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Development of a Canine Nociceptive Thermal Escape Model

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INTRODUCTION

Validated behavioral models of acute nociception in the rodent have become an essential component of both mechanistic and pharmacologic research. Thermal, mechanical, or electrical stimuli are applied, and a behavioral endpoint signifying a spinal reflex or higher order nociceptive processing is assessed. The well-validated rodent thermal escape model incorporating hind paw withdrawal to a radiant light stimulus (Hargreaves model) (Hargreaves, et al., 1988) has been used extensively. This model is based on the principle that a thermal stimulus sufficient to activate nociceptors on small sensory afferent fibers when applied to the hind paw skin surface of unanesthetized animals produces an organized escape response. This model has proved sensitive and specific in activating C-fibers when a logarithmic rate of rise in contact surface temperature corresponds to a linear rate of rise at cutaneous tissue nociceptors (Yeomans and Proudfit, 1994). Selective stimulation of nociceptive input combined with a defined behavioral withdrawal response facilitates evaluation of both parenteral and neuraxial analgesics. Analgesics which selectively inhibit afferent traffic in small sensory neurons reliably lengthen withdrawal latencies. Conversely, analgesics acting on pathways involved with chronic inflammatory or neuropathic pain may not alter acute thermal withdrawal latencies (Yaksh, 1999). Clinically, acute thermal pain is of minimal importance, but as a mechanistic screening tool for analgesic action, thermal nociception can yield clinically useful results. Acute nociceptive models validated for large animal species, however, are limited. Previous work has typically employed a local cutaneous muscle reflex evoked by a focal thermal stimulus applied to the lower back (Allen and Yaksh, 2004). Our interest in such drug assessment issues in a canine model and our experience with the rodent Hargreaves paradigm led us to develop and validate a parallel model for dogs. Such a thermal nociceptive screening tool in a large animal model may be useful in identifying analgesics which are efficacious in humans and in veterinary practice. Here we report development and validation of a canine hind paw thermal escape model patterned after the rodent paw stimulator and the effect of analgesics on withdrawal latencies.

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METHODS

All protocols were approved by the Institutional Animal Care and Use Committee of the University of California, San Diego.

Animals

Twelve destination bred beagle dogs [mean 13.9 kg (11.0 – 15.8 kg), mean 16.4 months (12 – 21 months), 8 males, 4 females] were used in this study. Three additional animals (1 male, 2 females) enrolled in an unrelated study were used for the metatarsal paw pad thermocouple trials prior to humane sacrifice. All animals were deemed healthy based upon physical exam, complete blood count, and serum chemistry analysis. Animals were housed in an AALAC-approved vivarium and provided food and water *ad libitum*. Food, but not water, was withheld for 12 hours prior to all drug studies. Prior to entering these drug studies, animals underwent an extensive adaptation period (minimum of 2 weeks) to acclimatize them to the testing paradigm and additional handling.

Apparatus

A cart (Intermetro Industries, Welks-Barre, PA, USA) was customized to accommodate a fabric canine restraint sling over a wire grill on which a 45.7 × 76.2 cm, 0.3 cm-thick plate of glass was secured. Beneath the grill was a shelf holding the paired radiant lamps, device timer, and intensity control box. The lamps, timer, and intensity control device are components of a rodent thermal nociceptive testing device described by Dirig, et al. (1997). (Figure 1) Briefly, the intensity control box delivers variable amperage with an arbitrary scale of 0 – 100 to paired, calibrated, focused (5 mm aperture diameter) projection bulb lamps. A silent relay circuit between the lamp, control box, and timer is independently activated for each lamp by a foot pedal which is released by the researcher when the animal moves the hind paw being tested. The lamps were fixed 28 cm apart in an aluminum frame so the bulbs lie directly under the glass plate at one end of the cart and project vertically through the glass. The spacing and orientation was set to accommodate the natural stance and hind paw position of the dogs as they stood in the restraint sling so that the light beam was naturally focused on the anterior one-third of the metatarsal paw pad. All behavioral studies were carried out between 0800 and 1800 hours in a closed, normally lighted room with care taken to avoid extraneous auditory and handling stimulation.

