

A Method for Studying Cutaneous Pain Perception and Analgesia in Horses

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Pain perception and its alteration by analgesic drugs is difficult to measure in the horse. The latency to onset of flexion of a limb in response to a noxious thermal stimulus has been used as a nociceptive end point for analgesic studies in many species. While this method has been employed in the horse, it may be confounded by the spontaneous locomotor activity observed after administration of narcotic analgesics. Consequently, an alternative method of assaying narcotic analgesia that did not involve the equine locomotor apparatus was developed. This report describes the use of the heat-evoked skin-twitch reflex as a reproducible measure of pain threshold and its alteration by the narcotic analgesic fentanyl. This method is compared with the heat-evoked hoof-withdrawal reflex, and the apparatus necessary to elicit both reflexes in the horse is described. Fentanyl, administered at intravenous doses of 0.010, 0.005, and 0.0025 mg/kg, produced a dose-related prolongation of the skin-twitch reflex but failed to alter the latency to hoof withdrawal following noxious thermal stimulation. The skin-twitch reflex is therefore a more sensitive assay of narcotic analgesia in the horse than is the hoof-withdrawal reflex.

Key Words: Pain; Analgesia; Horses; Fentanyl

INTRODUCTION

The effects of drugs on pain perception in the horse have been difficult to measure. Aside from being large, cumbersome, and difficult to manage the horse is stimulated by narcotics, the most commonly used group of pain-relieving agents. For this and other reasons, studies of the effects of analgesics in the horse have not been as elaborate as in other species, despite their frequent use in pain management in horses. (For example, horses suffering from colic require analgesics to avoid the danger of thrashing, and race horses, due to rigorous training, are given varieties of pain killers.)

Two classes of drugs are used to treat pain in the horse: opiate analgesics and nonsteroidal antiinflammatory analgesics. Despite their long use as analgesics in horses, the opiates have not been systematically studied in this species.

Pain perception and its therapeutic modification in the horse have been measured subjectively and objectively. The majority of clinical studies employed subjective

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measures of pain, which include hoof compression (Szabuniewicz and Szabuniewicz 1975), needle probing (Kerr et al., 1972), digital pressure (Jones and Hamm, 1978), and scored clinical signs (Gideon, 1977). These techniques are inherently imprecise and make comparisons among observers difficult. Recently, Pippi et al. (1979a,b) have developed objective models for measuring superficial, visceral, and periosteal pain in ponies. While these studies ranked various agents according to their analgesic performance, results obtained were highly variable among subjects and between experimental days, and often represented responses obtained after only a single dose of a drug. Further, these studies were conducted primarily in ponies that appear to respond to narcotics in a different fashion than performance horses.

Recently, Tobin (1978) and Combie et al. (1979) have developed an assay for measuring the locomotor stimulation produced by the narcotic analgesics. These studies showed that narcotics elicit a reproducible trotting response that can be augmented in a dose-related manner. We have observed this trotting response in horses that were allowed to roam freely in box stalls and in horses that were confined to equine stockades.

Opiate analgesia in the rat, dog, and other species is often evaluated by measuring changes in latency to flexion-withdrawal reflex elicited by a noxious stimulus applied to an extremity (Woolfe, 1944; Andrews, 1974). Pippi et al. (1979a) and Kalpravidh et al. (1984a,b) reported that opiates prolonged the latency to flexion of the forelimb following a noxious thermal stimulus in ponies and horses. In an attempt to duplicate this, we observed that the stimulus-induced flexion of the forelimb was difficult to distinguish from the spontaneous locomotor movements produced by the narcotic, particularly at high doses. Since narcotic-induced locomotor stimulation limited the usefulness of changes in flexion-reflex latency as a measure of analgesia, an alternative method was developed. The following report discusses the use of the heat-evoked skin-twitch reflex as a reproducible measure of pain threshold and its advantages over the forelimb-flexion reflex as an assay for narcotic analgesia. Further, the design and application of the devices used to elicit nociceptive reflexes in performance horses are described.

METHODS

Two models of cutaneous pain perception were employed in the present study. Both models used radiant heat as the noxious thermal stimulus. Test areas chosen were the lateral aspect of the fetlock and the skin of the back between the scapulae or withers area. Radiant heat was delivered as a beam of focused light of fixed intensity, similar to that described by Hardy et al. (1940).

