Pathological pain is considered a disease and is defined as abnormal sensory processing as a result of tissue damage. Analgesics are administered to decrease the detrimental consequences of pain, obtund behavioral and stress-related changes, and decrease recovery time as well as to improve quality of life during the recuperative period.1–3 Treatment of pain in horses remains largely empirically based.

Opioids are believed to provide analgesia by binding to μ, κ, and δ opioid receptors located in the CNS and peripheral tissues.5 Regardless of demonstrated analgesic efficacy of opioid administration in horses,7 opioid use in this species remains controversial, in large part because of concerns regarding the development of adverse effects when high doses of opioids are administered. Examples of such adverse effects include excitement, increases in locomotor activity and muscle tone,7–12 cardiovascular stimulation,13 and decreases in gastrointestinal motility.14–17 The dose, route, and duration of opioid treatment likely influence whether adverse effects develop and the severity of those effects.8,10,17 As a consequence, opioids are typically withheld from horses or administered to them in conjunction with an α2-adrenoceptor agonist or phenothiazine tranquilizer.11,15

**Cardiorespiratory, gastrointestinal, and analgesic effects of morphine sulfate in conscious healthy horses**

Juliana P. Figueiredo, MV, MS; William W. Muir, DVM, PhD; Richard Sams, PhD

**Objective**—To compare the cardiorespiratory, gastrointestinal, analgesic, and behavioral effects between IV and IM administration of morphine in conscious horses with no signs of pain.

**Animals**—6 healthy adult horses.

**Procedures**—Horses received saline (0.9% NaCl) solution (IM or IV) or morphine sulfate (0.05 and 0.1 mg/kg, IM or IV) in a randomized, masked crossover study design. The following variables were measured before and for 360 minutes after drug administration: heart and respiratory rates; systolic, diastolic, and mean arterial blood pressures; rectal temperature; arterial pH and blood gas variables; intestinal motility; and response to thermal and electrical noxious stimuli. Adverse effects and horse behavior were also recorded. Plasma concentrations of morphine, morphine-3-glucuronide, and morphine-6-glucuronide were measured via liquid chromatography–mass spectrometry.

**Results**—No significant differences in any variable were evident after saline solution administration. Intravenous and IM administration of morphine resulted in minimal and short-term cardiorespiratory, intestinal motility, and behavioral changes. A decrease in gastrointestinal motility was detected 1 to 2 hours after IM administration of morphine at doses of 0.05 and 0.1 mg/kg and after IV administration of morphine at a dose of 0.1 mg/kg. Morphine administration yielded no change in any horse’s response to noxious stimuli. Both morphine-3-glucuronide and morphine-6-glucuronide were detected in plasma after IV and IM administration of morphine.

Few reports if any exist regarding the cardiorespiratory, gastrointestinal, and behavioral effects of IV or IM administration of morphine sulfate at doses of 0.05 and 0.1 mg/kg to conscious horses. Only 1 study has been conducted to compare the analgesic effects of a 0.05 mg/kg dose of morphine administered intra-articularly and IV in conscious horses with experimentally induced synovitis. However, in that study, analgesic effects were not evaluated until 2 hours after morphine administration.

The purposes of the study reported here were to determine cardiorespiratory, gastrointestinal, behavioral, analgesic, and adverse effects of administration of 2 IV and IM doses (0.05 and 0.1 mg/kg) of morphine sulfate in horses and to determine plasma concentrations of morphine and 2 of its known major metabolites: M-3-G and M-6-G. We hypothesized that current clinically recommended doses of morphine sulfate would provide analgesia without clinically relevant adverse effects.

**Materials and Methods**

**Animals**—The study involved 6 healthy adult horses (2 Thoroughbreds and 4 Standardbreds; 4 castrated males and 2 females) that ranged in age from 6 to 10 years and had a mean ± SD body weight of 534 ± 49 kg. Horses were judged to be healthy on the basis of results of physical examination, ECG, CBC, and blood biochemical analysis. All horses were allowed to acclimate to the test facility for at least 1 week before the study began. The study protocol was approved by the Institutional Laboratory Animal Care and Use Committee of The Ohio State University and conformed with the Ethical Guidelines of the International Association for the Study of Pain.

**Study design**—The study consisted of 5 trials in a randomized, masked crossover design. Horses received morphine sulfate or saline (0.9% NaCl) solution at the following doses: saline solution (0.6 mL/100 kg, IM [n = 3 horses] and IV [3]; control group [6]) and morphine sulfate (0.05 mg/kg, IM; 0.05 mg/kg, IV; 0.1 mg/kg, IM; and 0.1 mg/kg, IV [6 for each dose and route]). Morphine sulfate contains 75.2% morphine, so actual morphine doses were 0.0376 mg/kg (0.05 mg/kg as morphine sulfate) and 0.0752 mg/kg (0.1 mg/kg as morphine sulfate). Trials were separated by a minimum of 10 days, and all treatments were administered between 9 AM and 10 AM. Baseline values were obtained for all measured variables before treatment administration. Ad libitum access to food and water was allowed before and during each trial. Room temperature was controlled and set between 25° and 27°C.