Rate-of-rise of the uppermost glass surface temperature during lamp activation was measured using an implantable thermocouple microprobe (IT-18, 0.064 mm diameter Teflon®-coated, sealed tip, Type T, response time, 0.2 sec, Physitemp Instruments, Inc., Clifton, NJ, USA) attached to a microprocessor thermometer (Model HH23, Omega Engineering, INC., Stamford, CT, USA) placed at the center of the lamp beam. Thermocouple microprobes were regularly calibrated against a mercury thermometer in constant temperature water baths between 34 °C and 60 °C. The thermocouple microprobe was secured to the glass surface and covered with dark fabric to prevent light scatter. Left and right lamps were individually activated and temperatures recorded at 5 second intervals between 5 and 25 seconds after activation of the lamp. Trials with each lamp were repeated three times with approximately 20 to 40 second delays between trials to simulate the normal testing interval employed during animal experiments.

Characterization of heating profile

In order to correlate glass surface temperature with metatarsal paw pad tissue temperature, the same thermocouple microprobes were implanted in the sub-dermal fascia at the anterior center of the metatarsal pad under local anesthesia in sedated dogs. The sedated animals were placed in the testing sling and the center of both metatarsal pads with the implanted thermocouple was

centered over each lamp. As for glass surface rate-of-rise trials, each lamp was activated individually, and metatarsal pad fascia temperatures from 5 to 20 seconds after activation were recorded at 5 second intervals. Trials with each lamp were repeated three times with approximately 20 to 40 seconds between trials to simulate the normal testing interval employed during animal experiments. Following sacrifice, the metatarsal pad tissue from these animals was harvested to examine thermocouple position and to look for histologic evidence of thermal damage. Glass surface and metatarsal paw pad time-temperature curves for both lamps were generated on multiple days at a fixed lamp intensity (between 4.0 and 4.5 Amps, dial setting = 90) adjusted to achieve baseline withdrawal times of 6 to 12 seconds, and the cut-off time of 20 seconds corresponded to a glass surface temperature of $\leq 60^{\circ}\text{C}$. The stimulus cut off in the absence of a response was defined to be 20 seconds based on the absence of tissue injury after repeated exposure and calculated to be greater than 4 standard deviations (SD) from baseline latency. The lamp intensity was kept constant for all subsequent parts of the study and glass surface time-temperature curves were verified periodically. Time-temperature data was plotted and curve-fit using GraphPad Prism® software run on the Apple® Mac computer.

Determination of Baseline Stability

Dogs were acclimated to the testing environment and to the restraint sling prior to any testing. During training and baseline testing, one researcher stood at the dog's head to discourage the dog from turning or moving in the sling. A second researcher stood at the left side of the dog at the hindquarters with one hand placed gently on the anterior tibial or metatarsal region of each hind limb. This contact served to steady and calm the dog, and alert the researcher to weak or subtle withdrawal motions. Once the dog was standing quietly with one hind paw centered over each lamp, the researcher actuated the lamp under the paw to be tested using a foot pedal, and terminated the stimulus when the dog purposefully moved the tested hind paw. Each hind paw was tested twice in every trial; the order of testing between left and right paw was randomized and time between each test was at least 20 seconds. Thus, at any given observation, the response latency was assessed twice for both hind paws. Because left and right paw variation for a given animal was not significant, a mean of these 4 tests (2 left, 2 right) was calculated to give a representative latency. This single mean value was then taken to be the "response latency" of that animal at that time point. Additional environmental modifications, including consistent background noise (fan), regular but unpatterned speaking to the animal, and positive verbal reinforcement were used to alert the dog to the testing situation without alerting it to which paw was being tested or when testing had started or was completed. Left and right hind paw baseline latencies were recorded prior to all intravenous drug testing with 12 dogs completing a total of 50 trials. Linear regression curves were fit for all left and right paw trials and the slopes compared. Mean and standard deviations for left, right, and population withdrawal latency were calculated; significance was set at $p < 0.05$.

