

# Cervicomedullary Intrathecal Injection of Morphine Produces Antinociception in the Orofacial Formalin Test in the Rat

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**Background:** High cervical and medullary drug delivery has been advocated for the treatment of refractory head and neck pain in humans. Currently, parallel models in animals have not been developed to support this methodology. We combined an accepted animal model of pain of cranial origin with a novel technique of neuraxial drug delivery to address this issue.

**Methods:** Male Wistar rats were implanted with intrathecal catheters that were advanced cephalad through a lumbar guide cannula to the high cervical spinal cord. The orofacial formalin test was used to assess antinociception. Vehicle or morphine (1, 3, 6, 10, 30  $\mu$ g) was injected intrathecally followed 10 minutes later by injection of formalin solution, 2.5%, into the vibrissal pad. Motor assessment and hemodynamic and respiratory blood gas measurements were evaluated in a separate group of animals.

**Results:** Intrathecal morphine produced a dose-dependent decrease in the first and second phases of the behavioral response ( $P < 0.05$ ). The ED<sub>50</sub> (95% confidence limits) values for the first and second phases were 6.65  $\mu$ g (3.52–14.9  $\mu$ g) and 3.40  $\mu$ g (2.37–4.61  $\mu$ g), respectively. Ten micrograms intrathecal naloxone antagonized the morphine effect ( $P < 0.05$ ). Significant cardiovascular and respiratory depression was observed. No significant motor dysfunction was observed.

**Conclusions:** Cervicomedullary injection of morphine produced antinociception in the orofacial formalin test in the rat. This animal model may be useful to assess analgesics designed for parallel clinical application in humans.

THE management of chronic head and neck pain remains a significant therapeutic challenge. The advent of spinal and intracerebroventricular analgesic delivery systems has improved the care of patients with painful conditions that are refractory to more traditional approaches.<sup>1</sup> Appellgren *et al.*<sup>2</sup> reported the clinical application of high cervical intrathecal and intracisternal administration of analgesics for the treatment of refractory head and neck pain. Currently, there are no animal models that allow the systematic assessment of the efficacy and toxicity of putative analgesics by this method. Thus, one aim of the current investigation was to develop an animal model that may serve to guide the

rational choice of analgesics for the treatment of refractory head and neck pain in humans.

Neurons whose somata reside in the trigeminal ganglion provide sensory innervation of the mystacial (whisker) pad *via* the infraorbital branch of the maxillary division of the trigeminal nerve. The central processes of these neurons terminate in the trigeminal subnucleus caudalis, which also has been designated the medullary dorsal horn. Despite the fact that opioids modulate electrophysiologic responses<sup>3–5</sup> and cellular activity<sup>6</sup> of medullary dorsal horn neurons, behavioral correlates are rare.<sup>7</sup> In the current investigation, a novel method of high cervical/medullary drug delivery was combined with the orofacial formalin test to assess the role of  $\mu$ -opioid receptors in trigeminally mediated phasic and tonic pain in the awake, unrestrained rat. We used the orofacial formalin test in this investigation because it has been reproduced by several independent investigators<sup>8–10</sup> and is considered a valid animal model of nociception mediated by cranial afferents. The fact that the upper cervical and medullary dorsal horns contain opioid receptor<sup>11</sup> and receive sensory input from the mystacial pad<sup>12</sup> provides the rationale for our approach.

## Materials and Methods

### Animals

Ninety-five male Wistar rats (weight, 250–300 g) (Harlan Industries, Indianapolis, IN) were used for these experiments. Rats were housed individually and maintained on a 12-h light-dark cycle with access to food and water *ad libitum*. Experimentation conformed to the "Guide for Care and Use of Laboratory Animals" published by the National Institutes of Health and the guidelines for the ethical use of animals in pain research published by the International Association for the Study of Pain. In addition, experimentation was approved by the Johns Hopkins University Animal Care and Use Committee (Baltimore, Maryland). All animals were given a single formalin injection and were used only once in this investigation.

### Drug

One to 30  $\mu$ g morphine sulfate (GMW 360; Infumorph, Madisonville, KY) and 10  $\mu$ g naloxone hydrochloride (GMW 364; Dupont, Wilmington, DE) were administered by intrathecal injection. Formalin, 2.5%, was diluted from stock formaldehyde solution,<sup>13</sup> 37%, and administered by subcutaneous injection. Vehicle and drug

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dilutions were accomplished with sterile preservative-free saline (Abbot Laboratories, North Chicago, IL).

