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Nociceptive threshold and analgesic response to morphine in aged and young adult rats as determined by thermal radiation and intracerebral electrical stimulation

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Abstract

The present experiment compared the nociceptive threshold and analgesic response to morphine in young (4–5 months) and aged (24 months) rats using peripheral thermal stimulation and intracerebral electrical stimulation. Responses to thermal stimuli were assessed using both the classical tail-flick procedure in which latency of response is the dependent variable and a new method in which threshold in calories of heat is the dependent variable. In the intracerebral nociceptive threshold procedure, electrical stimuli were delivered via an electrode implanted in the mesencephalic reticular formation (MRF), a pain pathway, and the animals were trained to terminate the stimulation by turning a cylindrical manipulandum embedded in one wall of the experimental chamber. For the classical tail-flick method, the aged rats required a greater intensity of stimulation to produce a basal response latency that was between 2.5 and 3.5 s. Using the new psychophysical method for determining the tail-flick threshold, the aged rats' basal thresholds were significantly higher than that of the young rats. However, the basal thresholds obtained by direct stimulation of the MRF failed to show a significant age effect, suggesting that the registration of pain is not different between young and aged rats. These age-related differences in baseline tail-flick response may be due to changes in the spinal reflex associated with aging. Although, there was no difference in the analgesic effects of morphine between young and aged rats using the latency of the tail-flick response, evidence for decreased analgesic response was seen using the tail-flick threshold measure and the intracerebral stimulation threshold method.

Keywords: Nociception thresholds; Pain; Tail-flick; Brain stimulation; Analgesia; Morphine aging

1. Introduction

There has been concern that pain is undertreated in the elderly (Ardery et al., 2003; Gagliese and Melzack, 1997; Sauaia et al., 2005). This problem may arise, in part, from a limited understanding of the actions of opioid analgesics in the elderly. There is a common belief held by many clinicians, supported, for the most part, by considerable clinical experimental evidence, that aged patients have higher pain thresholds than the young and are more responsive to the analgesic action of opiate drugs (Gibson and Helme, 2001; Macintyre and Jarvis, 1995). However, Gaston-Johansson et al. (1999) in reviewing pain management in older adults, concluded that the undertreatment of pain in the older patient is a significant problem. The extent of the difference between the aged and young is often a function of the type of clinical pain (Helme et al., 2004) or pain scale used (Gagliese and Katz, 2003). In the evaluation of the intensity of clinical pain patients are often asked to judge the intensity of their pain on a 10 point scale with a score of 10 being the worst pain they ever felt or could imagine. This makes the assumption that a score of 10 in the aged is equal to 10 in the young. Given these problems, greater experimental evidence is needed before the actions of opiate drugs in the aged can be considered to be adequately characterized.

Animal models of the effects of aging on opioid analgesia have been used to determine if the efficacy and potency of opioid agents such as morphine are altered with age. However, these experiments have given mixed results (Gagliese and Melzack, 2000). Differences obtained are often a reflection of

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the pain model used. Although a variety of nociceptive stimuli are used, (e.g., thermal radiation, mechanical pressure, electrical stimulation) most studies of responses to nociceptive stimuli use an unconditioned withdrawal response as the dependent variable. The two most common experimental procedures for the study of pain and analgesia are the "tail-flick" (D'Amour and Smith, 1941; Ness and Gebhart, 1986) and the "hot-plate" procedure (Eddy and Leimbach, 1953). In the former, radiant heat is focused on the tail of a rodent and the latency of a reflexive withdrawal of the tail is the dependent variable. In the latter, rodents are placed on a "hot-plate" and the latency of a withdrawal response (e.g., raising a rear paw) is the dependent variable. In both of these procedures a fixed nociceptive intensity is used. The tail-flick response is believed to involve both spinal (Borszcz et al., 1990; Douglass and Carstens, 1997; Irwin et al., 1951; Kauppila et al., 1998; King et al., 1997; Ness and Gebhart, 1986), and supraspinal levels (Carstens and Douglass, 1995; Jensen and Yaksh, 1986; Kauppila et al., 1998; King et al., 1997) of the central nervous system.

In determining the analgesic effects of drugs using the tail-flick procedure, the stimulus intensity is typically adjusted for each animal so that the baseline latencies are approximately equal for all animals (Crisp et al., 1994; Kramer and Bodnar, 1986; McLaughlin and Dewey, 1994; Sawamura et al., 2002). This adjustment of the intensity may have its limitations in comparisons between experimental groups with significantly different baseline intensity thresholds. In an experiment where baseline latency levels of response, using the tail-flick procedure, were reported, an increase in response latency was found in 25 month old rats compared to three month old rats (Akunne and Soliman, 1994). However, Islam et al. (1993) found six month old rats had longer latencies versus 12, 18, and 24 month old rats. Results from an experiment by Crisp et al. (1994) indicated that 25–26 month old rats had shorter latencies than 15–16 month old rats but no difference from five to six month old rats. Further, some experiments have found no differences in assessments of baseline response latency with age (Hamm and Knisely, 1986; Hamm et al., 1986). An additional experiment, in which pain thresholds were determined by electric shock stimuli and vocalization, rather than thermal radiation, found no significant age differences (Goicoechea et al., 1997).

