinal dorsal horn neurons, higher doses of morphine (cumulative maximum dose, 3.5 mg/kg iv) only rarely inhibited Vi/Vc corneal units in this or a previous study (Meng et al. 1998). The exact mechanisms that underlie morphine enhancement of Vi/Vc corneal units remain uncertain. Microinjection of morphine into the Vc/C1 transition region could mimic the effect of systemic morphine on some Vi/Vc units, suggesting a possible action via intermuscular projections in Vsp (Meng et al. 1998). Systemic morphine also could act locally to disinhibit interneurons at the Vi/Vc transition region or to activate opioid-dependent descending control pathways from higher brain areas. Systematic testing of possible peripheral effects of morphine on corneal afferent fibers has not been reported; however, in humans topical morphine reduced the pain associated with corneal injury without affecting corneal sensitivity in uninjured patients (Peyman et al. 1994). Recent anatomic evidence indicates some opioid receptor activity in corneal nerves (Wenk and Honda 1999). Thus it cannot be excluded that a portion of the morphine-induced inhibition of Vc/C1 units could occur through action at peripheral sites, whereas enhancement of Vi/Vc unit activity by morphine likely occurs via central mechanisms.

**Projections of Vi/Vc and Vc/C1 corneal units**

If the contribution of second-order neurons to the various aspects of nociception (e.g., sensory-discrimination, motor reflexes, autonomic reflexes) is determined in part by the strength of projections to specialized brain areas (see Laird and Cervero 1991; Price and Dubner 1977), then identification of the efferent projection targets of Vi/Vc and Vc/C1 corneal units may aid in defining their roles in corneal nociception. The efferent projections of Vi/Vc and Vc/C1 corneal units were markedly different as determined by antidromic activation methods. With an electrode array implanted in the contralateral thalamus directed at sites within the PO group, VPM and SM, it was determined that 7 of 19 Vc/C1 corneal units projected to the PO group and none of 30 units projected to the VPM or SM. By contrast, none of 21 Vi/Vc corneal units were antidromically activated from sites in the contralateral thalamus. These results were similar to those of a previous study in which >80% of Vc/C1 corneal units, but none of the Vi/Vc units, were antidromically activated from sites in the parabrachial complex and Kolliker-Fuse nucleus (Meng et al. 1997). Recently, we have combined c-fos immunocytochemistry and retrograde tracing methods to determine whether Vi/Vc and Vc/C1 corneal units project to the contralateral thalamus (Bereiter et al. 1999). Numerous Fos-positive/FluoroGold double-labeled neurons were found in lamina I at the Vc/C1 transition after FluoroGold injection into the caudal PO group confirming the results of antidromic activation; however, some double-labeled cells also were seen at the Vi/Vc transition. Numerous double-labeled cells were seen at the Vi/Vc, but not at the Vc/C1 transition after FluoroGold injections into SM. No Fos-positive neurons were seen to project to VPM from the Vi/Vc or Vc/C1 transition regions. The two main conclusions drawn from this study were that Vc/C1 corneal units project selectively to the PO group and, second, neither Vi/Vc or Vc/C1 corneal units project significantly to the contralateral VPM. This finding differs...
FIG. 10. Response properties of a Vc/C1 corneal unit that was activated antidromically from the caudal posterior thalamic group (POc) contralateral to the recording site. A: criteria to establish antidromic activation. a: antidromic spike (*) occurred a constant latency after stimulus onset (downward arrow, 3 sweeps; 2.8 ms latency). b: orthodromic spike (solid dot) occurring within a critical time interval before stimulation prevented antidromic spike (*). c: orthodromic spike (solid dot) occurring outside critical time window did not collide with antidromic spike (*). d: antidromic responses (*) followed high-frequency stimulation (200 Hz, 0.1-ms pulse, 5 pulses). B: location of antidromic stimulation sites in caudal posterior thalamic group (POc). APT, anterior pretectal n.; MG, medial geniculate n.; POc, caudal posterior thalamic n. C: response properties indicated this neuron as a corneal unit with a WDR-like convergent cutaneous RF on the lower eyelid. This unit encoded CO2 concentration. D: location of cutaneous RF on the lower eyelid and recording site in the superficial laminae at the Vc/C1 transition region.
from results seen in the cat in which corneal units were recorded throughout the VPM (Hayashi 1995). Anterograde tracer injections into superficial laminae of Vc in the rat were reported to label terminals in PO and VPM of contralateral thalamus (Iwata et al. 1992). However, others have reported that injection of FluoroGold into VPM of the rat did not retrogradely fill neurons in those regions of the Vsp that corresponded to the location of Vi/Vc or Vc/C1 corneal units (Dado and Giesler 1990). It is not certain why Vi/Vc corneal units could not be antidromically driven from the PO group or SM despite anatomic evidence of such efferent projections (Bereiter et al. 1999; Dado and Giesler 1990; Yoshida et al. 1991). Possibly, these projections involve sparse or very fine terminal regions in the thalamus. Also, some corneal-responsive neurons recorded at the Vi/Vc transition may be local circuit neurons. In recent preliminary studies, we have found several Vi/Vc corneal units that could be activated antidromically from sites in or near the superior salivatory nucleus/facial motor nucleus region (Bereiter et al. 1999). This is consistent with results from Pelligrini et al. (1995) in the guinea pig indicating direct projections from Vi/Vc units to the facial motor nucleus in control of eye blinks. The function of the POc in sensory processing remains uncertain. The POc has been referred to as a phylogenetically primitive thalamic processing area because it is not somatotopically organized (see Diamond 1995). Many POc neurons have large cutaneous RFs (Perl and Whitlock 1961) and receive convergent input from multiple sensory systems (LeDoux et al. 1987). Because stimulation of sites in the POc antidromically activated mainly nociceptive neurons in the cervical dorsal horn (Dado et al. 1994), this suggested a significant role for the POc in pain processing.