**Instrumentation**—For each trial, horses were held in a stockade and quietly stood unrestrained with no signs of discomfort. One to 2 mL of mepivacaine hydrochloride was injected SC over the left and right jugular veins and left carotid artery. Intravascular catheters (14 gauge and 5.25 inches) were percutaneously placed in the left and right external jugular veins before each treatment for IV drug administration and blood sample collection, respectively. Only the right jugular vein was catheterized when a treatment was administered IM.

The right carotid artery, which had previously been surgically elevated to a subcutaneous position, was also percutaneously catheterized (19-gauge, 12-inch catheter) for collection of arterial blood samples and direct measurement of systemic arterial blood pressure. The catheter was connected to a calibrated strain gauge transducer, which was positioned at the level of the scapulohumeral joint (zero pressure point) and connected to a multiparameter monitor. A base-apex ECG machine was used to monitor HR and heart rhythm.

**Assessment of gastrointestinal motility**—Gastrointestinal motility was evaluated through intestinal auscultation in accordance with a published scoring system for horses (Appendix 1). Auscultation was performed over each of 4 abdominal quadrants (left and right fossa paralumbaris and left and right ventral abdominal regions) for 2 minutes (total, 8 minutes). Scores from each quadrant ranged from 0 (total lack of intestinal sounds) to 4 (loud and frequent sounds). The total score ranged from 0 (total lack of intestinal sounds) to a maximum score of 16 (loud and frequent sounds in all abdominal quadrants). Intestinal sounds were judged to have returned to baseline when a score of 12 was achieved. Frequency of intestinal sounds was also counted during an 8-minute period of auscultation.

**Behavioral assessment**—Behavioral attitudes of horses before and after drug administration were scored on a 4-point scale, with 0 representing sedated and 3 representing violent or extremely anxious (Appendix 2).

**Assessment of analgesia**—For thermal threshold testing, 2 rectangular areas (approx 15 X 25 cm) of the skin of the right dorsocostal region (over the latissimus dorsi and serratus dorsalis muscles) and the right shoulder region (over the supraspinatus and deltoideus muscles) were shaved of hair and a heat-generating source was applied. The device consisted of a halogen bulb (5 V; 50 W) housed in a metal cylinder (height, 3 cm; diameter, 5.7 cm). The heat intensity increased in a linear, steady manner (approx 2 W/s). A thermometer was inserted inside the metal cylinder adjacent to the skin surface to measure skin temperature. Degree of analgesia was inferred from the thermal threshold latency time, which was the interval (in seconds) from illumination of the heat lamp until purposeful movement of the horse (defined as any movement of the head toward the stimulus or movement of the body away from the stimulus). When no response was obtained, the thermal stimulus was terminated after 23 seconds or when the skin temperature increased to > 50°C.

For electrical threshold testing, the hair around the coronary band of the hind limbs was clipped, and stimulus electrodes were taped to the region. An electrical nerve stimulator was used to generate an electrical stimulus through the electrodes placed on the coronary band of one of the hind limbs. The electrical current was increased linearly (1 mA/s) until the horse kicked, which was considered a positive response. The current was terminated at 25 seconds or 25 mA if the horse did not respond. The latency time to electrical threshold
was defined as the interval (in seconds) from current initiation until a positive response was detected.

**Data collection**—Behavior was evaluated, and HR, respiratory rate, SAP, DAP, MAP, rectal temperature, and thermal and electrical thresholds were determined 30 minutes before treatment (baseline) and 15, 30, 45, 60, 90, 120, 180, 240, 300, and 360 minutes after treatment. Arterial blood samples were collected in heparinized syringes and immediately analyzed for pH, PaO₂, and PaCO₂ 15 minutes before treatment (baseline) and 15, 30, 45, 60, 90, 120, 180, 240, 300, and 360 minutes after treatment. Gastrointestinal motility was assessed in the morning before treatment and 1, 2, 3, 4, 5, 6, 8, 12, 24, 36, and 48 hours after treatment.

Horses were observed for signs of abdominal discomfort, and total fecal output was collected and weighed for 48 hours before and 48 hours after treatment, at 24-hour intervals. Signs of adverse effects were recorded as well as the time at which they were detected.

**Analysis of plasma morphine and morphine metabolite concentrations**—Venous blood samples for determination of plasma morphine, M-3-G, and M-6-G concentrations were collected from the right external jugular vein catheter into evacuated glass tubes containing EDTA as anticoagulant. The samples were placed on ice, then centrifuged for 15 minutes at 4,000 X g. Plasma was separated and stored frozen at –80°C until analysis. Blood samples were collected 30 minutes before and 2, 5, 10, 15, 30, 45, 60, 75, 90, 120, 150, 180, 240, 360, 480, 720, 1,440, 2,160, and 2,880 minutes after morphine or saline solution administration.