Regarding the analgesic response to morphine, a number of investigators have reported diminished analgesia in older rats and mice (Kavaliers et al., 1983) as measured by the tail-flick method (Kramer and Bodnar, 1986; McLaughlin and Dewey, 1994), the "hot-plate" procedure (Kavaliers et al., 1983), tail immersion in warm water (Jourdan et al., 2002), and jump-threshold to electric shock (Kramer and Bodnar, 1986). In one of these experiments, the direction of the difference depended on the post-injection time of testing (Kramer and Bodnar, 1986). The direction of an age-related difference has also been shown to depend on the dose of the drug (Spratto and Dorio, 1978). Results in which aged animals were consistently more sensitive to morphine than younger cohorts were reported by Islam et al. (1993) and Spratto and Dorio (1978) using the tail-flick procedure and Saunders et al. (1974), using response to foot-shock. Other investigators have found no age-related differences between young and aged rats (Akunne and Soliman, 1994; Van Crugten et al., 1997a).

Almost all of the previous tail-flick experiments comparing analgesic responses of young and aged rats used a fixed intensity of thermal radiation with latency of response as the dependent variable. However, the integrity of the tail-flick reflex response may be compromised in the aged rat, and consequently latency of response may not be a valid measure for the comparison of the sensitivity of animals in different age groups to painful stimuli. Because of this possibility we employed a threshold method that was independent of speed of response. This new tail-flick method varied the intensity of stimulation and the dependent variable was the absolute psychophysical threshold in units of heat (cal). Both baseline sensitivity to thermal radiation and the analgesic effects of morphine were examined in this study using this threshold procedure. The results of this approach were compared with those obtained using the classic tail-flick approach.

Another issue that has not been addressed by prior studies is whether there are age-related differences in sensitivity to nociceptive stimuli and to the analgesic actions of morphine at the supraspinal level as compared to differences that might exist at lower levels of the nervous system. In order to bypass the possible differences in response to peripheral stimulation between young and aged rats, an additional experiment was performed employing a method in which aged and young rats were intracerebrally implanted with stimulating electrodes in the mesencephalic reticular formation (MRF), a pain pathway, and the psychophysical thresholds for centrally delivered electrical stimuli were determined. In this experiment the analgesic effects of morphine also were determined in both the aged and young rats. Thus, the combined experiments allowed a comparison of peripheral and central nociceptive stimulation as well as the analgesic response of morphine in young and aged rats.

2. Materials and methods

All procedures used in this study were approved by the Boston University School of Medicine Institutional Animal Care and Use Committee (IACUC).

2.1. Animals

Male Brown Norway/Fischer 344 F1 hybrid rats (F344/BNF1) (Harlan Sprague Dawley, Indianapolis, IN) were used in these experiments. Responses to peripherally delivered stimuli were evaluated in two tail-flick experiments in which either tail-flick latencies or tail-flick thresholds were determined. A brain-stimulation escape paradigm was employed for the third experiment, evaluating analgesic responses using intracerebral stimulation.

Sixteen animals were used in the completion of the tail-flick stimulus intensity threshold ($n=8$/group) experiment. These animals were also used in the tail-flick latency experiment, but because of the loss of two animals only 7 animals were used per

group. Animals were aged 5 and 24 months and had mean body weights of 348.6 grams (g)±26.5 SD and 629.8 g±39.9 SD, respectively. Eleven animals were used in the brain-stimulation escape experiment. These animals were placed into one of two groups, aged 4 ($n=5$) and 24 months ($n=6$), at the start of the experiment with mean body weights of 362.3 g±58.2 SD and 474.3 g±44.9 SD, respectively.

All animals were singly-housed in standard plastic cages in a temperature-controlled (20–22.2 °C) colony outside of the experimental environment. The lights in the colony were maintained on a 12-h light/dark cycle. Lights were turned on at 0700 h and turned off at 1900 h. Animals had access to standard rat chow (18% protein, Harlan) and water ad libitum.

2.2. Apparatus

Latency of tail-flick responses to thermal radiation was measured with a Model 33 Tail-Flick Analgesia Meter (IITC Inc., Life Science Instruments, Woodland Hills, CA). The apparatus was equipped with a 150-W halogen quartz light source adjacent to a small cooling fan. During experiments, a light beam was focused through a lens located 6.7 centimeters (cm) from the base of the apparatus. The base of the tail-flick meter was equipped with an acrylic plate upon which animals were restrained. The plate was designed with a central groove where tails were placed. During operation, the light was focused directly onto the tail and the induction of the reflexive tail withdrawal resulted in a disruption of the light beam automatically stopping the apparatus's timer (Akunne and Soliman, 1994; D'Amour and Smith, 1941; Irwin et al., 1951). Response latencies were displayed digitally to 0.01 s. The unit was equipped with a manual dial that varied the intensity of the stimulus through nine steps that were linearly related to calories of heat. This was confirmed by measuring the calories of heat absorbed by 2 milliliters (ml) of water over a 5 s period using a K-Thermocouple Thermometer, Model HH-603 (Omega Engineering, Inc., Stamford, CT), equipped with a beaded-wire probe. Absorbed calories of heat increased in proportion to intensity setting levels.