Drug Studies

Study animals received intravenous bolus doses of hydromorphone (Dilaudid HP®, 10 mg/mL, Abbott Laboratories, Chicago, IL, USA), morphine (Infumorph®, 25 mg/mL, Baxter Healthcare Corp., Deerfield, IL, USA), fentanyl (fentanyl HCL solid, donated by NIDA and compounded aseptically in-house to 0.1 mg/mL in sterile water), dexmedetomidine (Precedex® 0.1 mg/mL, Hospira, Inc., Lake Forest, IL, USA), acepromazine (Promace®, 10 mg/mL, Ft. Dodge Animal Health, Fort Dodge, IA, USA), buprenorphine (Buprenex®, 0.3 mg/mL, Reckitt & Colman Pharmaceuticals, Richmond, VA, USA), butorphanol (Torbugesic®, 10 mg/mL, Ft. Dodge Animal Health, Ft. Dodge, IA, USA), and sterile saline at the doses listed in Table 1. Doses were selected to reflect doses which are considered to be clinically comparable (e.g. equi-analgesic) in common veterinary usage for dogs.

Researchers were not blinded to individual treatments, but: i) treatment order was randomized for the population of dogs tested; and, ii) data compilation was not undertaken until completion of a series. A minimum wash-out period of 48 hours between administrations was observed, and animals were fasted overnight prior to all drug administrations. Left and right hind paw baseline latencies were recorded prior to drug administration. The test drug was delivered over approximately 20 seconds via direct venipuncture of the left or right cephalic vein. Hind paw withdrawal latencies were assessed at 5, 15, 30, 60, 120, 240, and 480 minutes after drug administration as described for baseline measurements.

Behavioral effects secondary to drug treatment were noted. The animal's arousal status following drug administration was considered an important component of the model. This was quantified at each observation time on a -3 to +3 score of profound sedation (-3) to normal (0) to profound agitation (+3) in increments of 0.5 as previously described (Yaksh et al., 2000).

Human studies

To consider the human psychophysics of the test system, the thermal escape latency of 4 human volunteers was assessed. Here each subject placed his or her thenar eminence of the left and right hand on the glass plate over the respective lamp stimulus site and thermal stimuli were then applied to either hand in the same paradigm as was employed in the dog. The volunteer was instructed to respond verbally when the "stimulus was first detected" and then to withdraw when the sensation was judged to be "intense". The time (in seconds) of both detection and withdrawal was recorded. The mean of 4 stimulus applications for each observer was used as their respective response latency.

Statistics

For analysis of analgesic effects, the group mean and standard error of the mean (SEM) of the individual dog response latencies in seconds were plotted against time. For statistical analysis, the percent of maximum possible effect (% MPE) was calculated for each dog based on the formula: $\% \text{ MPE} = [(\text{mean latency} - \text{mean baseline}) / (\text{cut-off} - \text{mean baseline})] \times 100$ where cut-off latency was 20 seconds. The group means and SEMs were then calculated based on the individual animal %MPE values. Additionally, the area under the % MPE vs. time curve was calculated for each animal and this value used to calculate the mean $\pm$ SEM of the area under the curve (AUC) for each treatment. Analysis of the arousal scores were performed with the means $\pm$ SEM of the observed arousal score plotted against time. For statistical comparisons, the cumulative arousal score for each animal was calculated and expressed as a percent of the maximum possible cumulative arousal score. A 1-way Analysis of Variance statistic (ANOVA) was used to compare treatment groups. If statistically significant, *post hoc* analyses (Tukey's multiple comparison test) were used for group comparisons. For hydromorphone, a dose response curve was prepared based on the mean % MPE observed at 15 minutes. For these data, a best fit linear regression line was calculated and the extrapolated dose yielding 50% of maximum effect (ED50) with 95% Confidence Intervals (CI) was derived. A power analysis was performed on baseline data to determine animal numbers necessary for assessing group latency differences in this paradigm using the "2 Sample Means Power and Sample Size calculator" JMP Software (JMP®, Version 5) by SAS Institute Inc., Cary, NC, USA.