Plasma morphine, M-3-G, and M-6-G concentrations were determined by means of liquid chromatography–mass spectrometry. The routine volume for drug extraction was 1 mL of plasma. Glass centrifuge tubes (5 mL) containing 0.5 mL of saline solution were prepared for each of the experimental, calibrator, and control samples. Calibrators and positive control samples were supplemented with independently prepared working standard solutions containing mixtures of morphine, M-3-G, and M-6-G that were prepared daily from stock standard solutions.

Working standard solutions at 0.05, 0.5, and 5 ng/µL for preparation of calibrators were routinely prepared by serial dilution of the stock standard solutions. Eight calibrators at concentrations of 1, 5, 10, 20, 50, 100, 300, and 500 ng/mL were also prepared for each of the 3 analytes in the control samples were prepared for each set of samples. Working standard solutions at 0.4 and 4 ng/mL were then prepared for the positive control samples. Positive control samples were prepared at 2, 5, 20, 40, 200, and 400 ng of analyte/mL. A 50-µL aliquot of the internal standard mixture containing morphine-d₃, M-3-G-d₃, and M-6-G-d₃ each at 0.4 ng/µL, was added to each calibrator, control sample, and study sample. Control horse plasma (1 mL) was added to each of the calibrator and control samples containing the saline solution supplemented with the analytes and internal standards.

Duplicate 1-mL aliquots of study samples were transferred via pipette into tubes containing 0.5 mL of saline solution that had been supplemented with the internal standard mixture. A 1-mL aliquot of 0.1M HCl was added to each tube, and the contents were mixed with a vortex device for approximately 10 seconds before extraction was attempted.

Solid-phase extraction columns for each calibrator, control sample, and study sample were placed in a pressure processor containing solid-phase extraction columns that had been washed with 1 mL of methanol followed by 1 mL of 0.1M HCl. The study samples, control samples, and calibrators were loaded onto the columns and aspirated after 5 minutes. The columns were then washed with 1 mL of 0.1M HCl and dried with nitrogen at 172.4 kPa (as shown on a pressure gauge) for 10 minutes. After drying, each column was eluted with 2 mL of 5% (vol/vol) ammonium hydroxide in methanol. The extracts were evaporated to dryness under nitrogen in an evaporator. The dried extracts were then dissolved in 300 µL of 0.1% formic acid in water. A portion of each extract was transferred to a 250-µL polypropylene autosampler vial.

All extracts (20-µL aliquots of each) were assayed with a liquid chromatography system and analyzed on a linear ion trap mass spectrometer operated in positive electrospray ionization mode. The system was equipped with a C₁₈ (2.1 × 20-mm, 5-µm) guard column and C₁₈ (2.1 × 150-mm, 5-µm) analytic column. The column compartment was maintained at 16°C.

The liquid chromatography instrumental method consisted of gradient elution with a constant flow of 200 µL/min. The mobile phase was a mixture of 0.1% formic acid and methanol-acetonitrile (50:50 [vol/vol]) containing 0.1% formic acid. The starting conditions (2% methanol-acetonitrile–formic acid solvent) were maintained for 0.5 minutes after sample injection. The mobile phase composition was increased linearly from 2% methanol-acetonitrile–formic acid solvent at 0.5 minutes to 70% methanol-acetonitrile–formic acid solvent at 8 minutes and was held there for 3 minutes before the system was re-equilibrated to starting conditions for 9 minutes. The retention times of morphine, M-3-G, and M-6-G were 7.4, 7.70, and 7.13 minutes, respectively, under the conditions of analysis.

Mass spectrometric data were acquired via tandem mass spectrometry scan for protonated molecular ions (m/z, 286 [morphine] and 289 [morphine-d₃]) with a collision energy of 40% and protonated molecular ions (m/z, 462 [M-3-G and M-6-G] and 465 [M-3-G-d₃ and M-6-G-d₃]) with a collision energy of 35%. One mass spectrometer tune file was used for analysis of all analytes. Area counts (m/z, 201) produced from the ions (m/z, 286 and 289) were monitored for morphine determinations. Area counts (m/z, 286 and 289) produced from ions were also monitored for M-3-G (m/z, 462) and M-6-G (m/z, 465) determinations. Concentrations of the 3 analytes in the control samples and study samples were determined from the slope and intercept of the line produced by linear regression analysis of the calibrator data, which was weighted as the reciprocal of the squared analyte concentration in the corresponding calibrator. The method was validated, and the lower limits of quantitation were determined for each analyte.