The Fetlock Model

Elicitation of the classic flexion-withdrawal reflex was performed by directing a beam of focused light onto the fetlock area (metacarpophalangeal joint) of the forelimb. Because the horse can react with considerable force to a noxious stimulus, it was necessary that the heat-generating apparatus be easily removed from the site of stimulation after a response. Further, to assure a consistent stimulus, the distance between lamp and fetlock had to remain constant. To meet these requirements,

the hand-held heat lamp shown in Figure 1 was developed. The stimulus was generated as a beam of light emanating from a 500-W incandescent projection lamp that was focused through a condensing lens. The light beam formed a 2.5-cm² image of intense light approximately 24.5 cm from the lens. The distance to the fetlock area and the location of the image was calibrated by means of the probe shown in Figure 1, which deliberately underestimated the focal length by 2.0 cm. The lamp and countdown timer (Cole-Parmer, Chicago, IL) were simultaneously started by pressing the ON switch located in the handle of the heat lamp. The lamp was extinguished and the timer was arrested when the OFF switch was pressed. The cycle could be repeated by pressing the reset switch. The entire apparatus was connected to the control console shown in Figure 2 (left side). A line from the console to a physiograph registered the time during which the lamp was illuminated and allowed correlation of the pain stimulus with other physiologic parameters. The entire hand-held apparatus was constructed from aluminum tubing and weighed approximately 400 g.

Withers Model

Because locomotor activity was observed following narcotic administration, it appeared that the fetlock model would be inadequate for the quantitation of narcotic analgesia. Consequently, a radiant heat device was developed to elicit a nociceptive reflex that did not require participation by the horse's locomotor appa-

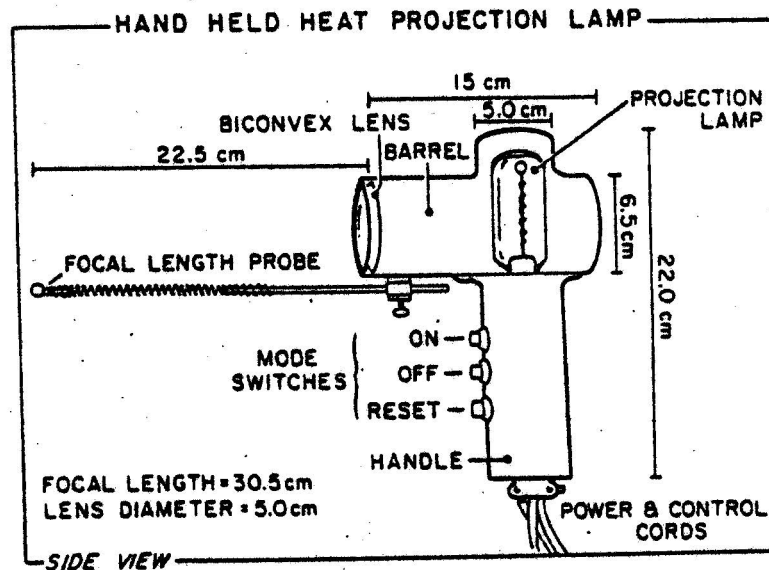


FIGURE 1. Drawing of the hand-held heat projection lamp used in the fetlock model. The lamp is directed toward the fetlock and positioned approximately 2 cm from probe tip to skin surface. The lamp is operated manually by pressing the appropriate mode switch. A line from the handle to the control console (Figure 2) operates the timer and permits bulb intensity adjustment via the rheostat.