### *Surgical Preparation*

Rats were anesthetized with halothane and placed on a stereotaxic table. A 2.5 cm by 2.5 cm area of skin overlying the lumbosacral spine was shaved with an electric razor. The animal was positioned such that the lumbar spine was flexed to maximize the opening of the intervertebral space at L5-L6 in a manner similar to the method of Storkson *et al.*<sup>14</sup> The skin was prepped with isopropyl alcohol solution, 70%. A skin incision was made first by a 16-gauge needle paramedian to the L5-L6 interspace and extended rostrally by a No. 10 blade just enough to accommodate passage of the blade itself. A periosteal elevator was used to undermine the skin in a rostral and lateral direction from the incision. A 22-gauge angiocatheter was advanced through the incision and lumbar paraspinal musculature into the lumbar subarachnoid space, and the needle was withdrawn. A sterile, 24-cm-long polyethylene catheter (model PE-10; Intramedic, Clay Adams, Parsippany, NJ) was advanced through the angiocatheter 10.5 cm to the high cervical spinal cord followed by removal of the angiocatheter. A loose knot was tied in the exposed portion of the PE-10 catheter at the skin exit site, and the shape of the knot was reinforced with acrylic cement (methyl methacrylate polymer; Lang Dental, Wheeling, IL). An 18-gauge Crawford needle (model B-D; Franklin Lakes, NJ) was used to tunnel the exposed end of the PE-10 catheter subcutaneously to exit the skin at the level of the cervicothoracic junction. The catheter portion with the acrylic knot was positioned subcutaneously within the undermined pocket. The exposed length of catheter was pulled gently so that the acrylic knot was not directly beneath the original incision. The catheter was flushed with preservative-free saline through a 29-gauge needle and heat sealed.

### *Behavioral Assessment (Formalin)*

Animals were allowed to recover 4 to 7 days before testing during the light phase between 9:00 AM and 3:00 PM. Room temperature was maintained at 23–24°C. Each animal was habituated for 20 min in a 30 cm by 30 cm by 30 cm, clear plastic testing chamber before the experiment. A mirror was placed at a 45° angle underneath the testing chamber to provide an unobstructed view of the animal. Handling times were minimized during the injection process. Testing was accomplished in a quiet, dimly lit room. The testing chamber was thoroughly cleaned with water and mild detergent between animals. Rats were given a 10- $\mu$ l intrathecal injection of either vehicle (saline) or morphine (1–30  $\mu$ g) followed by 15  $\mu$ l sterile saline to flush the catheter. A subset of morphine-treated rats was given 10  $\mu$ g intrathecal naloxone 10 min before either the first phase or

the peak of the second phase for the antagonist study. This sequence of injections ensured that the time course of the behavioral response and the peak effect of each drug would coincide. Ten minutes after vehicle or drug, each rat was given an injection of formalin, 2.5%, into the C1-D1 to C3-D3 region<sup>15</sup> of either the right or left vibrissal pad. A 29-gauge needle was used for formalin injection to minimize procedural stress. Rats were returned to the testing chamber, and the number of seconds spent rubbing the ipsilateral face was recorded for 15 intervals of 3 min each in real time with a stopwatch.

### *Motor Assessment and General Behavior*

A separate group of animals was given intrathecal drug or vehicle and was subjected to the following tests: catalepsy test, righting reflex, forelimb placing, forelimb grasp, cornea reflex, and inclined plane. Because the orofacial formalin model requires an integrated behavioral response, these tests were used to distinguish antinociception from generalized sedation or motor dysfunction. To test for catalepsy (loss of spontaneous mobility),<sup>16</sup> the forepaws of the rat were placed on a horizontal bar 8 cm above the table surface. Animals were considered cataleptic if they remained in position for longer than 10 s. The righting reflex was tested by positioning the animal on its dorsal surface. The test result was considered normal if the animal returned to upright positioning within 2 s. To test for forelimb placing,<sup>17</sup> the dorsal-lateral surface of the animal's forepaw was gently brushed along the edge of a table. The test result was considered normal if the animal placed the ventral surface of the forepaw onto the table surface. The cornea reflex was tested by gently applying a cotton-tipped applicator to the cornea, and the normal response consisted of a blink reflex. To test the forelimb grasp reflex,<sup>18</sup> the animal was suspended by the tail and the ventral surface of the forepaw was touched with a pencil. The test result was considered normal if the animal grasped the pencil. For the inclined plane test,<sup>19</sup> the rat was placed horizontally along an inclined surface and the ability of the animal to negotiate a 45° inclined plane was recorded.