**Statistical analysis**—Physiologic variables, borborygmus frequency, thermal and electrical threshold la-
tency times, electrical threshold, and fecal output were compared among treatments by use of a 2-way ANOVA for repeated measures, followed by Bonferroni correction for multiple comparisons and appropriate posttests (t-tests). Categorical data were analyzed with Friedman and Dunn posttests. Values of P < 0.05 were considered significant. All parametric data are reported as mean ± SD. Nonparametric data are reported as median and interquartile range.

Nonlinear regression analysis was used to evaluate plasma concentration-versus-time data by use of a pharmacokinetic analysis software program.1 Curves for plasma morphine, M-3-G, and M-6-G concentration versus time after IV and IM administration were also performed on the data (results not reported here).

**Results**

Behavior—All 6 horses remained calm, periodically ate hay, and had no signs of abnormal behavior at any time during or after any treatment (saline solution [IM or IV] or morphine sulfate [0.05 and 0.1 mg/kg, IM or IV]). The median behavioral attitude score was 1 during the baseline period. No significant changes in

### Table 1—Mean ± SD values of cardiovascular variables in 6 horses before (baseline) and at various points after receiving each of saline (0.9% NaCl) solution [0.6 mL/kg, IM and IV] or morphine sulfate (0.05 and 0.1 mg/kg, IV and IM) in a randomized crossover study.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment Baseline</th>
<th>MAP (mm Hg)</th>
<th>Respiratory rate (breaths/min)</th>
<th>Arterial pH</th>
<th>Paco2 (mm Hg)</th>
<th>Rectal temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (beats/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline solution</td>
<td>31 ± 3</td>
<td>—</td>
<td>28 ± 1.1</td>
<td>73 ± 2.3</td>
<td>36.8 ± 0.4</td>
<td>37.5 ± 0.2</td>
</tr>
<tr>
<td>0.05 mg/kg, IM</td>
<td>34 ± 3</td>
<td>35 ± 3</td>
<td>38 ± 0.4</td>
<td>38 ± 0.3</td>
<td>37 ± 0.4</td>
<td>37.5 ± 0.2</td>
</tr>
<tr>
<td>0.05 mg/kg, IV</td>
<td>31 ± 3</td>
<td>37 ± 1.8 *</td>
<td>38 ± 0.4</td>
<td>38 ± 0.3</td>
<td>33 ± 0.5</td>
<td>37 ± 0.2</td>
</tr>
<tr>
<td>0.1 mg/kg, IM</td>
<td>33 ± 4 101.5 *</td>
<td>42 ± 1.7 *</td>
<td>38 ± 0.4</td>
<td>38 ± 0.3</td>
<td>31 ± 0.5</td>
<td>37 ± 0.2</td>
</tr>
<tr>
<td>0.1 mg/kg, IV</td>
<td>33 ± 4 101.5 *</td>
<td>42 ± 1.7 *</td>
<td>38 ± 0.4</td>
<td>38 ± 0.3</td>
<td>31 ± 0.5</td>
<td>37 ± 0.2</td>
</tr>
<tr>
<td>SAP (mm Hg)</td>
<td>127 ± 11</td>
<td>—</td>
<td>126 ± 6</td>
<td>127 ± 10</td>
<td>123 ± 8</td>
<td>37.5 ± 0.2</td>
</tr>
<tr>
<td>0.05 mg/kg, IM</td>
<td>136 ± 15</td>
<td>138 ± 19</td>
<td>127 ± 13</td>
<td>123 ± 12</td>
<td>112 ± 10</td>
<td>37.5 ± 0.2</td>
</tr>
<tr>
<td>0.05 mg/kg, IV</td>
<td>113 ± 15</td>
<td>117 ± 12</td>
<td>119 ± 12</td>
<td>117 ± 12</td>
<td>112 ± 10</td>
<td>37.5 ± 0.2</td>
</tr>
<tr>
<td>0.1 mg/kg, IM</td>
<td>151 ± 13</td>
<td>153 ± 12</td>
<td>143 ± 12</td>
<td>143 ± 12</td>
<td>134 ± 12</td>
<td>37.5 ± 0.2</td>
</tr>
<tr>
<td>0.1 mg/kg, IV</td>
<td>118 ± 13</td>
<td>119 ± 12</td>
<td>124 ± 12</td>
<td>124 ± 12</td>
<td>134 ± 12</td>
<td>37.5 ± 0.2</td>
</tr>
</tbody>
</table>

