The Medullary Dorsal Horn: A Target for the Expression of Opiate Effects on the Perceived Intensity of Noxious Heat

J.-L. Oliveras,¹ W. Maixner,² R. Dubner, M. C. Bushnell,³ D. R. Kenshalo, Jr., G. H. Duncan,³ D. A. Thomas, and R. Bates

Neurobiology and Anesthesiology Branch, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20892

We examined the effects of morphine microinjected into the medullary dorsal horn (MDH) on the ability of monkeys to detect temperature increases in the noxious heat range. Behavioral detection latency and the percentage of correct detections were used as measures of the perceived intensity of noxious heat stimuli. Three monkeys were trained to detect a change (T2) of 0.4, 0.6, or 1.0°C from a previous noxious heat level of 46°C (T1). Effects on attentional, motivational, and motoric aspects of the monkeys' behavior were assessed by having them detect innocuous cooling and visual stimuli in tasks of similar difficulty. Morphine (1, 3, and 10 µg) microinjected into the MDH produced a dose-dependent and stimulus-intensity-dependent increase in the latency to detection of the T2 stimuli. These effects were opiate receptor-mediated since they were antagonized by systemically administered naloxone (0.5 mg/kg, i.m.) given 40 min after the microinjection of morphine. There were no effects of morphine on the behavioral detection latencies to the innocuous cooling and visual stimuli, indicating that the effects of morphine were modality-specific and independent of changes in motivation, attention, or motoric ability. These data demonstrate a pharmacologically specific effect of opiates on the perceived intensity of noxious heat stimuli at the earliest central relay pathway transmitting noxious information.

The mechanism of action of opiates and the sites where opiates produce their analgesic effects remain topics of current interest. Beecher (1959, 1966) proposed that opiates decrease pain perception by altering the emotional reaction to a noxious stimulus without altering its perceived intensity. In contrast, human psychophysical studies demonstrate that opiates diminish the perceived intensity of noxious tooth-pulp stimulation with variable effects on the perceived unpleasantness (Gracely et al., 1979, 1982).

Although the CNS sites where opiates diminish the perceived intensity of a noxious stimulus have not been identified, one likely site is the dorsal horn of the spinal cord and medulla. Biochemical studies have shown the dorsal horn to be rich in

opiate receptors (Atweh and Kuhar, 1977) and opioid peptides (Glazer and Basbaum, 1981; Hökfelt et al., 1977; Hunt et al., 1981; Ruda, 1982). Electrophysiological studies provide evidence that systemic and local administration of opiates depress the responses of dorsal horn neurons to noxious stimuli (for a review, see Besson et al., 1979; Dubner and Bennett, 1983; and Yaksh, 1981). A number of studies have shown that opiates impair behavioral responses to noxious stimuli via a spinal mechanism (for a review, see Yaksh, 1981; Yaksh and Rudy, 1978). Finally, the medullary and spinal dorsal horns contain neurons that encode the intensity of noxious stimuli (see Dubner and Bennett, 1983; Willis and Coggeshall, 1978). The activity of these neurons correlates strongly with the perceived intensity of noxious heat stimuli in behaving monkeys (Bushnell et al., 1984; Hoffman et al., 1981; Maixner et al., 1986).

Although some of the above studies have shown that opiates produce a behavioral analgesia at the spinal level, they have not examined whether these effects are related to changes in the perceived intensity of noxious stimuli. The behavioral reactions observed may have resulted from changes in attentional, motivational, or motoric processes. In the present studies, we have examined morphine's effects in a behavioral task that distinguishes between these components of behavioral responses to noxious stimuli. Monkeys were trained to detect noxious heat stimuli (Bushnell et al., 1985; Maixner et al., 1986). Morphine was administered into the medullary dorsal horn, and we examined its ability to alter stimulus detection. We used behavioral detection latency, or reaction time, as a measure of the intensity of sensation. Reaction time is a feature of behavior that covaries with parameters of sensory stimulation even without specific training (Moody, 1970). Animal psychophysical studies have established a relationship between response latency and parameters of sensory stimulation in a number of sensory modalities (Stebbins, 1970) and recently we have shown that behavioral detection latency covaries with small temperature increases in the noxious heat range (Kenshalo et al., 1985). It appears that detection latency is a reliable measure of the perceived intensity of noxious heat stimuli. In addition, we have used the percentage of correct detections as another index of sensory function. Effects on attentional, motivational, and motoric aspects of the monkeys' behavior were assessed by having the monkeys detect innocuous cooling and visual cues in addition to noxious heat stimuli. By using innocuous cooling cues, we were also able to assess the submodality specificity of morphine's action. We report that morphine administered in the medullary dorsal horn impairs the detection and discrimination of noxious thermal stimuli in a specific, dose-dependent manner without altering motivation, attention, or motoric ability. A preliminary report of this material has appeared elsewhere (Oliveras et al., 1986).

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Correspondence should be addressed to Dr. Ronald Dubner, National Institute of Dental Research, National Institutes of Health, Building 30, Room B-20, 9000 Rockville Pike, Bethesda, MD 20892.

¹ Present address: INSERM, 2, rue d'Alesia, Paris, France.

² Present address: Dental Research Center, Room 03, Bldg. 210H, University of North Carolina, Chapel Hill, NC 27514.

³ Present address: Département de Stomatologie, Faculté de Médecine dentaire, Université de Montréal, Montréal, Québec H2V 3B5, Canada.

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Figure 1. Drawing of the experimental situation and diagram of the temporal sequence of events associated with the performance of the thermal and visual detection tasks. On *innocuous cooling* and *light* trials, the final T2 intensity was adjusted so that its difficulty was equivalent to that of the 0.4°C heating stimulus.