RESULTS

Validation of apparatus

The average glass surface temperature for both lamps, the average temperature at the dermofascial region of the metatarsal paw pad directly overlying the lamp, and the rates-of-change recorded over a 20 second heating cycle are displayed in Figure 2. As indicated, over

time after initiation of the heating cycle, the glass temperature rose from a mean of 26.6 ± 0.7 °C to 56.5 ± 0.8 °C at 20 seconds. The corresponding change in paw thermocouple temperature rose from a mean of 33.5 ± 0.5 °C to 42.0 ± 0.8 °C at 20 seconds. The glass surface data fit an exponential curve while the paw pad data fit a linear curve.

Response morphology

The withdrawal response was characterized by a shifting of the weight bearing to the contralateral paw and a transient, regulated, elevation of the stimulated paw several cm above the stimulus surface. The response was not accompanied by vocalization or by any persistent signs of agitation. Even with repeated testing, no dog displayed consistent signs of any aversive conditioning to the test environment.

Baseline withdrawal latency

Figure 3 displays the baseline response latencies assessed in 12 animals over an interval of 12 months. As indicated, the slope of the best fit regression lines for the left and right paw was not statistically significant indicating no systematic change in the test system over time in the absence of treatment. Based on these observations, the population baseline response latency with SD was 9.3 ± 1.7 seconds.

Consideration of baseline latency in the context of the thermal heating profile (see Figure 3) indicates that the glass temperature at the time of escape was on the order of 49.6 °C. Assessment of thermocouple temperature in the fascia deep to the dermo-epidermal layer of the metatarsal pad showed a temperature of 36.6 °C. It is believed that the thermal nociceptors mediating the evoked response lie more superficially than the tissue in which the thermocouple was placed.

Effects of opiates on thermal escape latencies

The intravenous delivery of several opiates at doses considered to be clinically equi-analgesic resulted in a time dependent increase in the thermal escape latency in the dog. Figure 4 presents the peak % MPE vs. time for morphine, buprenorphine, butorphanol, fentanyl and hydromorphone. All drugs showed a rapid effect with peak actions observed within 15 (butorphanol, fentanyl, hydromorphone) to 30 (morphine) minutes except for buprenorphine, which had a delayed onset and peak effect of 60 and 120 minutes, respectively. Plotting the peak effects, it was observed that these doses produced similar peak effects of between 60 and 90% of MPE. Statistical comparisons showed that all were different from saline ($p < 0.05$) but not each other. Duration of analgesic effect, represented by the area under the % MPE vs time curve (AUC), yielded a rank order of morphine = hydromorphone > butorphanol > buprenorphine > fentanyl = saline.

The ability to demonstrate a dose dependency in nociceptive escape effects was established with IV hydromorphone. As shown in Figure 5, doses between 0.03 and 0.2 mg/kg resulted in a progressive increase in magnitude and duration of effect. Plotting the dose effect curve indicated a statistically significant slope with an ED50 value of 0.05 mg/kg and a 95 CI of 0.03 – 0.08 mg/kg.

Effects of sedative agents on thermal escape latencies

A second set of drug studies examined the effects of the non-opiate, alpha 2 agonist dexmedetomidine and the phenothiazine sedative acepromazine. As indicated in Figure 6, dexmedetomidine, but not acepromazine, resulted in a rapid and significant increase in the thermal escape latency, which was statistically different from saline ($p < 0.05$).

Effects of drugs on arousal

All of the agents at the doses employed produced varying depression of arousal as compared to vehicle. Figure 7 displays the time course of this depression for all treatments. Calculation of the cumulative loss of arousal over the first 240 minutes following drug administration indicates that morphine, buprenorphine, butorphanol, fentanyl, and the two higher doses of hydromorphone produced depression that was significant as compared to vehicle, while acepromazine and the lowest dose of hydromorphone had non-significant levels of depression with respect to saline. In contrast, dexmedetomidine produced a potent depression which was statistically greater ($p < 0.01$) than all other agents at the dose employed.