Prior to any experimental procedures, each rat was handled by the experimenter for a minimum of 30 min per day for seven days. At the conclusion of each handling session, the body weights of each rat were recorded. The tail of each animal was measured for length, in cm, and three anatomical tail locations were identified for analgesic testing. Given that there is some level of variability in individual rat tail lengths, with aged rats typically having longer tails, testing locations were proportionally defined such that the anatomical locations tested would correspond to distances of five, eight, and 11 centimeters (cm) from the proximal tail end of a "standard" rat. The determination of these particular locations was based on a standard rat tail length of 17 cm. These sites were clearly marked with black ink, a technique often employed (Crisp et al., 1994; Goettl et al., 2000; Irwin et al., 1951; Tsuruoka et al., 1988). Prior to testing, rats were exposed to a restraining towel, which was employed for all tests. Rats had a minimum of two days of restraint experience before experiments commenced. This was done to habituate animals to the restraining method prior to actual testing.

2.3. Tail-flick procedure and preliminary testing

Initial tests were conducted to determine the appropriate stimulus intensity to use for each animal in the experiment in which latency of response was the dependent variable. Intensities at which a tail-flick response occurred with a latency of 2.5 to 3.5 s were determined for each animal. One test was performed at each blackened anatomical location for each stimulus setting (1 through 10 on the manual dial). To avoid excessive heating of the tail, testing was conducted at each location at 10 min intervals. Prior to testing, animals were handled briefly for one to two minutes and placed in the restraining cloth.

With the exception of two rats that died, one aged and one young, before the completion of the experiment, all animals in the tail-flick experiment underwent testing using two methods to determine sensitivity to the radiant heat stimulus, the standard tail-flick procedure, in which latency of response was the dependent variable and an alternative psychophysical procedure in which intensity of the stimulation was the dependent variable. Half of the animals were first tested in the latency experiment followed, on separate days, by testing using the psychophysical threshold method while the other animals were tested in the opposite order.

2.3.1. Latency tail-flick method

Maintaining fixed stimulus intensity settings for each animal, tail-flick response latencies were determined following the administration of subcutaneous (sc) injections of either bacteriostatic sodium chloride (0.9% saline, 1 ml/kg) (Abbott Laboratories, North Chicago, IL) or morphine sulfate (NIDA, Bethesda, MD) dissolved in bacteriostatic saline.

Animals were restrained on the apparatus and underwent three trials at both 15 and 60 min post-injection, one test at each tail site. Trials were limited to 10 s in duration and a minimum 30 s inter-trial interval between tests allowed sufficient time for cooling of the apparatus. Also, to further insure sufficient time for cooling, all animals completed their first trial before the second trial commenced, e.g., in a given testing group, each rat underwent a tail-flick test at a single tail point. When all rats in that particular group had been tested at a single tail location, additional trials proceeded at the next tail location. This prevented individual animals from undergoing consecutive tail-flick tests. Values for tail-flick latencies were determined by finding the mean of three trials, one for each anatomical tail location. Animals not responding to the stimulus within the allotted 10 s time interval were assigned a response latency of 10 s. The dependent variable was the difference between each animal's mean baseline saline response latency and that obtained after each dose of morphine.

Animals were tested on the tail-flick procedure twice, at 15 and 60 min, following either saline or morphine administration. The baseline response latencies from each tail-flick test, following saline treatment (15 and 60 min), were averaged to

yield a final baseline (mean) response latency for each animal. Injections of saline and morphine sulfate (5.0 and 10.0 mg/kg) were administered subcutaneously (sc) following a crossover design. Only eight animals (4 young and 4 aged) received a 2.5 mg/kg dose. Animals receiving the 2.5 mg/kg morphine dose had already completed a morphine regimen that included doses of 5.0 and 10.0 mg/kg. A minimum of 72 h elapsed between injections. The testing procedure following injections remained the same, as described above.

2.3.2. Threshold tail-flick method

Using a modification of the classical psychophysical method of limits, individual animals underwent a series of tests on the tail-flick apparatus employing stimulus intensity thresholds as the dependent measure. This methodology resembles previously reported designs utilizing foot-shock thresholds (Bonnet and Peterson, 1975; Crocker and Russell, 1984; Kramer and Bodnar, 1986; Markowitz et al., 1976) and intracerebral stimulation (Hubner and Kornetsky, 1992; Izenwasser and Kornetsky, 1988, 1990; Sasson and Kornetsky, 1983, 1986; Sasson et al., 1986; Unterwald et al., 1987). Tail-flick tests were conducted on three different locations on the tail. An ascending series of stimulation intensity was delivered to each tail location. The first series of tests was conducted at the most proximal of the three tail points, the next at the second most distal location, and the third series at the most distal location. The first trial of each series was performed at the lowest intensity of stimulation. Subsequent trials were conducted at the next highest level. Stimulation was limited to five s in duration with 10 s inter-trial intervals. In a given testing group, all animals completed the first testing series before beginning a second series, at the next tail point, to allow cooling of the apparatus and tails between trials.

Responses were classified as either positive (+) or negative (−). A positive response was defined as the reflexive withdrawal of the tail within the five second fixed duration. Conversely, a negative response was defined as the absence of withdrawal during the five second interval. If, in a given series, two consecutive stimulus intensity settings prompted a tail-flick response within five seconds, the remaining higher intensity settings were assumed to result in withdrawal responses, thus negating the need to be tested and decreasing the number of stimuli necessary to determine the intensity threshold. Testing proceeded to the second and finally, the third testing series. As in the first testing series, the delivery of stimuli in the second and third series followed an ascending pattern of stimulus intensities. Between series, animals were returned to their cages for two and one-half minutes. Stimulus intensity thresholds for each series were determined as the midpoint value between the intensity setting that generated the first positive response and the intensity setting that immediately preceded it, which by definition was a negative response. The mean threshold for an animal was the mean of the thresholds determined for the three series.