Materials and Methods

Three macaque monkeys (2 *M. mulatta*, 1 *M. fascicularis*) were used. Although these animals were fluid-restricted in order to motivate them to perform the behavioral tasks, they maintained a constant body weight between 4 and 5 kg, and normal food intake. Their fluid intake was earned as they performed the various behavioral tasks shown in Figure 1. Thermal stimuli were delivered via a 1-cm-diameter contact thermode positioned on the maxilla adjacent to the ala of the nose. A visual cue was presented as a small white light contralateral to the thermode placement, and was positioned on the top of the panel in front of the monkey. Monkeys performed the behavioral tasks while sitting in a primate chair (3 hr/d). At all other times, monkeys were housed in their home cages.

Noxious heat, innocuous cooling, and visual-detection tasks

Preliminary training procedures. The monkeys initiated a behavioral trial by depressing an illuminated button located on the lower part of the panel. The monkeys first were trained to press the illuminated button until a bright light, located on the upper part of the panel, was illuminated 3–9 sec after trial initiation. If the monkeys released the panel button, they were rewarded with fruit juice, and a new trial was presented 15.0 sec later. When the monkeys performed the visual task at better than 95% accuracy, their responses were transferred to cooling and noxious heat stimuli as described previously (Hoffman et al., 1981).

Final behavioral paradigm. The final tasks are shown in Figure 1. The different thermal and visual stimuli were delivered under computer control (Digital PDP 11/34). In the noxious heat task, panel-button press resulted in an increase in temperature from a baseline of 38°C to a noxious heat level of 46°C (T1). The monkeys could escape the 46°C

Figure 2. Stability of detecting small incremental increases in noxious heating, innocuous cooling, and visual stimuli for monkeys *H* and *G*. The percentage of trials correctly detected over 10 consecutive control sessions remained constant and was approximately equivalent for the 0.4°C noxious heat T2 and the cooling and light stimuli.

stimulus by releasing the panel, but received no reward. A T1 temperature of 46°C is below pain tolerance levels, since monkeys escape this temperature with a probability of 0.3 or less (Dubner et al., 1976). Thermode temperature remained at 46.0°C for a random time period of 3–9 sec, and then was heated an additional 0.4, 0.6, or 1.0°C (T2). If the monkeys released the button within 2.4 sec of the onset of T2, they were rewarded with 0.3 cm³ of fruit juice. The monkeys performed a similar innocuous cooling-detection task. Following panel-button press, the thermode decreased in temperature from 38 to 36°C (T1). After a variable time period of 3–9 sec, the thermode decreased an additional 0.5°C (T2). Panel-button release within 2.4 sec of T2 provided a juice reward. Finally, the monkeys performed a visual-detection task. Following panel-button press, a light signal (L1) of suprathreshold intensity was illuminated. After a 3–9 sec random time period, the light increased in intensity (L2). As in the thermal-detection tasks, panel release within 2.4 sec resulted in a juice reward.

Two measures of behavioral performance were used in these studies: the mean latency to detection (mLat) of correctly detected trials (i.e., the panel button was released within 2.4 sec of stimulus onset), and the percentage of trials not detected (% ND, i.e., the panel button was released after 2.4 sec of stimulus onset). The stimuli used in these studies were suprathreshold. The overall % NDs for experimental control sessions (i.e., no drug treatment) were 5.9, 0.3, and 0.0% for 0.4, 0.6, and 1.0°C T2s, respectively. The corresponding mLat for correctly detected trials over the same sessions were 0.780 ± 0.008 sec, 0.684 ± 0.004 sec, and 0.658 ± 0.003 sec. The intensities of the innocuous cooling cue and the visual cue were adjusted to the same degree of behavioral difficulty as the 0.4°C thermal stimulus. Figure 2 shows that their degree of difficulty remained constant over a number of experimental sessions.

Microinjection procedures

When the monkeys performed the behavioral tasks at better than 95% accuracy, the first 4 cervical vertebrae were fused to the occipital bone, as described previously (Hoffman et al., 1981). This procedure prevented spinal cord and brain stem movements during medullary dorsal horn (MDH) neuronal recordings and microinjection procedures. A stainless steel chamber was also implanted in order to support the microelectrode drive and the microinjection cannula guide (Hoffman et al., 1981). The MDH was systematically explored with platinum-coated tungsten microelectrodes (1–2 M Ω at 1000 Hz) in order to define the MDH region that represented the skin around the upper lip.

Morphine sulfate (1, 3, or 10 μ g) dissolved in saline (0.9%) vehicle was microinjected into MDH sites from which thermally sensitive nociceptive neurons were recorded. The monkeys received only 1 morphine microinjection per week and each dose was given once to each monkey to minimize the possibility of tolerance effects (Yaksh, 1983). Morphine sulfate and saline were delivered in a volume of 0.2 μ l over

Figure 3. Average peristimulus histograms obtained from the responses of a wide-dynamic-range neuron to a 46.0°C T1 stimulus and T2 stimuli of 0.4, 0.6, or 1.0°C. Twelve trials were averaged to construct the histogram associated with the 38–46.0°C (T1) stimulus. Four trials were averaged to construct each histogram associated with the 0.4, 0.6, or 1.0°C increase (T2) in heating. Arrows indicate time of occurrence of each stimulus. *P*, The press of the illuminated panel button, which was followed 0.5 sec later by the onset of the T1 stimulus. *L*, Time of occurrence (12 trials, range shown by brackets) of illumination of the panel button, indicating to the monkey that he could initiate the trial. *R*, Release of the panel on the 4 trials (brackets indicate range) for each T2 stimulus. The mean behavioral detection latencies were 0.96, 0.77, and 0.68 sec on the 0.4, 0.6, and 1.0°C trials, respectively. The stippled area in the face drawing represents the neuron's receptive field to low-threshold mechanical stimulation. The insert of the medullary dorsal horn illustrates the location of the microinjection cannula.

a period of 2 min via a 32-gauge stainless steel cannula connected to a 1 μ l Hamilton syringe, driven with a micropump.