**Functional significance**

The results of the present study confirm and extend previous findings (Meng et al. 1997, 1998) to conclude that corneal-responsive neurons at the Vi/Vc and Vc/C1 transition regions serve different functions in corneal nociception. Corneal units at the Vc/C1 transition behaved, in most respects, similar to spinal dorsal horn nociceptive neurons. Vc/C1 corneal units received convergent cutaneous input that could be classified as nociceptive (i.e., WDR or NS), were always inhibited by morphine, and often displayed efferent projections to supraspinal regions associated with higher-order processing of nociceptive input (parabrachial complex or thalamic PO group). These features were consistent with the hypothesis that Vc/C1 corneal units mediated the sensory-discriminative aspects of corneal pain as well as the autonomic reflex adjustments that accompany pain sensation. Despite similar stimulus-response functions to CO2 pulses, Vi/Vc corneal units displayed unique properties not seen at the Vc/C1 transition or the spinal dorsal horn. Vi/Vc corneal units often had RFs restricted to the corneal surface, were facilitated by a broad range of morphine doses, and were rarely activated antidromically from sites within the medial or lateral sensory thalamus. The function of Vi/Vc units in corneal nociception remains uncertain. The available evidence suggests that Vi/Vc corneal units contribute to ocular-specific functions such as eye blink reflexes and lacrimation. However, the enhanced responsiveness of Vi/Vc units after morphine and the anatomic evidence of projections to the SM (Bereiter et al. 1999; Dado and Giesler 1990; Yoshida et al. 1991) is consistent with a role in recruitment of endogenous antinociceptive controls. These results support the notion that corneal chemosensory information is relayed without significant transformation to different levels of the trigeminal system. Last, evidence for differential roles of Vi/Vc and Vc/C1 neurons in corneal nociception is better supported by the response to morphine and efferent projections than by the encoding properties to chemical irritant stimuli.

We thank D. Bereiter and A. Benetti for excellent technical assistance and Dr. Ian Meng of the Department of Neurology, University of California, San Francisco, for valuable suggestions during the early stages of these experiments.

Address for reprint requests: D. A. Bereiter, Rhode Island Hospital/Brown University, Depts. of Surgery and Neuroscience, 222 Nursing Arts Bldg., Providence, RI 02903.

Received 2 April 1999; accepted in final form 1 July 1999.

### REFERENCES