### Table 2—Mean ± SD values of respiratory variables and rectal temperature of the horses in Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment Baseline</th>
<th>Respiratory rate (breaths/min)</th>
<th>Arterial pH</th>
<th>Paco2 (mm Hg)</th>
<th>Rectal temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline solution</td>
<td>14 ± 1</td>
<td>14 ± 4</td>
<td>15 ± 9</td>
<td>17 ± 7</td>
<td>17 ± 8</td>
</tr>
<tr>
<td>0.05 mg/kg, IM</td>
<td>10 ± 2</td>
<td>10 ± 2</td>
<td>12 ± 5</td>
<td>13 ± 5</td>
<td>14 ± 4</td>
</tr>
<tr>
<td>0.05 mg/kg, IV</td>
<td>17 ± 5</td>
<td>17 ± 6</td>
<td>18 ± 5</td>
<td>18 ± 5</td>
<td>19 ± 5</td>
</tr>
<tr>
<td>0.1 mg/kg, IM</td>
<td>10 ± 2</td>
<td>10 ± 2</td>
<td>12 ± 5</td>
<td>13 ± 5</td>
<td>14 ± 4</td>
</tr>
<tr>
<td>0.1 mg/kg, IV</td>
<td>17 ± 5</td>
<td>17 ± 6</td>
<td>18 ± 5</td>
<td>18 ± 5</td>
<td>19 ± 5</td>
</tr>
</tbody>
</table>

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*Value is significantly (P < 0.05) different from baseline value.
__ — Not measured. *Values with different superscript letters are significantly (P < 0.05) different between groups, with a < b.
behavioral score over time within or among treatments were observed.

**Cardiovascular variables**—Heart rate, SAP, DAP, and MAP increased unexpectedly for approximately 2 minutes immediately after administration of both IV doses of morphine sulfate (Table 1). The changes that followed IV administration of morphine sulfate at a dose of 0.1 mg/kg were greater, albeit not significantly, than those that followed IV administration of morphine sulfate at a dose of 0.05 mg/kg. There were no clinically important temporal changes in HR, SAP, DAP, or MAP for the remaining data collection periods for any treatment.

**Respiratory variables**—No changes in respiratory rate were observed in any group. The PaCO$_2$ was significantly higher than the baseline value at 30, 45, 60, and 360 minutes after IV administration of morphine sulfate at a dose of 0.05 mg/kg, but the difference was not considered clinically relevant (PaCO$_2 > 60$ mm Hg). The PaO$_2$ was significantly lower than the baseline value at 15, 30, 45, 60, 120, and 300 minutes after IM administration of morphine sulfate at a dose of 0.1 mg/kg, but remained within the range of reference values (83.6 ± 1.7 mm Hg) for adult horses (Table 2).

**Gastrointestinal variables**—The median baseline abdominal auscultation score was 16 for all treatments. No significant differences were detected for the 48-hour period after saline solution administration or after IV administration of morphine sulfate at a dose of 0.05 mg/kg. Following IM administration of morphine sulfate at doses of 0.05 and 0.1 mg/kg and IV administration at a dose of 0.1 mg/kg, the median abdominal auscultation score decreased for 60 minutes (Figure 1). The same pattern was observed for borborygmus frequency. Following IV administration of morphine sulfate at a dose of 0.1 mg/kg, intestinal sound frequency decreased for 120 minutes. Mean weight of feces collected at 24-hour intervals for 48 hours after drug administration did not change significantly from the weight recorded during the 24-hour period prior to drug administration (Figure 2).

The thermal threshold latency time did not change for any group for either the dorsocostal or the shoulder regions (Figure 3). Morphine administration was not followed by an increase in the electrical threshold latency time or the current at threshold (Figure 4).

Plasma morphine disposition after IV administration was characterized by a rapid distribution phase, followed by a slower elimination phase (Figure 5). The median plasma morphine concentration was > 8 ng/mL for all treatments, except after IV administration of morphine sulfate at a dose of 0.05 mg/kg and saline solution, at the point during which abdominal auscultation score and borborygmus frequency (60 minutes) were decreased. The median plasma morphine concentration was approximately 4 ng/mL at 60 minutes after IV administration of morphine sulfate.

![Figure 1](image1.png)

**Figure 1**—Median ± interquartile range abdominal auscultation scores (A) and mean ± SD number of borborygmus events in an 8-minute period (B) for 6 horses before (baseline) and at various points after receiving saline (0.9% NaCl) solution (0.6 mL/kg, IM and IV (squares)) and morphine sulfate (0.05 mg/kg, IM (black triangles); 0.05 mg/kg, IV (white triangles); 0.1 mg/kg, IM (black circles); and 0.1 mg/kg, IV (white circles)) in a randomized crossover study. *Indicated value differs significantly (P < 0.05) from corresponding baseline value. **Values with different superscript letters differ significantly (P < 0.05) between treatments, with a < b. BL = Baseline.

![Figure 2](image2.png)

**Figure 2**—Mean ± SD weight of feces produced before (negative time points) and after treatment administration for the horses in Figure 1. See Figure 1 for remainder of key.
at a dose of 0.05 mg/kg. Morphine was rapidly conjugated, and both M-3-G and M-6-G were detected in plasma samples (Figure 6).

Figure 3—Mean ± SD thermal threshold latency duration (dorsocostal [A] and shoulder [B] regions) for the horses in Figure 1. See Figure 1 for remainder of key.