The time course and effect of MDH-administered morphine on thermal and visual detection were evaluated in 3 monkeys. Each experimental session consisted of 18 sequential blocks of behavioral trials. Each block took approximately 8 min to complete and consisted of 20 randomly presented thermal and visual trials. Each block consisted of 4 visual trials, 12 noxious heat trials (4 each of 0.4, 0.6, and 1.0°C increments from 46.0°C), and 4 innocuous cooling trials (0.5°C decrements from 36.0°C). After placement of the microinjection system, the monkeys performed 2 blocks of behavioral trials. At the end of the second block of behavioral trials, either morphine or saline was microinjected into the MDH, as described above. Following microinjection of morphine or saline, the monkeys continued to perform 16 additional blocks of behavioral trials. On days without microinjections, the animals performed 18 behavioral blocks of trials to maintain their behavior and to monitor the degrees of difficulty of the different cues.

The ability of opiate-receptor blockade to diminish the effects of MDH-administered morphine was examined in 2 monkeys. Either 3 μ g (monkey A) or 10 μ g (monkey H) of morphine or saline (monkeys A and H) was microinjected into the MDH at the beginning of behavioral block 3. At the sixth block of behavioral trials, either naloxone (0.5 mg/kg) or an equal volume of vehicle (0.9% saline) was administered intramuscularly and the monkey continued to perform the remaining 12 blocks of behavioral trials.

In separate experiments, the time course and location of morphine-induced itching following MDH administration was observed in 2 monkeys (G and H). In these experiments, either 3 μ g (monkey G) or 10 μ g

(monkey H) was microinjected into the MDH. The animals were evaluated in their home cages over the next 120 min, which corresponded to the amount of time required to complete 15 behavioral blocks of trials. The number of scratches was monitored by 3 independent investigators. Rapid rubbing of the face or other body regions with either the dorsal surface of the hand or the nails was considered to represent a scratch. The number of scratches and their anatomical locations were monitored in 8 min epochs.

Data collection and analysis

Behavioral and neural data were collected, stored, and analyzed with the aid of Digital PDP 11/34 and PDP 11/70 computers. The following behavioral events were monitored: (1) the time from illumination of the lower panel button until panel press (i.e., trial initiation time); (2) the time from the onset of T2 until panel release (i.e., detection latency); (3) early panel releases (before T2) and misses (no detection—ND). Dose- and stimulus-intensity-dependent effects were determined from mLats on correct detection trials over blocks 3–18 from 3 monkeys. For time course data, averages of correctly detected latency values were calculated for each behavioral block of trials.

The effects of systematically administered naloxone and saline on the behavioral effects induced by MDH-administered morphine were evaluated between blocks 10 and 14. This time window began approximately 60 min after microinjection of morphine or saline into the MDH and approximately 30 min after the administration of naloxone. This time window was selected since it represents the period when morphine's effect on noxious heat detection had reached a plateau (Fig. 4).

Figure 4. Time course of the effects of MDH-administered morphine (●) or saline (○) on the average detection latency on correctly detected behavioral trials of 3 monkeys (A, H, G). Three panels (top to bottom) show effects of morphine (1, 3, and 10 μ g combined) and saline (NaCl 0.9%) administered into the medullary dorsal horn on the detection of 0.4, 0.6, and 1.0°C increases in heating from 46.0°C (T1). The bottom panel shows the effects of morphine and saline on the detection of a visual cue. Mean latency values were obtained by averaging individual latency values of correctly detected thermal and visual behavioral trials in each behavioral block of trials. For the *morphine* curves, a maximum of 36 individual latency values was used to determine each mean data point (e.g., 4 trials of a given stimulus per behavioral block of trials $\times$ 3 doses of morphine $\times$ 3 monkeys). For the *saline* curves, a maximum of 12 individual latency values was used to determine each mean data point (e.g., 4 trials of a given stimulus per behavioral block of trials $\times$ 1 dose of saline $\times$ 3 monkeys). Bars, SEM.

Dose, stimulus intensity, and treatment main effects were determined by ANOVA, using the proper factorial design. When appropriate, Duncan's Multiple-Range Test was used to determine which doses of morphine and stimulus intensities produced different mLat responses. Chi-square analysis was used to analyze percentage data. Significance was assumed at the $p < 0.05$ level.

Histological examination

The microinjection cannula placements were determined by examining the lower brain stem after tissue fixation (formaldehyde 4% and glutaraldehyde 0.2%) and staining (cresyl violet). Tissue was cut into 50 μ m sections and examined for the position of the cannula tip relative to the lamination of the MDH (for a description, see Gobel et al., 1977).