The treatment regimen for each animal included subcutaneous (sc) injections of saline (1 ml/kg) or morphine sulfate (5.0 and 10.0 mg/kg) administered following a crossover design. As in the latency method, only eight of the rats (4 young and 4 aged) received the 2.5 mg/kg dose. Testing methods were randomized such that half of the animals underwent testing in the latency measure procedure followed on separate days by the psychophysical threshold method and half vice versa. Drug treatment thresholds were converted to a difference score from the each animal's respective mean saline threshold. On testing days when animals were to receive morphine, a threshold was determined after first determining the threshold following the administration of saline. Fifteen minutes after the completion of the initial saline baseline for that particular test day, animals were treated with morphine and were tested at 15 and 60 min post-injection. As in the latency method, animals were restrained on the apparatus and testing between animals was staggered such that the apparatus, as well as the rats' tails, could cool prior to the onset of further testing. In addition to determining the threshold, for each positive response observed during testing, the response latency, in s, was recorded. As described above, if the animal did not respond within five seconds the trial was terminated.

2.4. Statistical analyses for tail-flick

Morphine treatment data for both the latency and the threshold tail-flick method were converted to a difference score from each animal's respective saline baseline measure. These scores were analyzed using SigmaStat® Version 2.03 software (SPSS, Inc., 1992–1997). Difference scores from saline baseline were analyzed using two-way repeated measure ANOVA's at both 15 and 60 min time periods. Post hoc comparisons were conducted using the Student–Newman–Keuls test. Comparisons between young and aged rats' baseline (saline) intensity thresholds and latency of response at threshold were analyzed using Student's *t*-tests.

2.5. Brain-stimulation nociceptive threshold

In this method, supraspinal nociceptive stimulation was delivered intracerebrally via bipolar electrodes implanted in the mesencephalic reticular formation (MRF), a pain pathway (Hubner and Kornetsky, 1992; Izenwasser and Kornetsky, 1988, 1990; Marcus and Kornetsky, 1974; Sasson and Kornetsky, 1983, 1986; Sasson et al., 1986; Unterwald et al., 1987). As in the tail-flick threshold method, the brain-stimulation method employed a similar modification of the classical psychophysical method of limits.

2.5.1. Surgical procedure

Rats were anesthetized with ketamine (100 mg/kg) and xylazine (6 mg/kg) (Sigma-Aldrich Co., St. Louis, MO) administered intraperitoneally (ip). Bipolar stainless steel electrodes (Plastics One Inc., Roanoke, VA) (0.125 mm in diameter), insulated except at the tips, were implanted bilaterally into the mesencephalic reticular formation using the following stereotaxic coordinates: −7.0 mm posterior to bregma, ±2.5 mm from midline suture, and −7.0 mm ventral to the skull surface. The electrodes were placed through small burr

holes in the skull and attached permanently by a supporting platform constructed of cranioplastic liquid and powder, mixed in a 1:1 ratio (Plastics One Inc., Roanoke, VA), and three stainless steel mounting screws, placed posterior to lambda and immediately anterior to bregma. Animals were given at least one week for postoperative recovery before behavioral training was begun.

2.5.2. Brain stimulation apparatus, training, and testing procedure

Animals were trained and tested in an acrylic chamber (20×20×34 cm). A cylindrical manipulandum (15 cm long and 7.5 cm in diameter) was located within one wall of the test chamber. Four equally spaced cams on one endplate of the manipulandum operated a microswitch so that turning the manipulandum a quarter turn or less resulted in immediate cessation of the electrical stimulation. A constant current stimulator (Med-Associates, Inc., St. Albans, VT) was used to deliver the stimuli which consisted of biphasic symmetrical rectangle pulses occurring at a frequency of 160 Hz with a pulse width of 0.2 milliseconds (ms), and an intervening delay of 0.2 ms between positive and negative pulses.

The initial training procedure consisted of shaping the behavior of the rat to turn the wheel manipulandum which terminated the stimulation. Within 4 or 5 training sessions (approximately 60 min each) animals were making discrete wheel turns to terminate the stimulus. At this point, the threshold procedure was instituted. In order to minimize the number of stimuli delivered to the animal, the initial intensity setting was at a subthreshold level based on the initial training of the respective animal. Starting at the initial stimulus level, the intensity was systematically increased with a step size of 2 microamps (μA) and a test block consisting of three trials at each stimulus intensity. The intensities were increased until the rat terminated the stimulus in a minimum of two out of three trials (scored +) at two consecutive intensities. This started a descending series of intensities with the repeat of the last intensity. When the rat failed to respond to two out of the three trials (scored −) at two consecutive intensities, the direction was again reversed. Threshold for each of the four columns was defined as the midpoint in intensity between the block scored (+) and the block scored (−). The threshold for each session was defined as the mean of the means of the four columns. It took approximately one hour to complete a session and sessions commenced 10 min after saline or morphine administration. In addition to the threshold measure, the experimental program allowed the recording of latencies of response at threshold.

After training was completed, rats were first tested daily after an injection of saline. Once stable thresholds were obtained (i.e., absence of a trend of consistently increasing or decreasing stimulation thresholds), testing with morphine (2.5, 5.0, and 10 mg/kg) began.