An important consideration is the relationship between depression of arousal and the degree of analgesia. In Figure 8, plotting the mean $\pm$ SEM arousal score at peak analgesia against the peak mean $\pm$ SEM % MPE produced by each treatment is shown. As indicated, there are three groupings: 1) acepromazine which produced no analgesia and modest sedation; 2) the opiate analgesics which produced significant analgesia with a degree of sedation which was similar to that of acepromazine and 3) dexmedetomidine which resulted in potent analgesia and a profound sedation.

Injury status of stimulated hind paw

As evidenced above, many drug treatments were found to elevate the hind paw withdrawal latency to the cut off of 20 seconds, corresponding to an approximate glass surface temperature of 56.5 °C. In spite of such repeated exposures, no evidence of paw injury, erythema, or edema were noted in any dog. This emphasized that the cut off value of 20 seconds was unaccompanied by injury with a stimulus intensity that normally evoked a paw withdrawal at approximately 9 – 10 seconds.

Model power calculations

Based on the baseline escape latency and standard deviation defined above (9.3 ± 1.7 sec), we calculated the number of animals necessary to define significant differences ($p < 0.05$) with different increases in latency using a 20 second cut off as the maximum possible effect (e.g. MPE = 100%). These results are presented in Table 2.

Human psychophysics

Application of the thermal stimulus to the thenar eminence of human observers resulted in a time dependent report first of “warmth” and then later of “intense” sensation. These results are presented in Table 3.

DISCUSSION

We have developed and validated a canine thermal escape model characterized by calibrated, stable thermal stimuli, reproducible glass surface heating curves, and an apparatus well-tolerated by animals for multiple testing sessions. The stimulus evokes a well defined withdrawal of the stimulated paw. The withdrawal has a complex morphology with altered weight bearing to lift the stimulated paw, and a discrete transient elevation. In humans, the parallel application of the stimulus to the thenar eminence of the palm resulted in a reliable report of warmth after approximately 5 seconds and a short intense thermal sensation with a mean latency of around 10 seconds. While these results may not exactly reflect upon the canine sensation given the difference in cutaneous thickness between the human hand and the dog paw pad, it suggests the likelihood that the animal is indeed responding to an aversive component of the stimulus. This parallel has been drawn by other researchers in the development of animal nociceptive models (Le Bars et al., 2001; Dixon, et al., 2002)

The thermally evoked response

The response employed in these studies was a simple withdrawal of the hind paw. When adapted, the animals showed little tendency for spontaneous stepping when the test was initiated. The testing of both hind paws in a pseudo random fashion at each observation interval was useful, as it reduced the likelihood of conditioning the withdrawal response to environmental cues. The animal was found to reliably respond with the paw being stimulated. The use of side by side stimulus lights simplified testing by precluding the need to move the lights during the testing to randomly stimulate the alternate paw. The multiple tests of either paw permitted the response measure to be based on four observations, reducing the influence of outliers. This use of either paw also reduced the likelihood of injury with many thermal exposures. As noted in Figure 2, examination of either paw over time revealed no systematic difference between left or right response latencies. It should be noted that the use of both the left and right paw for analgesic assessments is not likely to be mechanistically important when studying systemically delivered drugs, but it is a paradigm that has significance when the experimental manipulation, such as a local anesthetic block or an intrathecal drug, may influence one paw more than the other. Additionally, in canine models of unilateral inflammation (Hamilton, et al., 2005), the independent targeting of left and right paws provides an important attribute.

Test paradigm stability

In these studies, animals were initially adapted to handling and testing procedures. Once acclimated, they were entered into the study. Examination of baseline withdrawal latencies in these animals over an extended series of test epochs revealed no systematic change, emphasizing stability and robustness of the measure.