Results

Morphine microinjection sites

While the monkeys performed the task, electrophysiological exploration of the MDH was conducted in order to identify regions that contained thermally sensitive neurons. Morphine was microinjected into sites containing wide-dynamic-range neurons (i.e., neurons that respond to innocuous tactile stimuli, but that

Figure 5. Dose- and stimulus-dependent effects of microinjected morphine or saline on the detection of noxious heat stimuli. The three curves represent the effects of MDH-microinjected saline or morphine on the detection of 0.4, 0.6, or 1.0°C increases in heating from 46.0°C. Data points represent the average latency value obtained from 3 monkeys for correctly detected trials over behavioral blocks 3–18 (i.e., 16 contiguous blocks of behavioral trials). Thus, a maximum of 192 individual trials was used to calculate each average latency value (e.g., 4 stimulus presentations per block of behavioral trials $\times$ 16 blocks of behavioral trials $\times$ 3 monkeys). Bars, SEM.

respond maximally to noxious stimuli) and nociceptive-specific neurons (i.e., neurons that respond only to noxious stimuli). These neurons encode noxious heat intensity and may provide monkeys with information necessary to properly execute the detection task (Maixner et al., 1986). The responses of a wide-dynamic-range neuron during the noxious heat detection task are shown in Figure 3. This neuron had a receptive field that included the ophthalmic and maxillary divisions of the face. When the thermode temperature increased to 46.0°C, the neuron increased its frequency of firing from approximately 10 to 32 Hz. On separate trials, the neuron encoded, in a graded manner, the intensities of T2 stimuli of 0.4–1.0°C. Similarly, the behavioral detection latencies decreased as the magnitude of discharge increased. This site was chosen for microinjection of morphine in this monkey; histological examination revealed its location to be 2–3 mm caudal to obex in the lateral aspect of laminae V, close to the ventrolateral region of the superficial dorsal horn laminae (Fig. 3, inset). The microinjection sites in the other 2 monkeys were similar.

Effects of morphine microinjection on general behavioral performance

Following morphine administration into the MDH, the monkeys could always successfully perform the 18 blocks of behavioral trials. Minor differences in behavioral performance were noted. During control sessions, the monkeys failed to initiate 4.1% of the trials within 2.0 sec of lower-panel-button illumination, while during morphine sessions this increased significantly to 8.7% ($p < 0.005$). The frequency of early panel releases on noxious heat trials increased significantly ($p < 0.001$), from 6% during saline sessions to 19% during morphine (1, 3, and 10 μ g) sessions. This effect was not dose-dependent. It also was not stimulus-modality-specific, since a similar effect was observed during the performance of the innocuous cooling trials (saline: 12%; morphine: 23%; $p < 0.004$).

Table 1. Effects of MDH-administered morphine on % ND of noxious heat trials

Solution	% ND		
	0.4°C	0.6°C	1.0°C
Saline	8	1 ^b	0 ^b
1 μ g Morphine	27 ^a	4 ^{a,b}	1 ^{b,e}
3 μ g Morphine	31 ^a	9 ^{a,b}	1 ^{b,e}
10 μ g Morphine	25 ^a	16 ^{a-d}	10 ^{a-d}

Data were obtained from all 3 monkeys and analyzed using a chi-square test ($\alpha = 0.05$).

^a Statistically different from the saline value.

^b Statistically different from the 0.4°C value.

^c Statistically different from the 1 μ g morphine value.

^d Statistically different from the 3 μ g morphine value.

^e Statistically different from the 0.6°C value.

Time course of the effects of morphine microinjection

Figure 4 shows the time course of the effects of MDH-administered morphine (1, 3, and 10 μ g combined) on the mLats observed on correctly detected noxious thermal and visual trials in the 3 monkeys. Saline administered into the MDH did not significantly alter the mean detection latency or % ND to noxious thermal stimuli over the next 16 blocks of behavioral trials, as compared to the initial 2 blocks of behavioral trials. In contrast, morphine induced a long-lasting increase in mLats associated with the detection of the 3 noxious T2 stimuli. This effect developed gradually and reached a plateau after microinjection for all three T2s. A transient decrease in morphine's ability to alter the detection of 0.4°C T2s was observed between 32 and 56 min after microinjection. Morphine did not increase the mLats for the detection of the near-threshold visual cue. Notably, MDH morphine actually decreased ($p < 0.05$; t test) the average mLats on visual trials summed between 30 and 60 min after microinjection, as compared to values obtained following saline administration.

Dose- and stimulus-dependent effects of morphine microinjection

Morphine increased the mLats and % ND dose-dependently (Fig. 5, Table 1). For 0.4, 0.6, and 1.0°C, a significant effect of the morphine dose administered on the mLats associated with correctly detected trials was observed (ANOVA; 0.4°C, $F = 17.4$, $df = 3,582$, $p < 0.001$; 0.6°C, $F = 21.1$, $df = 3,697$, $p < 0.001$; and 1.0°C, $F = 30.5$, $df = 3,778$, $p < 0.001$). The mLats following the administration of either 1, 3, or 10 μ g of morphine were greater than those associated with saline (Duncan's Multiple-Range Test; $p < 0.05$) for all 3 stimulus intensities. Similarly, the mLats following 3 μ g of morphine were significantly greater than those associated with 1 μ g of morphine for all 3 stimulus intensities. The mLats following 10 μ g of morphine were greater than those associated with 1 μ g for 0.6 and 1.0°C T2s. As can be seen in Table 1, morphine also dose-dependently increased the % ND of 0.6 and 1.0°C T2s.

Morphine microinjection impaired the detection of noxious thermal stimuli in a stimulus-intensity-dependent fashion. As is shown in Figure 5 and Table 1, as the intensity of the T2 stimulus increased, the ability of morphine to increase mLats or the % ND decreased. Analysis of the data (morphine doses 1, 3, and 10 μ g combined) used to construct Figures 4 and 5 reveal a significant effect of stimulus intensity ($F = 113.3$, $df = 2,146$, $p < 0.001$) on the ability of morphine to increase mLats.