Figure 4—Effects of administration of saline solution and morphine sulfate on mean ± SD electrical threshold latency time (A) and current at electrical threshold (B) in 6 horses. See Figure 1 for remainder of key.

Figure 5—Median and interquartile range plasma morphine concentrations at various points after IV (A) and IM (B) administration of morphine sulfate to the horses in Figure 1. See Figure 1 for remainder of key.

Figure 6—Median and interquartile range plasma M-6-G concentrations at various points after IV administration of morphine sulfate (0.1 mg/kg [black squares] and 0.05 mg/kg [white triangles]) and plasma M-3-G concentrations after IV administration of morphine sulfate (0.1 mg/kg [black triangles] and 0.05 mg/kg [white circles]) to the horses in Figure 1.
Discussion

Intravenous or IM administration of morphine sulfate at doses of 0.05 and 0.1 mg/kg did not produce clinically important alterations in measured cardiopulmonary variables or behavior. Importantly, gastrointestinal motility was reduced for a short period but did not affect laxation or fecal quantity. Administration of morphine at the doses tested did not yield any demonstrable analgesic effects in response to thermal or electrical stimulation in healthy conscious horses with no signs of pain.

Morphine sulfate is administered to horses to produce analgesia. Dose recommendations have evolved on the basis of clinical impressions, subjective visual scoring systems, concerns for adverse effects, and dogma.13,15,21 We selected doses and routes of administration for morphine on the basis of current clinical recommendations2,13,21 and pharmacokinetic studies in humans,3,13 monkeys, dogs, pigs,12 and horses14–20 as well as older and more recent studies7,10,13,17,27–29 of morphine in horses. For example, Combie et al13 described increases in locomotor activity by counting the number of steps that horses took during a 2-minute period after morphine sulfate administration. Significant increases in locomotor activity occurred after IV administration at doses > 0.3 mg/kg, with marked increases at doses > 0.6 mg/kg; severe ataxia and collapse occurred at doses of 2.4 mg/kg. The IV administration of morphine sulfate at a dose of 1.0 mg/kg produced signs of colic after the third treatment.17

Studies13–28 in which morphine sulfate was administered within the dose range of 0.1 to 0.2 mg/kg revealed no increase in the incidence of postoperative complications such as colic or excitement in recovery. Horses that received morphine sulfate IV at doses < 0.1 mg/kg had better recovery scores, had fewer attempts to stand, and took a shorter time to stand after anesthesia.10 Therefore, morphine sulfate doses of 0.05 and 0.1 mg/kg were deemed reasonable to study because of their lack of adverse effects and potential benefit in recovery of anesthesia.

Opioid administration results in dose-dependent behavioral changes and increases in locomotor activity in horses. These effects have been attributed to the release of endogenous catecholamines, activation of dopaminergic receptors,6,11 and species differences in the distribution and density of opioid receptors in the CNS.9 The morphine metabolite M-3-G produces dose-dependent behavioral excitation when directly administered into the CNS of rats;4 however, in humans, IV administration of M-3-G30 or the presence of M-3-G in plasma derived from morphine metabolism31 does not always induce neuroexcitation and hyperalgesia. Regardless, a high plasma M-3-G concentration resulting from high doses or chronic administration of morphine may contribute to neuroexcitation33,33 and may help to explain why others29,30 have found minimal decreases or increases in the minimum alveolar concentration of inhalation anesthetics in horses, particularly when larger doses are administered. These previous studies in horses did not involve determination of plasma concentrations of M-3-G, making it difficult to determine the metabolite’s role as contributing to CNS excitation in horses.

Administration of morphine, meperidine, oxymorphone, or pentazocine increases HR, arterial blood pressure, and cardiac output, with no change in arterial blood pH, PaO₂, or PaCO₂, in adult horses with no signs of pain.13 Stimulated cardiovascular responses, such as an increase in HR, blood pressure, and cardiac output, were also detected in other studies33,36 of opioids in horses with no signs of pain and have been attributed to CNS excitation. Although no signs of increased locomotor activity were observed in any horse in the present study, HR and arterial blood pressure increased significantly for approximately 2 minutes after IV administration of morphine sulfate. The hemodynamic stimulation observed minutes after IV administration was probably the result of a high peak plasma morphine concentration (> 150 vs < 60 ng/mL for IV and IM administration, respectively), suggesting that increasing plasma concentrations of morphine or a metabolite produce CNS stimulation and increased sympathetic outflow.13

Decreased laxation, constipation, and colic are clinically important adverse effects associated with the administration of morphine to horses. In a previous study,17 morphine sulfate was administered to healthy horses IV at a dosage of 0.5 mg/kg every 12 hours for 6 days, resulting in a decrease in fecal moisture content in the gastrointestinal tract and propulsive motility for up to 6 hours after each dose. Concurrent administration of methylaltrexone, which is a peripherally acting opioid receptor antagonist, and morphine sulfate partially prevented the effects of morphine on the gastrointestinal tract in horses in another study.37 However, 3 retrospective studies16,27,38 designed to evaluate the incidence of postoperative complications and gastrointestinal effects in horses yielded a different conclusion, although the dosages administered were lower.