The order of dosing was random. Animals were tested daily, Monday to Friday. Saline was administered on Mondays, Wednesdays and Thursdays. On Tuesdays and Fridays, rats received morphine. In most cases, each rat received each morphine dose twice with the average of two treatments used for data analysis.

2.6. Histology

Upon completion of the experiment, animals were euthanized with pentobarbital. Brains were harvested and immersed in 2-methylbutane on dry ice for 5 to 10 min. Brains were removed from the 2-methylbutane and stored at −80 °C until sectioning with a cryostat. Sections (thirty-micron thickness) were placed on glass slides, treated with histological-grade xylenes, stained with cresyl fast violet (Cell Point, Gaithersburg, MD), and examined under a light microscope for verification of electrode placement.

2.7. Statistical analyses for brain stimulation

For each rat, all escape thresholds were converted to a *z*-score based on the mean and standard deviation of the respective mean saline score. This type of standard score could not be carried out for the tail-flick data because the repeated saline testing needed to make a *z*-score transformation of the data would likely damage the tail of the rat. Brain-stimulation escape data were analyzed using SigmaStat® Version 2.03 software. *Z*-scores were analyzed using a two-way repeated measures ANOVA. Post hoc comparisons were completed using the Student–Newman–Keuls test. Dose effects comparing morphine to saline were examined using one-way ANOVA's for each age group. Baseline stimulation thresholds as well as response latency at threshold were compared using Student's *t*-tests. In the evaluation of response latency at threshold, separate *t*-tests were conducted at each dose.

3. Results

The results of these experiments are presented in two sections, 1) a comparison of basal nociceptive thresholds and 2) the effects of morphine on these nociceptive thresholds.

3.1. Comparison of basal thresholds

Fig. 1a illustrates the mean basal intensity setting used in the latency tail-flick method that resulted in a basal response latency of 2.5–3.5 s for both aged and young rats; Fig. 1b illustrates the basal response threshold in calories of thermal radiation stimulation resulting in the tail-flick; and Fig. 1c illustrates the intracerebral basal response threshold in microamps.

3.1.1. Latency tail-flick method

The analysis of individual stimulus intensity settings required to yield response latencies in the range of 2.5 to 3.5 s (3.2±0.25 and 3.3±0.17 s for aged and young rats, respectively) revealed that aged rats responded at significantly higher baseline stimulus intensity settings than young rats ($t=-3.02$, $df=12$, $p<0.05$).

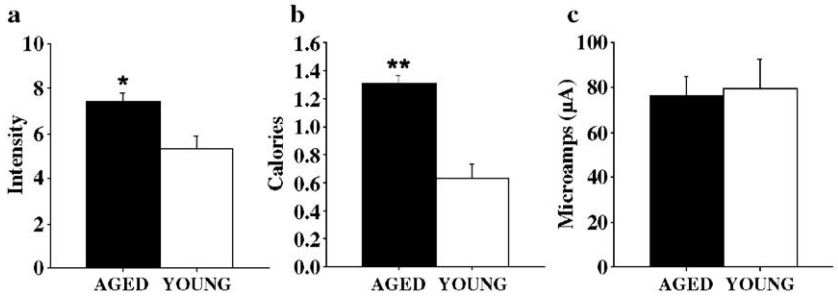

Fig. 1. (a) Stimulus intensity settings (±SEM) at which response latencies were equivalent between aged ($n=7$) and young ($n=7$) animals for the latency tail-flick method. (b) Mean (±SEM) baseline stimulus thresholds for aged ($n=8$) and young ($n=8$) animals for the threshold tail-flick method. (c) Mean (±SEM) baseline thresholds for aged ($n=6$) and young ($n=5$) animals obtained from the brain-stimulation escape procedure. $*p<0.05$ between aged and young animals. $**p<0.001$ between aged and young animals.

3.1.2. Threshold tail-flick method

Baseline stimulus thresholds, expressed in cal, were significantly higher in aged compared to young rats ($t=6.11$, $df=14$, $p<0.001$). Also, at threshold, aged rats exhibited a significantly longer response latency versus the young cohort, 3.0±0.15 and 2.3±0.14 s, respectively ($t=3.72$, $df=14$, $p<0.01$).

3.1.3. Brain-stimulation basal nociceptive threshold

Contrary to the findings using tail-flick, the baseline thresholds for young and aged rats were not significantly different (76.3±8.4 and 79.4±13.1 μA for aged and young rats, respectively).

3.2. Effects of morphine in aged and young rats on the three analgesic measures

3.2.1. Latency tail-flick method

Fig. 2a and b illustrates the difference in latency scores from saline baseline latency for young and aged rats at 15 and 60 min post-morphine treatment, respectively. At the 15 min test period, the ANOVA indicated a significant dose effect [$F(3,30)=26.789$, $p<0.001$] however there were no significant age [$F(1,12)=0.096$, $p=0.762$] or age × dose [$F(3,30)=0.674$, $p=0.575$] effects. Similarly, at the 60 min test period, a significant dose effect was observed [$F(3,30)=44.706$, $p<0.001$] with no significant age [$F(1,12)=0.902$, $p=0.358$] or age × dose [$F(3,30)=1.073$, $p=0.375$] effects. As indicated in Fig. 2, post hoc analyses indicated that at all doses and time periods, with the exceptions of the 2.5 mg/kg dose for both aged and young rats at the 15 min time period, and the 2.5 mg/kg dose for the aged at the 60 min time period, morphine caused significant increases in latency to respond.