Effects of systemically administered naloxone on morphine's action

The effect of systemically administered naloxone (0.5 mg/kg, i.m.) on morphine's ability to increase mLats of correctly de-

Figure 6. The effects of systemically administered naloxone on the increase in detection latencies associated with MDH-administered morphine irrespective of T2 stimulus intensity (i.e., 0.4, 0.6, and 1.0°C data combined). The open bar represents the mean response produced by intramuscularly (i.m.) administered injections of naloxone (0.5 mg/kg) alone; the stipple-shaded bar, the mean response produced by i.m. saline; the diagonal-shaded bar, the mean response produced by MDH-administered saline; the cross-hatched bar, the mean response produced by MDH-administered morphine (10 μ g, monkey H; 3 μ g, monkey A); and the solid bar, the mean response produced by MDH-administered morphine (10 μ g, monkey H; 3 μ g, monkey A) in the presence of naloxone (0.5 mg/kg, i.m.). Each bar was constructed by averaging detection latencies over behavioral blocks 10–14 (i.e., 5 contiguous blocks of behavioral trials when morphine was exhibiting its peak effect; see Fig. 4). Thus, a maximum of 120 values was used to calculate each average latency value (e.g., 12 stimulus presentations per block of behavioral trials $\times$ 5 blocks of behavioral trials $\times$ 2 monkeys). Top bars, SEM.

tected noxious thermal trials following administration into the MDH is shown in Figure 6. Analysis of these data (ANOVA) revealed a significant treatment effect ($F = 20.2$, $df = 4,578$, $p < 0.001$). Duncan's Multiple-Range Test revealed that naloxone treatment significantly attenuated the effects of MDH-administered morphine on noxious heat detection (0.4, 0.6, and 1.0°C data combined). MDH-administered morphine significantly elevated the mLats of correctly detected noxious thermal trials, compared to MDH-administered saline. Although naloxone had a tendency to decrease mLat values, compared to values obtained following systemic saline, this difference did not reach statistical significance. Naloxone had a tendency to decrease the % ND associated with MDH-administered morphine (5 vs 7.5%), but these values were not statistically different from one another.

Effects of morphine microinjection on cooling detection

Figure 7 shows the effects of MDH-administered morphine on the mLats associated with correctly detected cooling trials. Although a trend for morphine to increase the mLats associated with correctly detected cooling trials was observed, it did not reach statistical significance ($p < 0.07$), nor was it dose-dependent. The effect of morphine on cooling trials was greatest for the 1 μ g dose, in contrast to that on noxious heat trials, where its effect was greatest at the 3 μ g dose. Similarly, morphine administered into the MDH did not significantly alter the frequency of NDs as compared to MDH-administered saline on cooling trials. The % NDs following saline and 1, 3, or 10 μ g of morphine were 4, 9, 6, and 4%, respectively.

Scratching induced by MDH-administered morphine

As shown in Figure 8A, the scratches observed in each 8 min epoch occurred almost exclusively ipsilateral to the microinjection site. Morphine-induced itching began within 16 min of microinjection and the frequency of scratching remained constant during the subsequent 128 min of observation. The mean

Figure 7. The effects of MDH-administered saline or various doses of morphine on the detection of a 0.5°C cooling stimulus from 36.0°C. The curve for the detection of the 0.4°C increase in heating from 46.0°C is included for comparison (broken curve). Each data point was determined by averaging individual latency values from 2 monkeys (G, H) over behavioral blocks 3–18 (i.e., 16 continuous blocks of behavioral trials). Thus, a maximum of 128 trials was used to calculate each average latency value (e.g., 4 stimulus presentations per block of behavioral trials $\times$ 16 blocks of behavioral trials $\times$ 2 monkeys).

number of scratches for the 2 monkeys varied from 20 to 45 during each 8 min epoch. In Figure 8B, the percentage of total scratches in a given facial region over the time of observation is shown. These data show that itching occurred primarily around the eye and nose in regions that overlapped receptive fields of neurons in this area of the MDH (see Fig. 3). Nose and eye scratching were more frequent than scratching of other facial and body regions throughout the observation period. For other regions, such as the upper and lower lips, scratching developed later, reaching a maximum between 48 and 64 min after microinjection.

Discussion

We have shown that morphine microinjected into the medullary dorsal horn altered the monkeys' ability to detect noxious heat stimuli. Previous studies have demonstrated that detection latency is a reliable measure of the perceived intensity of a stimulus (Moody, 1970; Stebbins, 1970). The finding that behavioral detection latency covaries with small temperature increases in the noxious heat range (Kenshalo et al., 1985) has been confirmed in the present study. Mean detection latencies increased as a function of T2 magnitude. We also used the frequency of detection failure as an index of sensory function. The % ND decreased as a function of stimulus intensity. Morphine microinjection into the MDH altered these 2 measures of behavioral performance in a dose- and stimulus-dependent fashion. These findings suggest that morphine attenuates the perceived intensity of noxious heat stimuli by altering the sensory encoding process at the level of the dorsal horn.

The monkeys were able to perform the tasks with great accuracy and their behavior remained stable over time during control sessions. Morphine microinjection produced few effects on the monkeys' overall performance. The only general behavioral effects of MDH-administered morphine were an increase in the time to trial initiation and the frequency of early trial

Figure 8. The time course and somatotopic distribution of morphine-induced facial pruritis. *A*, Total number of scratches observed in 2 monkeys (H, G) during 15 observation periods lasting 8 min each. Scratches ipsilateral and contralateral to the microinjection site were determined. *B*, Relative percentages of scratches on different regions of the face ipsilateral to the microinjection site are plotted as a function of time. The drawing shows the different facial regions evaluated.

terminations during the performance of the noxious heat detection task. The increased frequency of early trial terminations most likely resulted from the monkey's search for new strategies to obtain liquid reinforcement as the detection of changes in noxious heating became more difficult. We have observed similar behavior in a monkey as it is trained to detect and discriminate new and more difficult sensory cues. The tendency towards early trial terminations introduces a guessing strategy that could result in correct responses on trials in which the monkey failed to detect the T2 noxious heat cue. Such behavior would tend to diminish the apparent effects of morphine on both mLat and % ND values.