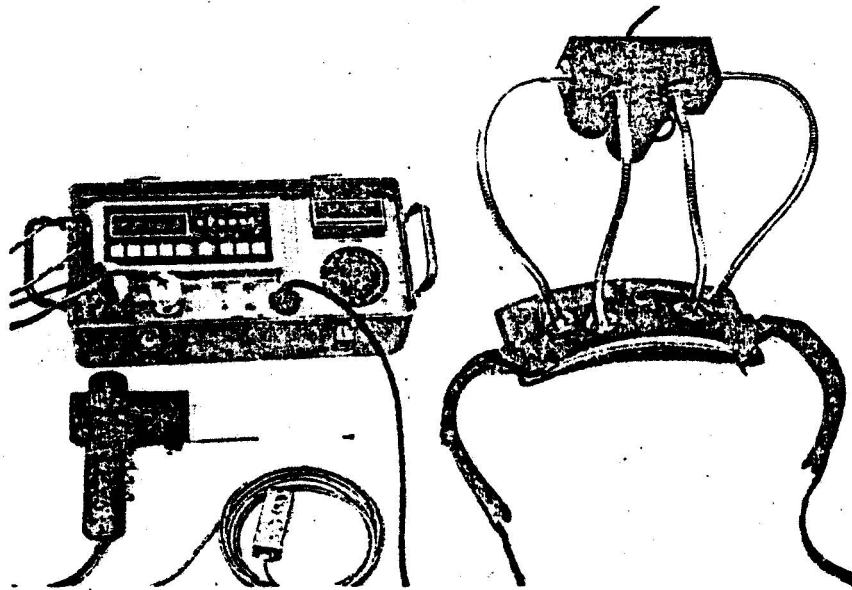


FIGURE 2. Apparatus for withers and fetlock pain models. Upper left: Control console (timer left, rheostat right). Lower left: hand-held heat lamp. Bottom center: hand-held control console for withers-mounted heat lamp. Right: Withers-mounted heat lamp attached to surcingle.

ratus. Noxious stimulation of the skin around the withers (dorsal intrascapular surface) produces reflex contraction of the cutaneous trunci muscle. This reflex could be elicited by manually probing the area with a needle or blunt instrument. It was also observed naturally following intradermal introduction of the proboscis of biting insects. A similar response was obtained following application of noxious radiant heat to the withers.

The resultant skin-twitch reflex represented a clearly identifiable end point that we used as a measure of cutaneous pain threshold. The intrascapular skin-twitch reflex is highly developed in the dog and has been used for years to assay the effects of the narcotic analgesics in that species (Martin and Jasinski, 1977).

To circumvent positional changes associated with narcotic-induced locomotor stimulation, it was necessary to modify the hand-held heat lamp. The modified device consisted of a dual heat lamp attached by flexible gooseneck connectors to a surcingle (girth with dorsal pad) (Figures 2 and 3). The lamp was stabilized by attaching it to a steel plate implanted in the surcingle. When mounted along the dorsal surface of the horse, the lamp remained at a fixed distance caudal to the withers. Spontaneous or drug-induced postural changes did not influence the lamp-to-withers distance. One lamp consisted of a projection bulb and condensing lens that delivered the radiant heat stimulus. The second lamp, located adjacent to it, produced nonfocused light when illuminated and served to reduce the possibility of operant conditioning. Both lamps were controlled remotely from a hand-held

console with ON-OFF and reset mode switches. A secondary toggle switch could be engaged to select between focused and nonfocused light, allowing for randomization of the stimulus order (Figures 2 and 3). The mode switches engaged and arrested the same countdown timer used with the hand-held lamp.

Intensity Control

The intensity of radiant thermal stimuli was controlled by a rheostat and digital readout located on the right side of the control console shown in Figure 2. Bulb voltages of 100 v for both the hand-held lamp and the withers-mounted lamp generally produced baseline reflex latencies of approximately 6 and 9 sec, respectively. Skin temperatures typically obtained at the time of skin twitch ranged from 43.2 to 49.3°C with a mean (\pm SEM) of $45.3 \pm 0.8^\circ\text{C}$. This mean value was similar to that reported as being noxious in humans ($44.3 \pm 0.5^\circ\text{C}$) when radiant heat was applied to the forehead (Hardy et al., 1952). All thermal stimuli were terminated when the duration of the stimulus exceeded the pretreatment or baseline latency by more than 1.8 times. This precaution was taken to prevent irreversible tissue damage due to excessive thermal stimulation. Selection between hand-held and withers-mounted heat lamps could be made by means of a toggle switch located on the control console (Figure 2).

The target tissue area in each animal was blackened with India ink to assure that the amounts of radiant heat absorbed by the skin of horses of differing colors was similar.