3.2.2. Threshold tail-flick method

Fig. 3a and b presents the difference scores from baseline threshold for young and aged rats at 15 and 60 min post-morphine treatment. The ANOVA indicated a significant dose effect at both the 15 min [$F(3,34)=12.526$, $p<0.001$] and 60 min [$F(3,34)=40.495$, $p<0.001$] time periods. Although there was not a significant age × dose effect at either the 15 min [$F(3,34)=0.211$, $p=0.888$] or 60 min [$F(3,34)=2.198$, $p=0.106$] time periods, at 60 min, there was a significant age effect [$F(1,14)=11.81$, $p=0.003$] that was not seen at the 15 min time period [$F(1,14)=1.273$, $p=0.275$]. As indicated in Fig. 3a, at the 15 min time period, morphine significantly raised the threshold only at the 10.0 mg/kg dose for both aged and young rats; however at the 60 min time period the threshold was significantly raised at all doses in the young rats but only at the 10.0 mg/kg dose in the aged rats and there was a significant

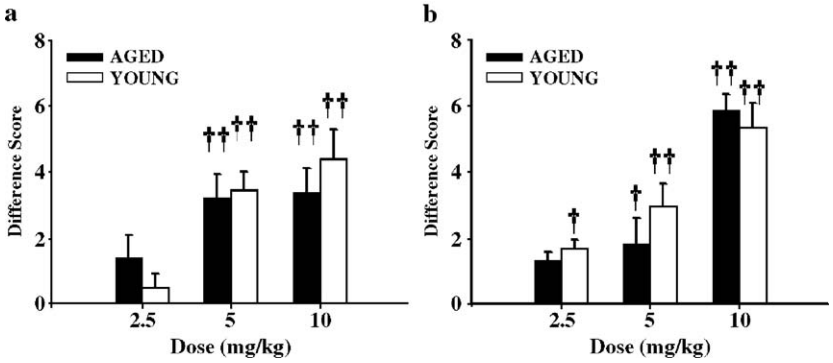

Fig. 2. (a) Mean (±SEM) difference scores (s) from saline baseline latency for aged and young animals for the latency tail-flick method at 15 min post-morphine treatment. (b) Mean (±SEM) difference scores (s) from saline baseline latency for aged and young animals at 60 min post-morphine treatment. Note: $n=7$ for group size except $n=4$ at the 2.5 mg/kg dose. $^{\dagger}p<0.05$ for within-group comparison between saline and treatment. $^{\dagger\dagger}p<0.001$ for within-group comparison between saline and treatment.

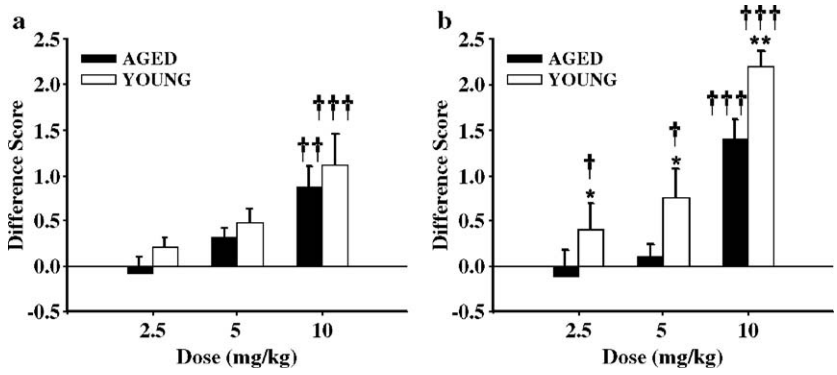

Fig. 3. (a) Mean (±SEM) difference scores (cal) from saline baseline threshold for aged ($n=8$) and young ($n=8$) animals for the threshold tail-flick method at 15 min post-morphine treatment. (b) Mean (±SEM) difference scores (cal) from saline baseline threshold for aged and young animals at 60 min post-morphine treatment. Note: $n=8$ for group size except $n=4$ at the 2.5 mg/kg dose. $*p<0.05$ between aged and young animals. $**p<0.01$ between aged and young animals. $^{\dagger}p<0.05$ for within-group comparison between saline and treatment. $^{\dagger\dagger}p<0.01$ for within-group comparison between saline and treatment. $^{\dagger\dagger\dagger}p<0.001$ for within-group comparison between saline and treatment.

difference between the young and aged at all three dose treatments.

3.2.3. Brain-stimulation nociceptive threshold

Fig. 4 illustrates effects of morphine expressed as a *z*-score threshold transformation of both aged and young rats. The ANOVA yielded a significant dose effect [$F(3,25)=20.688$, $p<0.001$] but no significant age [$F(1,9)=1.569$, $p>0.2$] or age × dose effects [$F(3,25)=0.949$, $p>0.4$]. As shown, post hoc analysis indicated significant threshold raising effects for all doses in the young and for only the two higher doses in the aged. At threshold there was no significant drug or age effect for latency of response to terminate stimulation (Fig. 5).

3.2.4. Histology

Using the atlas of Paxinos and Watson (1986), electrode placements were verified. Histology was not available for two animals, one due to illness and the second due to destruction during slide preparation. Examination of brain sections indicated that the tips of stimulating electrodes were located in the mesencephalic reticular formation (MRF) in the remaining animals (Fig. 6). There were no differences in placements between the aged and young.