### *Hemodynamic and Arterial Blood Gas Analysis*

A subset of rats with intrathecal catheters was anesthetized with halothane and implanted with femoral artery catheters using aseptic technique. The arterial catheter was tunneled subcutaneously, exteriorized dorsally at the mid-thoracic region, and connected to a swivel adapter that allowed unrestricted movement of the animal. Rats were allowed to recover for a minimum of 4 h after halothane anesthesia. Animals were given 10  $\mu$ g intrathecal morphine (ED<sub>90</sub> for phase 2) or vehicle followed 10 min later by injection of formalin, 2.5%, in the vibrissal pad. Hemodynamic (Windograf 900, Gould, Valley View, OH) and arterial blood gas (Chiron Diag-

nostics, East Walpole, MA) measurements were taken at baseline, 30 min after drug injection (peak of the second phase), and 60 min after drug injection (end of nocifensive period).

### Postmortem Analysis

Rats were euthanized by a lethal dose of halothane anesthesia. Cervical laminectomy and suboccipital craniotomy were performed using blunt dissection techniques. The location and patency of subarachnoid catheters were verified after injection of 10–20  $\mu$ l Evans blue dye followed by surgical exploration of the catheter tip. Only rats which demonstrated postmortem staining of cervicomedullary tissue and catheter tip position between C1 and C4 were used for data analysis.

### Statistical Analysis

The duration of time in seconds rubbing the vibrissal pad was recorded for each animal for 15 intervals of 3 min each. Time-response data are expressed as mean  $\pm$  SEM for each interval. The first phase was defined as the time period of vibrissal rubbing during the first 3-min interval. The second phase was defined as the time period of vibrissal rubbing between the 5th and 15th 3-min intervals. The sum of first- and second-phase behavioral responses was calculated for each animal. Dose-effect data are expressed as the mean  $\pm$  SEM for the first and second phases. Dose-effect relations were constructed by least-squares linear regression analysis. The dose producing 50% maximal effect ( $ED_{50}$  [95% confidence limits (CL)]) was determined for the first and second phases according to the methods described by Tallarida and Murray.<sup>20</sup> Dose-effect and catheter position data were analyzed by one-way analysis of variance. Heart rate, mean arterial pressure, and arterial blood gas data were expressed as the mean  $\pm$  SEM and analyzed by two-way analysis of variance for repeated measurements. *Post hoc* multiple-means comparisons were made by Bonferroni method. Student *t* test was used to compare independent samples. Pearson product-moment correlation coefficient was used to determine the strength of the relation between catheter tip position and drug effect. The Skewness-Kurtosis test was used to verify normality of data. Values of  $P < 0.05$  were considered statistically significant.

## Results

### Effect of Formalin, 2.5%, in Saline-treated Rats

The injection of formalin, 2.5%, produced the characteristic biphasic behavioral response in saline-treated rats (fig. 1). First-phase responses began almost immediately (within 5–10 s) upon return to the testing chamber and were generally restricted to the first 3-min interval. The mean ( $\pm$  SEM) response for the first phase was 41.2

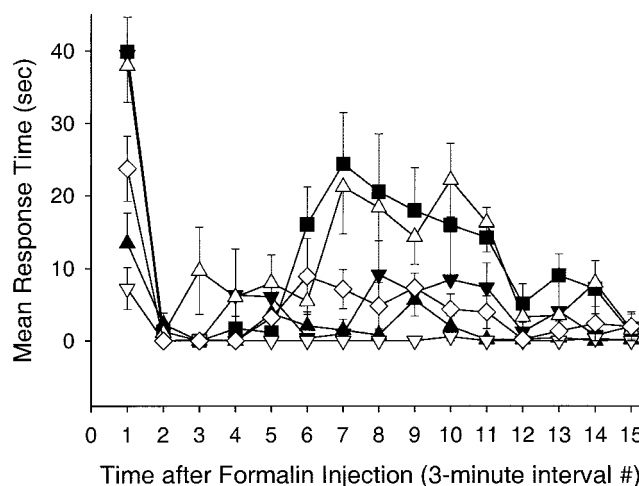


Fig. 1. Effect of cervicomedullary intrathecal injection of morphine (inverted open triangle = 30  $\mu$ g; closed triangle = 10  $\mu$ g, open diamond = 6  $\mu$ g, inverted closed triangle = 3  $\mu$ g, open triangle = 1  $\mu$ g) or vehicle (closed square) on formalin-induced behaviors. Data are presented as the mean  $\pm$  SEM for 6–8 animals for morphine and 12 animals for vehicle and represent the duration of time rubbing the vibrissal pad versus time (0–45 min) after formalin injection. Designation of statistical significance for data points (see Results) was omitted for illustrative clarity.