One study27 in which the incidence of adverse effects associated with the intraoperative use of morphine was evaluated in horses found that 1 dose (0.1 to 0.17 mg/kg, IV) did not result in an increased risk of gastrointestinal discomfort during the first 4 days after anesthesia. A large-scale study26 in which morphine sulfate was administered (0.1 to 0.15 mg/kg, IV) failed to show an increased risk of postanesthetic colic, in agreement with findings of a study27 of perioperative morphine administration in horses. Another large-scale retrospective study25 found a 4-fold increase in the risk of colic in horses that received morphine sulfate (0.08 to 0.3 mg/kg, IV), compared with the risk in horses that received no opioid, although the effect of dose was not specifically investigated.

In the present study, abdominal auscultation scores and borborygmin frequency decreased for 1 hour after IM administration of morphine sulfate at a dose of 0.05 mg/kg and after IV and IM administration at a dose of 0.1 mg/kg, indicating that gastrointestinal activity was decreased. The median plasma morphine concentration was > 8 ng/mL at 1 hour when morphine sulfate was administered IM at a dose of 0.05 mg/kg and IV and IM at a dose of 0.1 mg/kg. Intravenous administration of morphine sulfate at a dose of 0.05 mg/kg did not result in a significant decrease in abdominal auscultation scores and borborygmin frequency at any observa-
tion point. Our findings suggest that a decrease in abdominal auscultation scores or borborygmic frequency occurred when plasma morphine concentrations were between 4 and 8 ng/mL. Although auscultation of intestinal sounds is a subjective measurement, the method has been commonly used to evaluate the effects of drugs on gastrointestinal motility and appears to be a clinically useful tool for assessing gastrointestinal function.\(^\text{17,19,53}\)

Decreases in gastrointestinal sounds have been associated with the presence of abdominal discomfort as well as changes in gastrointestinal mechanical and electrical activity and transit time.\(^\text{14,17}\) None of the horses in the present study developed signs of colic or abdominal discomfort, and all of the horses maintained a constant degree of fecal output, compared with values before treatment. Therefore, the short-lived decrease in intestinal sounds that resulted from a low dose of morphine would likely not cause colic in healthy conscious horses with no signs of pain.

The lack of measurable analgesic effects of morphine in the present study was unexpected. Opioid binding sites have been identified in the CNS and peripheral nerves of horses,\(^\text{5,6}\) and analgesia has been achieved in horses after systemic and regional administration of opioids.\(^\text{7,12,18,39–43}\) The lack of detectable analgesia may have been a result of the dose, pharmacodynamic variations in the horses’ response to opioids, method used to evaluate analgesia (sensitivity and specificity), or lack of pain in the healthy horses used.

Administration of high doses of morphine sulfate (0.66 mg/kg) to horses reportedly provides 30 and 60 minutes of superficial and visceral analgesia, respectively. Retrospective and prospective clinical studies conducted to evaluate the analgesic and perioperative effects of low doses of morphine sulfate (0.1 to 0.2 mg/kg) in horses reveal few adverse effects but fail to demonstrate appreciable analgesia. Findings in most of these studies\(^\text{22–30}\) were confounded by the coadministration of other drugs. Plasma concentrations of morphine that were attained in the present study reportedly achieve analgesia in humans and dogs;\(^\text{24,44–45}\) however, horses may have a different plasma concentration–response relationship for morphine, compared with that in humans and dogs.

An effective pain model should fulfill the following criteria: the endpoint or pain-perception threshold should be readily identifiable, qualitatively similar among subjects, stable over time, and reproducible within subjects; the pain stimulus should be administered repeatedly without producing clinically relevant tissue damage; the pain model should be sufficiently sensitive to detect clinically relevant signs of pain and dose-related effects of putative analgesics without interference from the adverse effects of those agents;\(^\text{34}\); and the pain model should mimic clinical signs of pain.\(^\text{46,47}\) Electrical current threshold is an easy and effective method to detect analgesia in horses after systemic\(^\text{48}\) and epidural\(^\text{49}\) administration of opioids and \(\alpha\)-receptor agonists. Kamerling et al\(^\text{12}\) showed that changes in the latency of the skin twitch response to a noxious but constant thermal stimulus are an objective and repeatable means of assessing cutaneous analgesia. They also demonstrated a dose-dependent antinociceptive effect on the basis of increases in thermal thresholds following fentanyl administration (2.5 to 10 \(\mu\)g/kg) in conscious horses with no signs of pain. The method used in the present study for thermal noxious stimulation did not induce burns or visible epidermal damage.