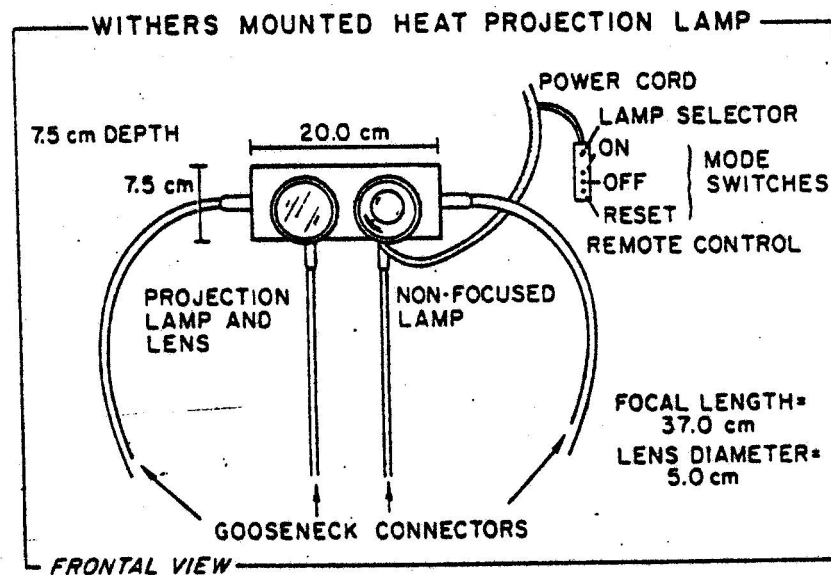


FIGURE 3. Drawing of withers-mounted heat projection lamp. Focused light is emitted from the bulb and lens assembly on the left side. Nonfocused light is emitted from the bulb on the right side. Gooseneck connectors are mounted on the steel reinforced surcingle (not shown). Lamps are selected and regulated by the hand-held remote control unit (right side).

Drug Studies

Mature thoroughbred and standardbred mares and geldings (400–550 kg) were used. All horses were kept at grass, but they were put in stalls 24 hr prior to the experiment and confined to equine stockades at the time of the experiment. Fentanyl, a short-acting opiate, was administered i.v. to eight horses at doses of 0.0025, 0.005, and 0.010 mg/kg along with saline as a control vehicle, according to a Latin square crossover design. Forelimbs- or hoof-withdrawal reflex latencies and skin-twitch reflex latencies were determined every 5 min for 30 min, then at 45 and 60 min after drug or saline. Response latencies for each horse were summed over the 60-min posttreatment period. A mean response latency (\pm SEM) was then determined for the eight experimental subjects at each dosage and saline over this time period. Regression lines and correlation coefficients were obtained from these values using a linear regression program (T1-58C Calculator, Texas Instruments, Lubbock, TX). A *t*-statistic was calculated from the correlation coefficient (*r*) using the formula $t = \sqrt{vr^2/1-r^2}$. A dose-response relationship was considered significant when a 95% or greater confidence interval was obtained for the *t*-statistic.

Fentanyl was generously supplied by McNeil Laboratories, Fort Washington, PA.

RESULTS

Fentanyl produced a significant dose-related prolongation of the skin-twitch reflex latency ($r = 0.999$, $p < 0.05$) as seen in Figure 4. However, a similar dose-related prolongation of the hoof-withdrawal reflex latency was not observed ($r = 0.286$, NS). In both assays, control pretreatment baselines and postsaline responses were stable over time. Restlessness and spontaneous locomotor activity was observed

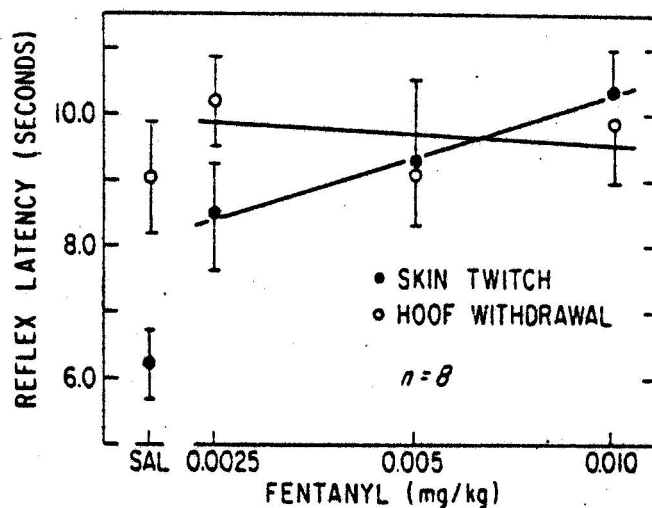


FIGURE 4. The effects of three doses of fentanyl and saline on skin-twitch and hoof-withdrawal reflex latencies. Points represent mean latency (\pm SEM) obtained from observations averaged over 60 min posttreatment, for eight horses.