As in any behavioral paradigm, the adaptation of the animal to the system and handling as well as the stability of the testing environment is crucial. Doubtless, the small native variation observed in these studies reflects upon the prior adaptation and the attention paid to handling. Although the animals were not specifically selected for their demeanor, it is anticipated that should an animal show a high degree of agitation and respond poorly to handling, that animal would not be incorporated in the study sequence.

Device parameters

For any given device, the stimulus applied to the plantar nociceptors is a function of the current supplied to the bulb and the surface on which the paw is resting. The use of an incandescent source was chosen for its ease of implementation and safety. Increasing current will increase the amount of energy applied to the paw and the rate of temperature rise. Thus, escape latency is a function of both of these parameters (Yarnitski et al., 1992; Yeomans and Proudfit, 1994). Accordingly, in developing this system, once an appropriate current-response latency was determined, this current/heating curve was retained for the duration of the study. Laser sources have been described and further development of this model might benefit from this modification (Tzabazis, et al., 2005).

Contact surface temperature can influence response latency (Dirig et al., 1994). Although glass temperature was not specifically controlled as in other rodent models, room temperature was tightly maintained and glass temperature varied little (see Figure 1).

In the present study, the baseline response latency observed at around 9 – 10 seconds corresponded to a glass temperature of approximately 50 °C. In an effort to measure temperature nearer the paw pad nociceptor, parallel readings were taken with a needle thermocouple. Unexpectedly, these readings showed a mild increase over time, but temperatures at the baseline response latency were only around 37 °C, below the temperature

at which most mammalian cutaneous thermo-nociceptors respond (Tominaga and Catarina, 2004). We conclude that these data indicate that the intraplantar thermocouple lay deep to the nociceptors influenced by the local skin heating, which generally terminate at the dermo-epidermal junction in the dog paw pad (Evans and Christensen, 1979).

Paradigm Power

An important issue in defining a stimulus exposure paradigm is that it results in no injury with repeated maximum exposure (cutoff) and that the maximum latency (at cutoff) is a minimum of greater than 4 standard deviations from baseline. This emphasizes that, should cutoff be observed after treatment, we can assert that the treatment had a significant effect at some alpha level less than $p < 0.01$. In other words, a model would be fatally flawed if baseline variance is such that the cutoff is less than that required to show a statistical difference. Importantly, in this model, the 20 second cutoff was not associated with any evidence of tissue injury. Power analyses (Zar, 1984) indicate that it is reasonable to assume that a treatment or dose which produces a behaviorally relevant effect (e.g. an increase of 50% or 75% above baseline thresholds) could be shown to be statistically significant with 6 and 4 dogs, respectively, in each treatment group. The importance of having such estimates of model power has been emphasized when developing comparisons between treatments in veterinary models. (Hofmeister et al., 2007)

Drug effects

Analgesia—In the present studies, we examined the analgesic effects of a variety of agents with known clinical utility on experimental thermal escape latency. The actions were assessed at doses which are widely considered to be therapeutically relevant and approximately equi-analgesic based on clinical efficacy in procedures such as ovariohysterectomy or fracture repair (Pascoe, 2000, see also Table 1). As indicated, these opiates and the alpha 2 agonist, dexmedetomidine, resulted in maximal or near maximal increases in latency. This activity provides some degree of validation that agents with the ability to produce a significant increase in this model will have clinical utility, side effects notwithstanding. As expected, the model was robust in defining dose dependency with hydromorphone. The dose-effect relationship and ED 50 values defined in this model are interestingly comparable to those reported for cats using a thermal stimulation model (Wegner et al., 2007).

The durations of action of the agents tested were generally comparable to that expected clinically. The one exception was hydromorphone. The perceived clinical duration of action for hydromorphone at 0.1 mg/kg, and corresponding dosing interval employed, is 4 hours (Pettifer and Dyson, 2000). However, experimentally, hydromorphone has a significant antinociceptive effect for at least 6 – 7 hours (Wegner et al., 2004; Machado, 2006). As noted here, the effects of the higher doses of hydromorphone were comparable in duration to those observed for morphine.