Three limiting factors were evident with the analgesic model used in the present study. Opioids are selective for mainly C-fiber–mediated pain conduction\(^\text{47}\) but do not block other sensory functions such as touch, hearing, proprioception, and innocuous environmental temperature. Given that horses are particularly sensitive to nonnoxious tactile stimuli, the presence of such stimuli might have influenced the results, causing morphine to appear ineffective. Secondly, the previously described effect of learning, leading to recognition and subsequent avoidance of the stimulus (ie, learning effect),\(^\text{46}\) may have influenced results.

Thirdly, experimental nociceptive pain models typically evaluate threshold or endpoint responses (escape and withdrawal reflex) to various modalities of transient acute noxious stimulation (eg, thermal, mechanical, or electrical stimulation or cecal inflation). Therefore, such models only explore a limited portion of the multidimensional processes involved with clinical signs of pain.\(^\text{47}\) For instance, clinical inflammatory pain is often continuous, does not produce a simple acute response, and causes increased sensitivity to pain stimuli (hyperalgesia) or to innocuous stimuli (allodynia). In addition, drugs may change in action when pathophysiologic conditions such as chronic or inflammatory pain are present owing to upregulation or downregulation of peripheral or central receptor expression (eg, \(\mu\)-opioid receptor expression in inflamed joints).\(^\text{46}\)

Multimodal and multitissue testing models have been proposed as methods to assess analgesic drug efficacy because various receptor types and mechanisms are activated, resembling clinical pain.\(^\text{50}\) In the present study, antinociceptive effects were evaluated by means of electrical and thermal stimulation; however, only superficial pain was assessed. A systematic review of the human medical literature concluded that morphine had antinociceptive effects in various experimental pain modalities but produced a more robust antinociceptive response in deep pain models, compared with the response with models in which the pain stimulus was applied to the skin. The reason for this could be that deep pain is often higher in intensity than is superficial pain. Studies\(^\text{19,42}\) involving horses show that intraarticular administration of morphine yields analgesia in a lipopolysaccharide-induced synovitis model. This type of model produces moderate to severe lameness, and investigators can assess the degree of lameness as well as pain-related behavior for the duration of the induced inflammation. Therefore, although thermal and electrical stimuli are effective in assessing analgesic effects of opioids in horses with no signs of pain, the model reported here may be limited in predicting the analgesic effect of morphine in horses with naturally occurring pain.

The present study demonstrated that IV or IM administration of morphine sulfate at doses of 0.05 and
0.1 mg/kg resulted in minimal and transient, if any, cardiorespiratory, gastrointestinal, behavioral, and analgesic effects in healthy conscious horses with no signs of pain. It also demonstrated that measurable plasma concentrations of morphine metabolites (M-3-G and M-6-G) were achieved. No adverse effects were noticed at the doses used. Future studies should be conducted to investigate the role of morphine metabolites and the analgesic effects of morphine in horses with conditions that naturally cause pain.

References

31. Barleit SE, Cranmad T, Smith MT. The excito-atory effects of M3G are attenuated by LY274614, a competitive NMDA receptor antagonist and by midazolam, an agonist at the benzodiazepine site on the GABAA receptor complex. Life Sci 1994;54:687–694.


Appendix 1
Scoring system used to evaluate gastrointestinal motility in horses by means of intestinal auscultation.

<table>
<thead>
<tr>
<th>Score</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No intestinal sounds auscultated within a quadrant</td>
</tr>
<tr>
<td>1</td>
<td>Low-pitched crepitation-like sounds audible within a quadrant (1 sound/min)</td>
</tr>
<tr>
<td>2</td>
<td>Long and loud gurgling sounds audible within a quadrant (1 sound/min)</td>
</tr>
<tr>
<td>3</td>
<td>Long and loud gurgling sounds audible within a quadrant (&gt; 1 sound/min)</td>
</tr>
</tbody>
</table>


Appendix 2
Scoring system used to assess behavioral attitude of horses before and after drug administration.

<table>
<thead>
<tr>
<th>Score</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (sedated)</td>
<td>Response to auditory, visual, or tactile stimuli is less than typical; head down, with ears and lips drooping</td>
</tr>
<tr>
<td>1 (alert and relaxed)</td>
<td>Typical behavior; relaxed but responsive to external auditory, visual, and tactile stimuli; typical stance and head and lip positions</td>
</tr>
<tr>
<td>2 (anxious)</td>
<td>Settled but with signs of nervousness; shifts weight; flicks ears frequently</td>
</tr>
<tr>
<td>3 (violent or extremely anxious)</td>
<td>Faces, paws, shifts weight, flicks ears frequently; shifts tail, raises and lowers head or neighs intermittently, lunges, rears, strikes, or kicks</td>
</tr>
</tbody>
</table>