( $\pm 4.4$ ) s. Second-phase responses generally began during the 5th interval (16–18 min), peaked during the 7th interval (22–24 min), and generally dissipated before the 15th interval (43–45 min). The mean ( $\pm$  SEM) response for the second phase was 134.3 ( $\pm 16.1$ ) s. The interphase generally occurred between the second and fifth intervals. In general, animal behaviors were not continuous and individual animals commonly displayed a period of noxious face-rubbing followed by a period of inactivity. The intrathecal injection of 10  $\mu$ g naloxone did not have a significant effect on first-phase ( $46.5 \pm 7.0$  s) or second-phase ( $102 \pm 20.4$  s) responses compared with saline-treated animals ( $P > 0.05$ ).

### Effect of Morphine

The effect of morphine on the behavioral response to formalin is illustrated in figure 1. One-way analysis of variance indicated a statistically significant difference between the different treatment groups for the first ( $F_{5,37} = 7.84$ ,  $P < 0.05$ ) and second ( $F_{5,37} = 13.35$ ,  $P < 0.05$ ) phases. *Post hoc* analysis with Bonferroni correction of first-phase behavior demonstrated that 10  $\mu$ g ( $15.8 \pm 4.0$  s) and 30  $\mu$ g ( $7.24 \pm 2.9$  s) morphine significantly reduced first-phase responses compared with the response of saline-treated animals ( $41.2 \pm 4.4$  s) with an overall  $\alpha$  level of 0.05. One microgram ( $39.5 \pm 6.9$  s), 3  $\mu$ g ( $39.1 \pm 5.6$  s), and 6  $\mu$ g ( $23.7 \pm 4.5$  s) morphine did not have a significant effect. In contrast, *post hoc* analysis of second-phase responses demonstrated that 3  $\mu$ g ( $53.6 \pm 21.8$  s), 6  $\mu$ g ( $45.7 \pm 11.5$  s), 10  $\mu$ g ( $16.9 \pm 9.8$  s), and 30  $\mu$ g ( $1.11 \pm 0.9$  s) morphine significantly reduced second-phase responses compared



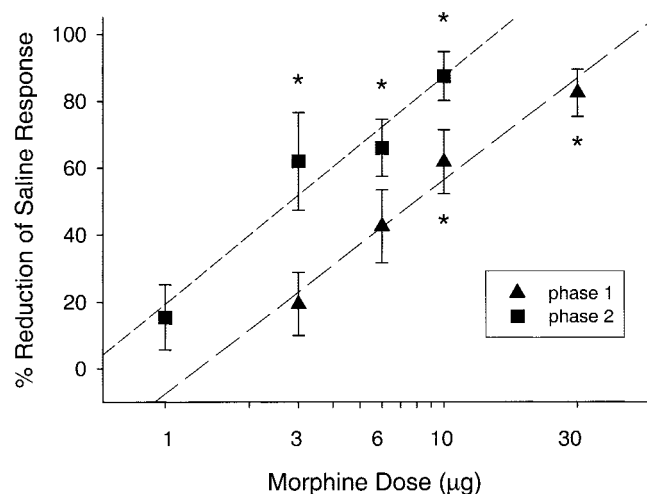


Fig. 2. Dose-effect relation of cervicomedullary intrathecal morphine during phase 1 (closed triangle) and phase 2 (closed square). Data are presented as the mean  $\pm$  SEM for 6–8 animals. Values statistically different from vehicle are indicated for  $P < 0.05$  (\*). Dashed lines were calculated by least-squares linear regression. Data on the y-axis were converted to percentage reduction of the saline response by the following formula:  $[1 - (\text{drug response}/\text{saline response})] \times 100$ .

with the response of saline-treated animals ( $134.3 \pm 16.1$  s) with an overall  $\alpha$  level of 0.05. One microgram morphine ( $137.9 \pm 21.0$  s) did not have a significant effect. The dose-effect relations are illustrated in figure 2. The  $ED_{50}$  (95% CL) values for the first and second phases were  $6.65 \mu\text{g}$  (3.52–14.9  $\mu\text{g}$ ) and  $3.40 \mu\text{g}$  (2.37–4.61  $\mu\text{g}$ ), respectively.

#### Effect of Naloxone

The injection of 10  $\mu\text{g}$  intrathecal morphine reduced both first-phase ( $15.8 \pm 4.0$  s) and second-phase ( $16.9 \pm 9.8$  s) responses after formalin injection (fig. 3). Ten micrograms intrathecal naloxone completely antagonized the effect of morphine during both phases (fig. 3). The mean values ( $\pm$  SEM) of first-phase ( $48.3 \pm 6.5$  s) and second-phase ( $115 \pm 22.3$  s) responses were significantly greater than those in morphine-treated rats ( $P < 0.05$ ) and statistically not different from values in saline-treated rats reflecting a return to baseline.