Arousal—As expected, the opiate analgesics, dexmedetomidine, and acepromazine produced varying degrees of sedation at the doses employed. As presented in Figure 8, at their equi-analgesic doses, opiates produced comparable degrees of sedation and this degree of sedation was similar to that produced by acepromazine. However, as indicated, acepromazine had no effect on the nociceptive threshold. This indicates that the opiate analgesic effects were independent of the modest sedation produced by these doses. Dexmedetomidine at an approximately equi-analgesic dose to the opiates showed a profound sedation score. This finding is consistent with the known sedative properties of alpha 2 agonists such as medetomidine and xylazine which are used in veterinary practice as sedative-analgesic pre-anesthetic agents. Figure 8 is useful as it demonstrates that the study paradigm can make a clear distinction between clinically useful classes of analgesic and sedative agents.

Interpretation of drug effects

We would stress that the model is useful in predicting the therapeutic utility of agents which have an effect upon acute small afferent evoked escape behavior. It is widely appreciated that many useful therapeutics such as NSAIDs or gabapentin have no effect in models of acute nociception as their effects relate to an action on models of central and/or peripheral facilitation. However, many classes of drugs, including opiates, alpha 2 agonists and local anesthetics do alter such afferent traffic (Yaksh, 1999). Thus, failure to see an analgesic signal does not therefore exclude the potential benefit of a particular class of agents. Indeed, activity in this model can likely predict therapeutic ratios for novel analgesics at doses which will be effective in acute, moderate to severe, clinically relevant pain states.

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Figure 1. Canine Thermal Escape System, consisting of a metal cart frame on which a fabric sling is suspended over the glass plate and grill on which the dog stands. Focused high-intensity lamps lie directly below the glass with positioning aids for placement of the dog's hind paws. Intensity control and timer lie below the grill and consist of a closed, silent relay so the dog is not alerted to testing. A foot pedal allows initiation and termination of lamp heating. See text for further details.

Figure 2.

Mean glass surface and paw pad temperatures as a function of time. The x-axis is the time in seconds from lamp actuation to termination for all testing. The left y-axis is the temperature in degrees centigrade measured by a needle thermocouple placed either on the glass surface directly over a lamp or inserted at the dermo-fascial region of the anterior metatarsal pad of the dog. The right y-axis is the rate of temperature change in °C over the time course of heating; dashed lines apply to the glass surface while solid lines apply to the paw pad. Open circles designate the mean glass surface temperature for all validation testing; closed circles designate the mean paw pad temperature of three dogs. Baseline withdrawal latency for the population of dogs tested is indicated by the solid vertical line.

Figure 3.

Left and right withdrawal latency measurements for 12 dogs, fifty baseline trials. The trial number is on the x-axis while the response time in seconds is on the y-axis. The best fit linear regression for all left (solid) and right (dashed) paw trials is indicated; slopes were not significantly different. Mean $\pm$ SD withdrawal times for the left paw, right paw, and population are listed below the plot.

Opiates

Figure 4.

Panels show analgesic effects of opiate analgesics. A) Time course of effect for intravenous morphine, 0.2 mg/kg hydromorphone, butorphanol, buprenorphine, and fentanyl with saline as a reference. The x-axis is the time in minutes from drug administration, the y-axis is the % MPE calculated for a mean of 4 to 6 animals per drug. B) Peak % MPE $\pm$ SEM of intravenous morphine, 0.2 mg/kg hydromorphone, butorphanol, buprenorphine, and fentanyl. All drugs beneath the overlapping dotted line were significantly ($p < 0.05$) different from saline, but not significant in effect from one another. C) Area under the analgesic effect-time curve (% MPE-time curve $\pm$ SEM) for intravenous morphine, 0.2 mg/kg hydromorphone, butorphanol, buprenorphine and fentanyl. Rank order of AUC effect is: Morphine=hydromorphone>butorphanol>buprenorphine>fentanyl>saline. In the histogram, agents not connected by a common overlapping dotted line indicate agents which differed significantly in duration of action ($p > 0.05$).

Figure 5.