Additional evidence for the pharmacological specificity of the observed effects on noxious heat detection is provided by the results of relatively low doses of naloxone (0.5 mg/kg i.m.). Naloxone reversed the effects of MDH-administered morphine, and the time course of this reversal was compatible with the pharmacokinetics associated with systemically administered naloxone. In the rat, peak naloxone brain levels are obtained approximately 30 min after administration and have a half-life of approximately 24 min (Misra, 1978).

The opiate receptor subtype that mediates morphine's effect on noxious heat detection is not known. It has, however, been established that μ -, δ - and κ -opiate receptors are localized in the spinal cord (Martin et al., 1976; Yaksh, 1983). It is likely that μ - and/or possibly δ -opiate receptors are responsible for the observed effects, since morphine is active at these 2 receptor sites ($\mu > \delta$; Wüster et al., 1978; Yaksh, 1983). Furthermore, these 2 opiate receptor subtypes are thought to influence reflex responses to noxious thermal heat in rats (Schmauss and Yaksh, 1984).

A number of findings support the view that morphine microinjection specifically impairs the detection of noxious heating without influencing attention, motivational factors, or gross motor ability. MDH-administered morphine did not alter the monkeys' ability to perform or initiate behavioral trials, nor did it produce a state of behavioral depression, which commonly follows the systemic or intrathecal administration of opiates (Hernandez and Appel, 1979; Vierck and Cooper, 1984; Yaksh, 1983). The tendency for the detection of the visual cue to improve following MDH-administered morphine also demonstrates that morphine did not impair noxious heat detection by altering attentional aspects of behavior. The improvement in performance in the visual detection task most likely resulted from an increase in the monkeys' vigilance as noxious heat detection became more difficult. In support of this view is that the detection of the 0.4°C T2 stimulus also improved transiently during this time period (Fig. 4). These findings suggest that this task is sensitive to attentional effects and that MDH-administered morphine may have actually improved the monkeys' vigilance during the task in spite of the depressive action of opiates on noxious heat detection. Such increased vigilance would tend to diminish the effects of morphine on mLat responses and the frequency of trials not detected.

These findings are in agreement with other studies that have demonstrated pharmacologically specific, antinociceptive effects of opiates administered into the MDH (Rosenfeld et al., 1983) or intrathecally (Yaksh, 1983; Yaksh and Rudy, 1978). These studies employed behavioral measures such as face rubbing, tail flick, or shock titration. However, such assays do not separate the effects of opiates on the sensory-discriminative (i.e., perceived intensity, duration, and location of the stimulus) aspects of pain from their effects on attentional, motivational, or motoric factors associated with behavioral performance. In contrast, we have designed a behavioral task that reveals that morphine alters the sensory-discriminative aspects of noxious heat without influencing these other aspects of the monkeys' behavior.

The inability of MDH-administered morphine to significantly alter cooling detection in a dose-dependent manner provides additional evidence that morphine exerts specific effects on the detection of noxious heat stimuli. This confirms previous findings that cooling and noxious heat stimuli are encoded by different neuronal systems in the dorsal horn (Dubner et al., 1975; Poulos and Molt, 1976). Our findings do not agree with those of Schwartz et al. (1978), who demonstrated that systemically administered morphine impaired cooling detection in monkeys. However, the relatively high doses of morphine (i.e., 1–10 mg/kg, i.m.) that they used may have influenced the general behavioral performance of the monkeys.

We also observed face scratching following MDH-administered morphine. Earlier reports described morphine-induced pruritis after intrathecal administration in both humans and monkeys (for a review, see Yaksh, 1981). Our findings demonstrate a specific pattern related to the microinjection site and the somatotopic organization of the MDH, whereas earlier studies described a general body pruritis. It is possible that morphine-induced itching may have influenced the detection of noxious heat by distracting the animals, but this is unlikely for a number of reasons. First, the scratching behaviors and morphine effects exhibited different time courses. Second, the monkeys were able to detect the onset of a difficult light stimulus and of a difficult thermal cooling stimulus that was also applied to the same area of the face. Finally, one would have expected heat detection to have improved, since itch induced by pharmacological means or pathological conditions lowers the thermal pain threshold (Hardy et al., 1967).

Compared to systemically or intrathecally administered morphine, MDH-administered morphine provides more direct evidence that opiates can impair the transmission of nociceptive

information at the dorsal horn level. A number of factors make it likely that morphine's effect on noxious heat detection resulted from a direct effect on MDH neuronal activity as opposed to diffusion to other loci outside of the MDH. We used a relatively small microinjection system (i.e., 32 gauge), compared to the dimensions of the MDH. In addition, we microinjected small doses and a very small volume (i.e., 0.2 μ l) into the MDH. Previous studies that used doses as large as 30 μ g and volumes as large as 5.0 μ l have reported that morphine spread at a rate of 4.0 mm/hr (Clark et al., 1983; Jaeger, 1965; Lomax, 1966; Weast, 1977; Yaksh and Rudy, 1978). The observation that MDH-administered morphine did not impair the detection of difficult cooling and visual stimuli also supports the view that the observed effects did not result from the diffusion to sites outside of the MDH. Finally, the time course and localization of morphine-induced scratching to the ipsilateral face provides strong functional evidence that morphine remained within the MDH over the 128 min observation period. The most intense scratching occurred around the eye and nose regions, further suggesting that morphine remained localized to the ophthalmic and maxillary representations within the MDH. Verification that the cannula was localized to the maxillary and ophthalmic distribution was provided by electrophysiological recordings in the region where morphine was microinjected (see Fig. 3). Itching of the upper and lower lips appeared approximately 50–60 min after microinjection. The approximate distance between the MDH representation of the eye/nose somatic regions and the lips is 2–3 mm (Kerr et al., 1968), suggesting a diffusion rate of approximately 3.0 mm/hr.