#### Effects on Motor Function and Generalized Behavior

There was no motor dysfunction or catalepsy observed after intrathecal injection of saline or 1, 3, and 6  $\mu\text{g}$  morphine. However, 10  $\mu\text{g}$  morphine produced catalepsy (1 of 6 rats) and loss of cornea reflex (1 of 6 rats), and 30  $\mu\text{g}$  morphine produced loss of righting reflex (1 of 5 rats). Forelimb placing, forelimb grasp, and inclined-plane angle were unaffected by all doses of morphine. Ten micrograms intrathecal naloxone did not affect performance on any test. Table 1 provides a summary of these results. Other stereotypic behaviors (such as chew-

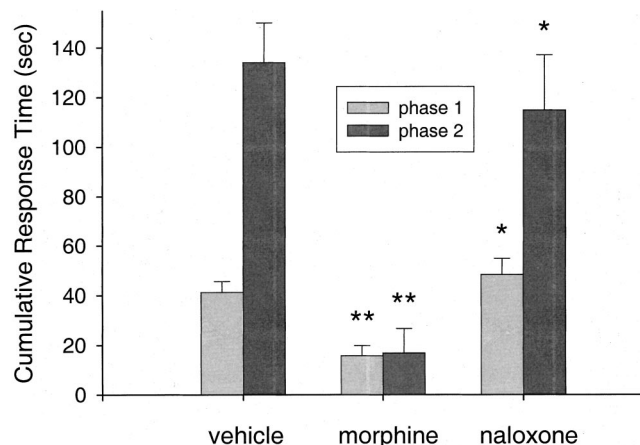


Fig. 3. Antagonism of intrathecal morphine antinociception by intrathecal naloxone. Histograms represent the mean  $\pm$  SEM for 12 animals for vehicle and 5–8 animals per drug and represent the cumulative response time rubbing the vibrissal pad after formalin injection during either the first or second phase. Values statistically different from vehicle are indicated for  $P < 0.05$  (\*). Ten micrograms naloxone significantly antagonized 30  $\mu\text{g}$  morphine antinociception ( $P < 0.05$  [\*\*]).

ing, licking, rearing) were observed but were not quantified.

#### Effects on Hemodynamics and Respiratory Blood Gas Values

Baseline heart rate, mean arterial pressure, pH, partial pressure of carbon dioxide ( $P_{\text{CO}_2}$ ), and partial pressure of oxygen ( $P_{\text{O}_2}$ ) in saline-treated and morphine-treated animals were not statistically different ( $P > 0.05$ ) (table 2). Two-way analysis of variance with repeated measures demonstrated a statistically significant treatment effect on  $P_{\text{O}_2}$  ( $F_{1,45} = 46.79$ ,  $P < 0.05$ ), pH ( $F_{1,45} = 11.31$ ,  $P < 0.05$ ),  $P_{\text{CO}_2}$  ( $F_{1,45} = 13.91$ ,  $P < 0.05$ ), and heart rate ( $F_{1,45} = 6.93$ ,  $P < 0.05$ ), as well as a significant treatment effect by time interaction on  $P_{\text{O}_2}$  ( $F_{2,45} = 14.18$ ,  $P < 0.05$ ), pH ( $F_{2,45} = 6.69$ ,  $P < 0.05$ ), and  $P_{\text{CO}_2}$  ( $F_{2,45} = 5.83$ ,  $P < 0.05$ ). Morphine caused a reduction in mean arterial pressure, but this effect lacked statistical significance ( $F_{1,45} = 2.42$ ,  $P > 0.05$ ). *Post hoc* comparisons demonstrated that 10  $\mu\text{g}$  intrathecal morphine produced

Table 1. Number of Animals Demonstrating Signs of Behavioral or Motor Impairment after Drug Treatment

	Incline Plane	Catalepsy Test	Forepaw Grasp	Forepaw Placing	Righting Reflex	Cornea Reflex
Saline						
10 $\mu\text{l}$	0/4	0/4	0/4	0/4	0/4	0/4
Morphine						
1 $\mu\text{g}$	0/4	0/4	0/4	0/4	0/4	0/4
3 $\mu\text{g}$	0/4	0/4	0/4	0/4	0/4	0/4
6 $\mu\text{g}$	0/4	0/4	0/4	0/4	0/4	0/4
10 $\mu\text{g}$	0/6	1/6	0/6	0/6	0/6	1/6
30 $\mu\text{g}$	0/5	0/5	0/5	0/5	1/5	0/5
Naloxone						
10 $\mu\text{g}$	0/4	0/4	0/4	0/4	0/4	0/4