Panels show dose dependent effects of hydromorphone. A) Time course of effect for three doses of hydromorphone with saline as a reference. The x-axis is time in minutes from drug administration, the y-axis is the % MPE calculated for a mean of 4 to 5 animals per drug; error bars are $\pm$ SEM. B) Dose-effect curve for hydromorphone. The x-axis is drug dose in mg/kg; the y-axis is peak % MPE. The mean peak % MPE $\pm$ SEM at 15 minutes post-administration is indicated along with the linear regression line for these values. The calculated ED₅₀ with 95% CI was: ED₅₀ 0.05 (0.03 – 0.08) mg/kg

Acepromazine and Dexmedetomidine

Figure 6.

Panels show analgesic effects of sedative agents. A) Time course of effect for intravenous acepromazine and dexmedetomidine with saline as a reference. The x-axis is the time in minutes from drug administration, the y-axis is the % MPE calculated for a mean of 5 animals per drug. B) Peak % MPE $\pm$ SEM of intravenous acepromazine and dexmedetomidine. Dexmedetomidine was significantly ($p < 0.001$) different from acepromazine and saline; the common overlapping dotted line indicates acepromazine was not significantly different from saline. C) Area under the analgesic effect-time curve (% MPE-time curve $\pm$ SEM) for intravenous acepromazine and dexmedetomidine. Rank order of AUC effect is: Dexmedetomidine>acepromazine>saline, but no difference was significant as indicated by a common overlapping dotted line.

Figure 7.

Panels show effects of agents on arousal. A) Time course of reduction in normal arousal state (sedation) for the first 240 minutes after drug administration. The x-axis is time in minutes, the y-axis is arousal score on a scale of -3 (deep sedation) to 0 (normal). Symbols for each drug represent the mean of 4 to 6 animals per drug; error bars are $\pm$ SEM. B) Maximum cumulative arousal reduction (sedation) over the first 240 minutes following drug administration. Arousal scores at each time point for all animals tested were summed and presented as a mean percent of maximum possible sedation $\pm$ SEM. Dexmedetomidine was significantly different from all other drugs ($p < 0.01$). As indicated by common overlapping

dotted lines, all other drugs were significantly different ($p < 0.05$) from saline except acepromazine and 0.03 mg/kg hydromorphone.

Figure 8.

Comparison of peak effect and peak sedation. The x-axis is the peak % MPE for all agents; the y-axis is the arousal reduction (sedation) score at the time of peak % MPE. Symbols represent the mean of 4 to 6 animals; error bars for both measurements are $\pm$ SEM. All drugs lying to the right of the 50% MPE line had significant ($p < 0.05$) analgesic effect with respect to saline. All drugs lying above the -1.5 arousal score line were not different ($p > 0.05$) in sedation from one another or from acepromazine, but were all significantly ($p < 0.01$) different from dexmedetomidine.

TABLE 1

Summary of the clinically useful, equi-analgesic doses employed for comparison in the present studies.

Drug	Dose (mg/kg)	Reference
Morphine	1.0	KuKanich et al., 2005
Acepromazine	0.1	Hofmeister and Egger, 2005
Buprenorphine	0.03	Slingsby et al., 2006
Butorphanol	0.4	Fox et al., 1998
Dexmedetomidine	0.01	Murrell et al., 2005
Fentanyl	0.01	Sano et al., 2006
Hydromorphone	0.2	Pettifer and Dyson, 2000

TABLE 2

nimal number or tests per group required to observe statistical significance at a given analgesic effect level vs. control.*

	Target % Maximum Possible effect			
	25%	50%	75%	90%
Group sample size	8	6	4	3

* Based on baseline escape latency and standard deviation defined above of 9.3 ± 1.7 sec, calculated assuming $\alpha = 0.05$; $\beta = 0.8$

TABLE 3Response latency (Mean $\pm$ SEM) for detection of “warmth” and “intense” sensation in human observers.

	Detected: Warmth	Detected: intense
Response latency (sec) [*]	5.9 $\pm$ 0.3	10.1 $\pm$ 1.9

^{*} N = 4