These data are the first to demonstrate that opiates reduce pain at the dorsal horn level by attenuating its perceived intensity. This is in contrast to the commonly held view that opiates reduce pain by diminishing the affective qualities of pain (Beecher, 1959, 1966). These findings are in agreement with recent human studies (Gracely et al., 1979, 1982) demonstrating that the opiate-receptor agonist fentanyl impairs the perception of the intensity of experimentally evoked tooth-pulp pain with variable effects on the perceived unpleasantness. It is unlikely that the monkey's detection latencies are a measure of the perceived unpleasantness of the T2 stimulus, since 46–47°C thermal stimuli are below the monkey's escape threshold (Dubner et al., 1976).

We conclude that opiates exert a pharmacologically specific effect on the sensory intensity component of pain at the earliest central relay pathway transmitting noxious information. It is generally accepted that the dorsal horn in the medulla contains the neural substrate encoding the intensity of noxious heat stimuli. The present findings support the conclusion that these neurons participate in the encoding process by which monkeys perceive the intensity of such stimuli (Bushnell et al., 1984; Hoffman et al., 1981; Maixner et al., 1986). Opiates appear to alter this encoding process.

References

- Atweh, S. F., and M. J. Kuhar (1977) Autoradiographic localization of opiate receptor in rat brain. I. Spinal cord and lower medulla. *Brain Res.* 124: 53–67.
- Beecher, H. K. (1959) *Measurement of Subjective Responses: Quantitative Effects of Drugs*, Oxford U. P., New York.
- Beecher, H. K. (1966) Pain: One mystery solved. *Science* 151: 840.
- Besson, J. M., A. H. Dickenson, D. LeBars, and J.-L. Oliveras (1979) Opiate analgesia: The physiology and pharmacology of spinal systems. In *Advances in Pharmacology and Therapeutics*, C. Dumont, ed., pp. 61–81, Pergamon, Oxford.
- Bushnell, M. C., G. H. Duncan, R. Dubner, and L. F. He (1984) Activity of trigeminothalamic neurons in medullary dorsal horn of awake monkeys trained in a thermal discrimination task. *J. Neurophysiol.* 52: 170–187.
- Bushnell, M. C., G. H. Duncan, R. Dubner, R. L. Jones, and W. Maixner (1985) Attentional influences on noxious and innocuous cutaneous