**Table 2. Hemodynamic and Respiratory Blood Gas Measurements in Saline- and Morphine-treated Animals**

	pH	Pco <sub>2</sub> (mmHg)	Po <sub>2</sub> (mmHg)	HR (beats/min)	MAP (mmHg)
Saline					
Baseline	7.47 (0.01)	29.9 (0.7)	88.3 (2.2)	495.0 (6.7)	113.2 (1.2)
t = 30 min	7.48 (0.01)	29.9 (1.0)	91.4 (1.5)	520.0 (10.0)	121.2 (2.8)
t = 60 min	7.47 (0.01)	27.1 (1.6)	92.9 (3.4)	490.0 (12.6)	116.0 (4.8)
Morphine					
Baseline	7.48 (0.01)	30.5 (1.2)	88.4 (1.8)	496.4 (7.4)	112.5 (2.1)
t = 30 min	7.45 (0.01)*	34.3 (1.9)	73.2 (2.2)*	488.2 (9.1)*	116.6 (2.5)
t = 60 min	7.40 (0.02)*	42.0 (3.0)*	62.0 (3.8)*	450.9 (12.2)	110.0 (2.8)

Data are expressed as mean  $\pm$  SEM for 6–11 animals per group. Values statistically different from vehicle (saline) are indicated for  $P < 0.05$  (\*).

Pco<sub>2</sub> = partial pressure of carbon dioxide; Po<sub>2</sub> = partial pressure of oxygen; HR = heart rate; MAP = mean arterial pressure.

significant reduction in heart rate ( $P < 0.05$ ) during the peak of the second phase (t = 30 min) but not at termination of the nocifensive period (t = 60 min). In addition, morphine produced significant reduction in Po<sub>2</sub> and pH ( $P < 0.05$ ) during the peak of the second phase and at termination of the nocifensive period, as well as significant increase in Pco<sub>2</sub> ( $P < 0.05$ ) at the end of the nocifensive period.

#### Position of Catheter Tip

All cervical catheters terminated in the high cervical spinal cord between C1 and C4 vertebral levels (table 3). Intrathecal injection of Evans blue dye resulted in staining of cervicomedullary tissue in all animals. The mean catheter tip position did not differ between groups ( $F_{5,37} = 0.80$ ,  $P > 0.05$ ). There was a positive but extremely weak correlation ( $r = 0.13$ ) between catheter tip position and drug effect that lacked statistical significance ( $P > 0.05$ ).

#### Neurological Injury and Catheterization Success Rate

Eighty-six rats were implanted with high cervical intrathecal catheters. Eleven animals (11 of 86 [12.7%]) displayed signs of neurologic injury (severe = barrel rolling; isolated = unilateral forelimb paresis) after catheterization. Postmortem investigation demonstrated intracranial catheter placement in animals with severe neurologic dysfunction (n = 4) but no identifiable cause in animals with isolated forelimb paresis (n = 7). Seventy-five rats were given intrathecal drug injection. Four animals (4 of 75 [5.3%]) were removed from the study after injection (postmortem) because the catheter tip was located caudal to the C4 vertebral level. Overall, 71 rats (71 of 86 [82.6%]) implanted with high cervical catheters met the criteria for data analysis.

## Discussion

The spinal cord and medullary dorsal horns are a significant site for integration and relay of somatosensory information. The study of spinal cord dorsal horn recep-

tor pharmacology has been accelerated by the advent of subarachnoid catheterization and injection techniques.<sup>21</sup> However, the study of receptor pharmacology of the medullary dorsal horn has been limited compared with its spinal counterpart. The paucity of information regarding receptor pharmacology as it relates to animal behavior does not appear to be related to lack of scientific interest because anatomic and electrophysiologic investigations predominate in reviews of trigeminal somatosensory processing.<sup>22</sup>

Methods currently used to deliver drug into the medullary dorsal horn parenchyma require the brief but concurrent administration of anesthesia.<sup>23</sup> Chronic cervical intrathecal catheterization does not require delivery of anesthetic at the time of behavioral investigation but may in fact direct drug away from the medullary dorsal horn itself.<sup>24</sup> As an alternative, intracranial microinjection techniques have been described for the delivery of drug into the brainstem.<sup>25</sup> In general, these methods are moderately intrusive to cranial or upper cervical tissue, which may lead to the sensitization of cervicotrigenic convergent neurons.<sup>26</sup> Theoretically, these concerns may limit the application of present methods of medullary drug delivery to the study of trigeminal recep-