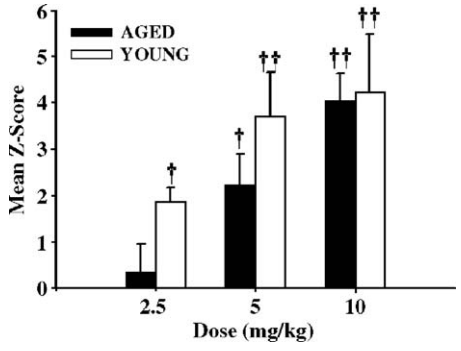

Fig. 4. Mean (±SEM) *z*-scores for thresholds obtained for aged and young animals following the administration of morphine for the brain-stimulation escape procedure. $^{\dagger}p<0.05$ for within-group comparison between saline and treatment. $^{\dagger\dagger}p<0.001$ for within-group comparison between saline and treatment.

4. Discussion

4.1. Nociception

The results of these experiments clearly indicate that the nociceptive threshold of aged rats is significantly higher than that of young rats using the tail-flick threshold method. However, when the periphery is bypassed by directly stimulating an intracerebral pain pathway (MRF), there is no difference in the nociceptive threshold between young and aged rats. Akunne and Soliman (1994) also found, using the tail-flick technique, that at a fixed intensity of stimulation, aged rats had longer latencies of response than did younger rats. However, contrary results were reported by Jourdan et al. (2000, 2002). They found that basal threshold to mechanical stimulation (paw pressure) was lower in the aged than in the young rats. Also, these investigators, using a tail immersion to a fixed thermal intensity paradigm, found no difference in latency of the tail-withdrawal response obtained using the tail-flick to radiant thermal stimulation.

The apparent anomaly of a difference between young and aged in nociceptive threshold to radiant thermal stimulation and

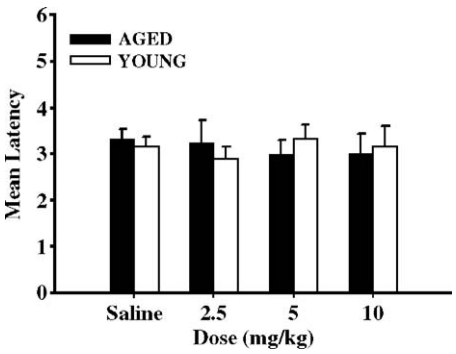

Fig. 5. Mean (±SEM) latencies to escape MRF stimulation for aged and young rats following either saline or morphine administration.

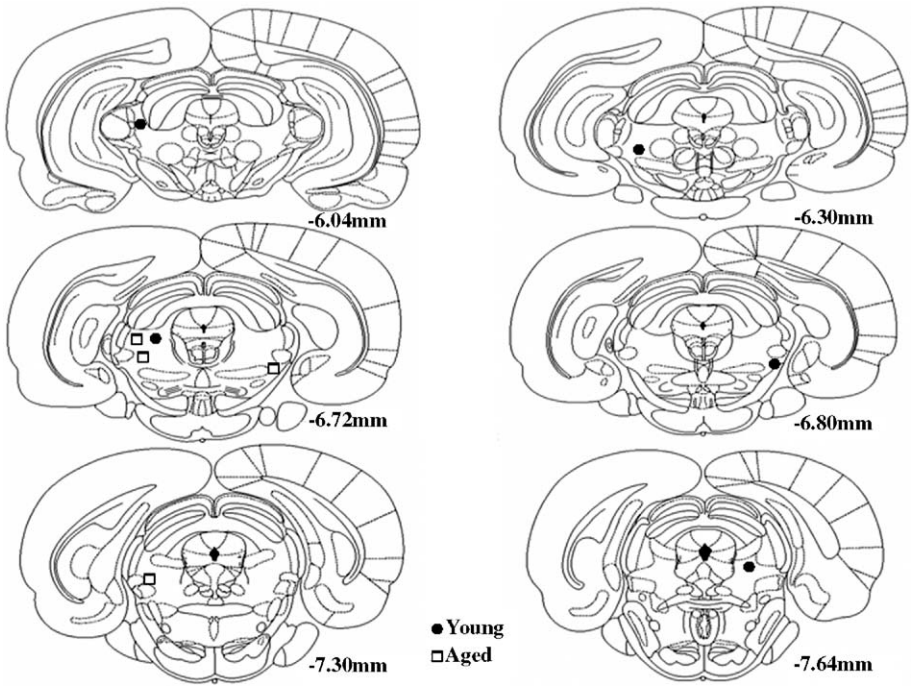

Fig. 6. Histological findings for the electrode tip placements. Diagrams were prepared using Paxinos and Watson (1997) as a guide for their preparation.