- heat detection in humans and monkeys. *J. Neurosci.* 5: 1103–1110.
- Clark, S. L., R. O. Edeson, and R. W. Ryall (1983) The relative significance of spinal and supraspinal actions in the antinociceptive effect of morphine in the dorsal horn—an evaluation of the microinjection technique. *Br. J. Pharmacol.* 79: 807–818.
- Dubner, R., and G. J. Bennett (1983) Spinal and trigeminal mechanisms of nociception. *Annu. Rev. Neurosci.* 6: 381–418.
- Dubner, R., R. Sumino, and W. I. Wood (1975) A peripheral cold fiber population responsive to innocuous and noxious thermal stimuli applied to monkey's face. *J. Neurophysiol.* 38: 1373–1389.
- Dubner, R., R. E. Beitel, and F. J. Brown (1976) A behavioral model for the study of pain mechanisms in primates. In *Pain: New Perspectives in Therapy and Research*, M. Weisenberg and B. Tursky, eds., pp. 155–170, Plenum, New York.
- Glazer, E. J., and A. I. Basbaum (1981) Immunohistochemical localization of leucine-enkephalin in the spinal cord of the cat: Enkephalin-containing marginal neurons and pain modulation. *J. Comp. Neurol.* 196: 377–389.
- Gobel, S., W. M. Falls, and S. Hockfield (1977) The division of the dorsal and ventral horns of the mammalian caudal medulla into eight layers using anatomical criteria. In *Pain in the Trigeminal Region*, D. J. Anderson and B. Matthews, eds., pp. 443–453, Elsevier, Amsterdam.
- Gracely, R. H., R. Dubner, and P. A. McGrath (1979) Narcotic analgesia: Fentanyl reduces the intensity but not unpleasantness of painful tooth pulp sensations. *Science* 203: 1261–1263.
- Gracely, R. H., R. Dubner, and P. A. McGrath (1982) Fentanyl reduces the intensity of painful tooth pulp sensations: Controlling for detection of active drugs. *Anesth. Analg.* 61: 751–755.
- Hardy, J. D., H. G. Wolff, and H. Goodell (1967) *Pain Sensations and Reaction*, pp. 220–221, Hafner, New York.
- Hernandez, L. L., and J. B. Appel (1979) An analysis of some perceptual effects of morphine, chlorpromazine and LSD. *Psychopharmacology* 60: 125–130.
- Hoffman, D. S., R. Dubner, R. L. Hayes, and T. P. Medlin (1981) Neuronal activity in medullary dorsal horn of awake monkeys trained in a thermal discrimination task. I. Responses to innocuous and noxious thermal stimuli. *J. Neurophysiol.* 46: 409–427.
- Hökfelt, T., A. Ljungdahl, L. Terenius, R. Elde, and G. Nilsson (1977) Immunohistochemical analysis of peptide pathways possibly related to pain and analgesia: Enkephalin and substance P. *Proc. Natl. Acad. Sci. USA* 74: 3081–3085.
- Hunt, S. P., J. S. Kelly, P. C. Emson, J. R. Kimmel, R. J. Miller, and J. Y. Wu (1981) An immunohistochemical study of neuronal populations containing neuropeptides or gamma aminobutyrate within the superficial layers of the rat dorsal horn. *Neuroscience* 6: 1883–1898.
- Jaeger, J. C. (1965) Diffusion from constructions. In *Studies in Physiology*, D. R. Curtis and A. K. McIntyre, eds., pp. 107–117, Springer, Berlin.
- Jaffe, J. H., and W. R. Martin (1980) Opioid analgesics and antagonists. In *The Pharmacological Basis of Therapeutics*, A. G. Gilman, L. Goodman, and A. Gilman, eds., pp. 494–534, Macmillan, New York.
- Kenshalo, D. R., Jr., R. Bates, and R. Dubner (1985) The effects of stimulus parameters and attention on the monkey's ability to detect small increases in temperature in the noxious range. *Soc. Neurosci. Abstr.* 11: 1178.
- Kerr, F. W. L., L. Kruger, H. O. Schwassman, and R. Stern (1968) Somatotopic organization of mechanoreceptor units in the trigeminal nuclear complex of the macaque. *J. Comp. Neurol.* 134: 127–144.
- Lomax, P. (1966) The distribution of morphine following intracerebral microinjection. *Experientia* 22: 249–250.
- Maixner, W., R. Dubner, M. C. Bushnell, D. R. Kenshalo, Jr., and J.-L. Oliveras (1986) Wide-dynamic-range dorsal horn neurons participate in the encoding process by which monkeys perceive the intensity of noxious heat stimuli. *Brain Res.* 374: 385–388.
- Martin, W. R., A. A. Eades, J. A. Thompson, R. E. Huppler, and P. D. Gilbert (1976) The effects of morphine- and nalorphine- in the nondependent and morphine-dependent chronic spinal dog. *J. Pharmacol. Exp. Ther.* 197: 517–532.
- Misra, A. L. (1978) Metabolism of opiates. In *Factors Affecting the Action of Narcotics*, M. L. Adler, L. Manana and S. Samarium, eds., pp. 297–343, Raven, New York.
- Moody, D. B. (1970) Reaction time as an index of sensory function. In *Animal Psychophysics: The Design and Conduct of Sensory Experiments*, W. C. Stebbins, ed., pp. 277–302, Appleton, New York.
- Oliveras, J.-L., W. Maixner, R. Dubner, M. C. Bushnell, G. H. Duncan, D. A. Thomas, and R. Bates (1986) Dorsal horn opiate administration attenuates the perceived intensity of noxious heat stimulation in behaving monkey. *Brain Res.* 371: 368–371.
- Poulos, D. A., and J. T. Molt (1976) Response of central trigeminal neurons to cutaneous thermal stimulation. In *Sensory Functions of the Skin in Primates*, Y. Zotterman, ed., pp. 263–283, Pergamon, Oxford.
- Rosenfeld, J. P., C. Prickel, and G. Broton (1983) Analgesia for orofacial nociception produced by morphine microinjection into the spinal trigeminal complex. *Pain* 15: 145–155.
- Ruda, M. A. (1982) Opiates and pain pathways: Demonstration of enkephalinergic synapses on thalamic projection neurons in the dorsal horn. *Science* 215: 1523–1525.
- Schmauss, C., and T. L. Yaksh (1984) *In vivo* studies on spinal opiate receptor systems mediating antinociceptive. II. Pharmacological profiles suggesting a differential association of μ , δ , and κ receptors with visceral, chemical and cutaneous thermal stimuli in the rat. *J. Pharmacol. Exp. Ther.* 228: 1–12.
- Schwartz, A. S., B. Woolf, C. Hedin, and P. Marchok (1978) Dissociation of discrimination of skin warming from skin cooling by morphine in monkeys. *Brain Res.* 156: 206–210.
- Skingle, M., and M. D. Tyers (1980) Further studies on opiate receptors that mediate antinociception—tooth pulp stimulation in the dog. *Br. J. Pharmacol.* 70: 323–327.
- Stebbins, W. C. (1970) *Animal Psychophysics: The Design and Conduct of Sensory Experiments*, Appleton, New York.
- Vierck, C. J., Jr., and B. Y. Cooper (1984) Guidelines for assessing pain reaction, and pain modulation in laboratory animal subjects. In *Advances in Pain Research and Therapy*, Vol. 6, L. Kruger and J. C. Liebeskind, eds., pp. 305–322, Raven, New York.
- Weast, R. C. (1977) *CRC Handbook of Chemistry and Physics*, p. F-62, CRC Press, Cleveland, OH.
- Willis, W. D., and R. E. Coggeshall (1978) *Sensory Mechanisms of the Spinal Cord*, Plenum, New York.
- Wüster, M., R. Schulz, and A. Hery (1978) Specificity of opioids towards the μ -, δ - and κ -opiate receptors. *Neurosci. Lett.* 15: 193–198.
- Yaksh, T. L. (1981) Spinal opiate analgesia: Characteristics and principles of action. *Pain* 11: 293–346.
- Yaksh, T. L. (1983) *In vivo* studies on spinal opiate receptor systems mediating antinociception. I. μ and δ receptors profiles in the primate. *J. Pharmacol. Exp. Ther.* 226: 303–315.
- Yaksh, T. L., and T. A. Rudy (1978) Narcotic analgesics—CNS sites and mechanism of action as revealed by intracerebral injection techniques. *Pain* 4: 299–359.