intermittantly in most horses following narcotic administration, especially at the 0.010 mg/kg dose. In some cases the heat-evoked hoof-withdrawal reflex could not be distinguished from narcotic-induced stepping. However, this occurred primarily during the first 5–15 min following drug administration and subsided thereafter.

As judged by inspection of the stimulus site, consistent responses to repeated stimulation was not associated with significant tissue damage.

Operant conditioning to presentation of the pain stimulus at the fetlock occurred in around 50% of the animals tested. Some began lifting the forelimb at the moment of lamp illumination. This response was extinguished by randomizing the presentation of painful and nonpainful light cues. However, only one animal became conditioned to thermal stimulation of the withers, and this was readily extinguished by randomizing the presentation of focused and nonfocused radiant light.

DISCUSSION

An effective pain model should fulfill the following general criteria: 1) an end point or pain-perception threshold should be readily identifiable, qualitatively similar among subjects, stable over time, and reproducible within subjects; 2) the pain stimulus should be administrable repeatedly without producing significant tissue damage; and 3) the pain model should be sufficiently sensitive to detect the dose-related effects of putative analgesics without interference from the side reactions of those agents. The skin-twitch reflex model fulfills these criteria. The reflex contraction of the cutaneous musculature in response to a noxious thermal stimulus is clearly identifiable, even to the untrained observer. The twitch is usually localized to the site of heat application in most horses, but it can involve rhythmic unilateral skin movement around the entire suprascapular area. The present studies have shown that noxious stimuli may be presented at regular intervals for at least 60 min without significant tissue damage. Response latencies have been consistent among horses and within experimental sessions. Further, the skin-twitch withers model is sufficiently sensitive to detect dose-related analgesia following administration of a prototypic narcotic analgesic. This agrees with the studies of Martin and Jasinski (1977) in which dose-related analgesia was produced by a variety of narcotics using the skin-twitch reflex in the dog. Studies in progress have shown that the fetlock model is quite sensitive to the analgesic effects of local anesthetics. Blockade of the nerves that innervate the lateral fetlock area by different local anesthetics produced analgesia. The fetlock model distinguished differences in onset and duration of action as well as potency between the anesthetics tested. However, the skin-twitch withers model is clearly superior as an assay of the analgesic effects of the narcotics which also produce locomotor stimulation.

The lack of a linear dose response to fentanyl using the hoof-withdrawal reflex latency reflects the inadequacy of this method as an assay of narcotic analgesia in the horse. The presence of narcotic-induced locomotor activity precluded the use of the forelimb flexion reflex and an accurate nociceptive endpoint in our studies. Using a similar heating device, Pippi et al. (1979b) electronically recorded movement of the thoracic forelimb in response to noxious thermal stimuli applied above the coronary band, as a nociceptive end point in ponies and later in horses (Kalpravidh,

1984b). Despite their reference to drug-stimulated locomotor effects, the narcotic analgesic, butorphanol, produced a dose-related prolongation in response latency in horses. This is difficult to reconcile in light of our observations with fentanyl on the hoof-withdrawal reflex.

Earlier studies by Pippi and Lumb (1979a) showed that intravenous fentanyl (0.22 mg/kg) produced analgesia in ponies using the heat-evoked forelimb flexion model. However, no dose relationship was tested. If it can be assumed that the ponies were not made recumbent by the dose of fentanyl employed in their study, then observations in ponies cannot be readily extrapolated to the horse. Intravenous doses of fentanyl 0.040 mg/kg or greater produced frank collapse of the horses used in our studies. Further, since no mention was made of locomotor stimulation following fentanyl administration in the ponies, it is assumed that this was not a confounding variable in the analgesia measurements. This further differentiates the two equine species in terms of their response to narcotics.

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