the absence of a difference to intracerebral stimulation as well as the longer latencies for the aged animals at threshold in the thermal radiation experiment, suggests the possibility that the relatively higher pain threshold in the aged rat, when peripherally stimulated, may be a function of some dysfunction in the response system. The tail-flick response is primarily a spinal reflex (Borszcz et al., 1990; Douglass and Carstens, 1997; Irwin et al., 1951; Kauppila et al., 1998; King et al., 1997; Ness and Gebhart, 1986) and there is evidence of age-related neurochemical changes in the spinal cord of the rat (Crisp et al., 1994; Goicoechea et al., 1997; Iwata et al., 2002; Ko et al., 1997) that could account for the difference in baseline threshold between the young and aged rats. Specifically, these previous experiments have demonstrated declines in serotonin (Goicoechea et al., 1997; Ko et al., 1997) and norepinephrine at the spinal level (Ko et al., 1997) with increases in age. In both of these experiments, aged animals were 25 to 26 months old, only slightly older than animals employed in the present investigation. Thus, the data suggest that the elevated threshold and longer latencies to respond of the aged rats compared to the young rats using the tail-flick may not be a function of a difference in the registration of the nociceptive stimuli but due to a compromised spinal reflex system and/or anatomical differences at the level of the tail. Gagliese and Melzack (2000), in a comprehensive review of age differences in nociception in the rat, reported that "…generally, with increasing age, the intensity or duration of stimulation required to elicit the response of interest increases…." They further suggest that there is considerable variability when techniques of fixed intensity are used, (e.g., tail-flick, jump-flinch test) and within these tests there may be differences between high intensity and low intensity. Goettl et al. (2000) demonstrated this latter point regarding stimulus intensity using the tail-flick paradigm in which aged animals exhibited a longer latency versus young animals at a "high intensity" setting but found no age differences when a "low intensity" setting was employed.

Although the present data suggest that the relative lack of sensitivity to the thermal stimulation by the aged rats might be due to differences in peripheral versus central stimulation; alternative hypotheses need to be considered. The observed age difference may simply be a function of a difference between thermal and electrical nociceptive stimulation and/or that the brain-stimulation technique requires the rat to make a conditioned instrumental response, while the tail-flick procedure, as well as most nociception procedures, employs an unconditioned response, often a simple reflexive withdrawal response.

Experiments that have used nociceptive stimulation have generally, but not consistently, observed age-related differences in basal response to the stimulus, with the aged subjects, both animal and human, having higher thresholds (Nicák, 1971; Hess et al., 1981; Buchsbaum et al., 1981; Zhang et al., 2002; Helme et al., 2004). However, Goicoechea et al. (1997), using a tail shock test, and Smith and Gray (2001), using a warm water tail-withdrawal paradigm, found no baseline difference between aged and young rats.

4.2. Analgesia

In the tail-flick procedure, using latency of response as the dependent variable there was no significant difference between young and aged rats in the analgesic effects of morphine at any dose studied. However, when the tail-flick threshold procedure was used, the aged rats were significantly less sensitive to the effects of morphine at the 60 min time period than the young rats. Confounding the interpretation of these finding is the

marked difference in basal threshold level of stimulation in both procedures. In the latency method the intensity was significantly increased for the aged rats so as to have baseline latencies identical between aged and young rats. Using the intracerebral electrical stimulation procedure, the baseline intensities were identical and although there was a trend for the aged animals to be less responsive to morphine than the young animals, there was no significant difference at any dose. However, the results do indicate significant morphine effects for the young animals at the lowest dose tested with no such significant morphine effects in the aged rats. Further, at the intermediate dose (5.0 mg/kg), where both young and aged rats had significant threshold raising effects, there is a trend demonstrating diminished morphine responsiveness in the aged cohort (Fig. 4).

The results of the difference in the effects of morphine between young and aged rats on the nociceptive thresholds of both the tail-flick threshold method and the intracerebral stimulation method suggest that aged rats are probably less sensitive to the analgesic effects of morphine than young rats. This decreased effect of morphine in the aged cohort is consistent with results from previous experiments in rats (Jourdan et al., 2000, 2002; Kramer and Bodnar, 1986; McLaughlin and Dewey, 1994) and mice (Kavaliers et al., 1983). Pharmacokinetic differences in metabolism of morphine between aged and young rats may account for age-related differences in sensitivity to morphine analgesia, however, previous studies indicate that changes in morphine pharmacokinetics may not occur with advancing age in rodents (Jourdan et al., 2002; Van Crugten et al., 1997a).

The present finding that morphine has less of an analgesic effect in the aged compared to the young rat is also contrary to clinical evidence that older individuals require less morphine versus younger individuals (Macintyre and Jarvis, 1995; Woodhouse and Mather, 1997). There is awareness by many clinicians, however, that pain management in the elderly is a major clinical problem (Cavalieri, 2002; Ferrell and Ferrell, 1991). Differences between the finding presented here and clinical findings may be related to differences in methodology and to differences in metabolism of morphine between the rat and humans. Most clinical studies have examined the effects of prolonged administration of opioids to patients with pain produced by different pathological conditions such as surgical and cancer pain while the present study has focused the acute effects of morphine on responses to discrete painful stimuli. Morphine is extensively metabolized to the 6-glucuronide metabolite in the human (Yeh et al., 1977) which has analgesic actions of its own (Hanna et al., 2005; Murthy et al., 2002; Skarke et al., 2003). In the rat, morphine is metabolized into the 3-glucuronide metabolite (Salem and Hope, 1997; Van Crugten et al., 1997b) and has demonstrated an antagonistic effect on morphine analgesia (Smith et al., 1990). Peterson et al. (1990) reported an increase in the 6-glucuronide metabolite with diminished creatinine clearance, following chronic morphine treatment. The mean age for this study was 68.5 years. These differences in metabolism may greatly influence the apparent potency of morphine in elderly whose renal function may be diminished when this drug is administered chronically and the morphine-6-glucuronide metabolite accumulates.

To the extent that the rat can model the human condition, the finding that the baseline threshold for nociceptive stimulation is not different in the aged versus the young rat when the periphery is bypassed, suggests that the belief that the aged patient feels less pain and requires less analgesic medication than the young patient needs to be reexamined.

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