**Table 3. Distribution of Catheter Positions by Treatment Group**

	Saline	30 $\mu$ g Morphine	10 $\mu$ g Morphine	6 $\mu$ g Morphine	3 $\mu$ g Morphine	1 $\mu$ g Morphine
Distribution	C2	C3	C4	C4	C3	C2.5
	C3.5	C3	C3	C4	C2	C4
	C3.5	C3.5	C1	C3.5	C4	C4
of catheters	C3	C4	C1.5	C2	C4	C2.5
	C1.5	C3.5	C3	C4	C2	C3.5
(cervical level)	C3		C4	C4	C4	C1.5
	C2		C2			
	C4		C4			
	C3					
	C3					
	C1					
	C4					
Mean (SEM)	2.8 (0.3)	3.4 (0.2)	2.8 (0.4)	3.6 (0.3)	3.2 (0.4)	3.0 (0.4)

Values represent cervical vertebral level of catheter tip position. The mean  $\pm$  SEM catheter tip position is presented for each treatment group.

tor pharmacology using animal behavioral models of nociception. Consequently, we developed a minimally invasive method of drug delivery to the cervicomedullary cerebrospinal fluid in the rodent that could be combined with an accepted model of cranial pain for the assessment of putative analgesics conveyed by trigeminal relays. This is important because the physiology<sup>27</sup> and pharmacology<sup>28</sup> of trigeminal pain processing may be different from their somatic counterparts.

In this investigation, we have reproduced the orofacial formalin test in Wistar (Harlan) rats. These formalin-evoked responses can be differentiated from the background level of spontaneous, nonnoxious face-grooming observed in naive animals.<sup>29</sup> The duration of the biphasic response in our study compares with results from previous investigation in Sprague-Dawley (Charles River; Iffa-Credo, France) rats.<sup>8-10,24,30</sup> However, the response amplitudes for second-phase responses in our study were not as great as those reported by previous investigators. These discrepancies may reflect strain differences<sup>31</sup> or methodological differences (lighting, noise, odors, handling stress) that can influence the formalin test itself.<sup>13,31</sup> In addition, we report a shorter onset to first-phase behaviors (5–10 s) *versus* that observed by other authors (15–30 s). Because volatile anesthetics delay the onset<sup>32</sup> and reduce the magnitude<sup>33</sup> of formalin-evoked responses, our findings may reflect the fact that we did not deliver an anesthetic before formalin injection.

The catheterization success rate in the present study (82.6%) compares with the catheterization success rate for lumbar intrathecal methods (70–90%).<sup>14</sup> Postmortem analysis demonstrated that catheters were distributed between the C1 and C4 vertebral levels, and injection of Evans blue dye verified staining of cervicomedullary tissue. This pattern of staining and distribution of catheter tips is consistent with the literature regarding the topographical organization of centrally projecting trigeminal sensory afferents from the mystacial vibrissae in the rat, which extend from the medullary to cervical dorsal horns as far caudal as C7.<sup>34</sup> Catheter tip positions did not differ significantly between treatment groups, and there was a weak correlation between catheter tip position and drug effect that lacked statistical significance. The relatively small anatomic distance between the C1 and C4 vertebral level (approximately 11 mm; 250- to 300-g rat) may have prevented our ability to demonstrate a significant effect based on catheter tip position.

Previous researchers have demonstrated that systemic,<sup>8,30</sup> intrathecal,<sup>24</sup> and peripheral<sup>30</sup> administration of morphine produces antinociception in the orofacial formalin test. Only the latter study<sup>30</sup> assessed and demonstrated the ability of naloxone to antagonize the effect of morphine. In the current study, we have demonstrated that the high cervical administration of morphine (3–30  $\mu$ g) produces dose-dependent, naloxone-revers-

ible antinociception after orofacial formalin injection. It is important that 10  $\mu$ g intrathecal naloxone alone had no effect on formalin-evoked behavior. This is consistent with the literature regarding the lack of effect of high-dose naloxone on formalin-evoked responses.<sup>35</sup>

In general, our results parallel similar investigation in the paw formalin test in the rat in which researchers have demonstrated that 0.1–10.0  $\mu$ g lumbar intrathecal morphine produces dose-dependent antinociception that is reversed by 10  $\mu$ g intrathecal naloxone.<sup>36</sup> The ED<sub>50</sub> (95% CL) values for the first and second phases in the paw formalin test were 3.7  $\mu$ g (1.6–8.1  $\mu$ g) and 3.8  $\mu$ g (2.6–5.4  $\mu$ g), respectively. The ED<sub>50</sub> (95% CL) values for the first and second phases in the current investigation were 6.65  $\mu$ g (3.52–14.9  $\mu$ g) and 3.40  $\mu$ g (2.37–4.61  $\mu$ g), respectively. The similarity in these findings confirms the robustness of the formalin model given the fact that different strains, behavioral end points, formalin concentrations, and injection sites were used (for review Abbott *et al.*<sup>31</sup>). Moreover, our results are consistent with previous research demonstrating the efficacy of a single 30- $\mu$ g dose of morphine injected into the high cervical intrathecal space in the second phase of the orofacial formalin test<sup>24</sup> or the dose-dependent effect of 2.5–10  $\mu$ g intracerebroventricular morphine in the paw formalin test.<sup>37</sup> Of interest, Aigouy *et al.*<sup>24</sup> reported that 30  $\mu$ g morphine had no effect on first-phase behaviors in the orofacial formalin model. This may reflect the fact that higher doses of morphine were required to maximally reduce first-phase responses compared with second-phase responses (in the current study). The fact that a higher dose of morphine was required in the previous study may be related to the catheterization technique itself (catheter tip aimed caudad rather than cephalad) or to methodologic differences previously discussed.<sup>13,31</sup>

Lumbar intrathecal injection of 10  $\mu$ g morphine had no effect on first- or second-phase responses. Thus, the dose-dependent effect of morphine was produced by a pharmacologic action of drug on rostral targets within the central nervous system. In this experiment in the current study, higher doses of morphine caused catalepsy and loss of the righting reflex and cornea reflex in some animals, which suggests that supraspinal targets were reached. Thus, site specificity cannot be determined precisely from the results of the current investigation despite the fact that the medullary dorsal horn is a logical site for opioid action.<sup>3-5,7,11</sup>

The reduction in formalin-evoked behaviors by morphine was not caused by overt motor dysfunction. However, we cannot rule out the contribution of catalepsy in the reduction in formalin-evoked responses because higher (10  $\mu$ g) doses of morphine decreased spontaneous movement in some animals. These observations are



consistent with previous reports of akinesia and catalepsy after slightly higher (30–60  $\mu\text{g}$ ) injected intracerebroventricular doses of morphine.<sup>38</sup> Also, formalin-evoked face-rubbing should be differentiated from the vigorous body-scratching and spontaneous agitation reported only after high-dose intrathecal injection of morphine.<sup>39</sup> Moreover, formalin-evoked face-rubbing should be differentiated from opioid-induced grooming, which occurs 40 min after intracerebroventricular injection of morphine.<sup>40</sup> In addition, the medullary dorsal horn itself is considered a site for opioid-mediated facial scratching and pruritus.<sup>23</sup> By these arguments, however, the morphine-mediated reduction of formalin-evoked responses observed in the current investigation may have been underestimated.

Ten micrograms intrathecal morphine produced a significant decrease in heart rate,  $\text{Pco}_2$ , and pH, as well as significant increase in  $\text{Pco}_2$ . In addition, the effects on respiratory blood gas parameters were time dependent and more pronounced at the end of the nocifensive period ( $t = 60$  min) compared with the peak of the second phase ( $t = 30$  min). This time dependency may be related to rostral redistribution of drug. As an alternative, ongoing nociceptive stimulation during the peak of the second phase may have prevented significant respiratory depression during this interval. In general, our results are consistent with the literature regarding the cardiovascular and respiratory depressant effects of opioids after short-term administration. It is important to note that chronic neuraxial administration of morphine to patients with chronic pain rarely is associated with significant cardiovascular or respiratory depression.<sup>41</sup>

The delivery of drug to the central nervous system has been described extensively in animals and humans.<sup>42</sup> In the current study, we have described a novel method for administration of drug to the high cervical and medullary cerebrospinal fluid that may facilitate animal behavioral investigation of trigeminal somatosensory pharmacology. The contributing effect of drug on more rostral brain sites cannot be excluded, and chronic infusion of analgesic will be required to determine whether side effects or toxic effects will limit the application of this method. To date, relatively little is known about the long-term effects of cervicomedullary infusion of analgesics in humans. Clinical reports of high cervical or intracisternal infusion therapy for the treatment of cranial pain have been sparse.<sup>2</sup> This probably reflects the widespread use of intracranial infusion methods<sup>43</sup> and the reported efficacy of lumbar spinal drug delivery for a subset of patients with head pain.<sup>44</sup> Further research in animals is required before the full potential of brainstem infusion techniques can be realized in the clinical management of complex craniofacial and cervical pain in humans.

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