THE OPPONENT CONSEQUENCES OF INTERMITTENT AND CONTINUOUS STIMULATION WITHIN THE RAT SPINAL CORD

A Thesis

by

DENISE ALEJANDRA PUGA

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2007

Major Subject: Psychology

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Approved by:

Chair of Committee, James Grau
Committee Members, Mary Meagher
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ABSTRACT

The Opponent Consequences of Intermittent and Continuous Stimulation within the Rat Spinal Cord. (August 2007)

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A substantial body of work exists to suggest that brain and spinal mechanisms react differently to nociceptive information. The current experiments were design to identify parallels and differences in the way the spinal cord processes nociceptive information, as compared to intact animals. In addition, pharmacological manipulations were employed to identify the opioid receptors activated by continuous shock, and to decipher at what synaptic level (e.g. pre or post synaptically) intermittent shock affects the release of endogenous opioids. A common dependent variable was used in all experiments to assess changes in nociceptive reactivity, the tail-flick test.

The results revealed that intermittent and continuous stimulation have an opponent relationship on nociceptive processing in the isolated spinal cord. Continuous stimulation (3, 25-s continuous 1.5 mA tail-shocks) induced an antinociceptive response that was attenuated by prior exposure to brief (80 ms) intermittent shock (Experiment 1). When intermittent shock was given after continuous shock, intermittent shock failed to attenuate continuous shock-induced antinociception (Experiment 2). The impact of intermittent shock on continuous-shock induced antinociception decayed after 24 hours (Experiment 3). Intermittent and continuous shock enhanced the antinociceptive

consequences of a moderate dose of systemic morphine (5 mg/kg) (Experiment 4). Continuous shock-induced antinociception was attenuated by equal molar concentrations of CTOP (μ opioid antagonist) and Nor-BNI (κ opioid antagonist), but not naltrindole (δ opioid antagonist) (Experiment 5). Intermittent shock failed to attenuate the antinociception induced by DAMGO (μ opioid agonist) or Dynorphin A (κ opioid agonist).

DEDICATION

I would like to dedicate this thesis, first and foremost, to my parents, Mr. and Mrs. Roberto and Alba Puga, for their infinite supply of love and guidance. I would also like to dedicate this thesis to my sisters, Christina and Pamela Puga, for allowing me the privilege and honor of being their “big sister”; and to my best friend, Eunice Saldaña, who has always been an outstanding accomplice and confidant.

ACKNOWLEDGEMENTS

I would like to thank Dr. James W. Grau for his support, guidance, and mentorship. Without his vision and empirical knowledge, this project would not have been possible. I would also like to thanks Drs. Mary W. Meagher and Rajesh Miranda for serving as committee members. Finally, I would like to thank Dr. Michelle A. Hook, Dr. Kyle M. Baumbauer, Dr. Stephanie N. Washburn, Russell Huie, and Kevin Hoy for providing an unbelievable working environment, where science is a joy to discover.

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INTRODUCTION

Exposure to aversive stimuli, such as electrical shock, induces an antinociceptive response in intact rats. This finding suggests that pain is not an inevitable consequence of noxious stimulation; instead, nociceptive information is filtered by mechanisms in the central nervous system (CNS) that can attenuate the perception of pain (Grau, 1987). Evidence indicates that the affective level of pain is inhibited by endogenous analgesic systems (Basbaum et al., 1977; Mayer et al., 1971); where, nociceptive information is blocked by supraspinal structures at the level of the spinal cord through both opioid and nonopioid analgesic systems (Akil et al., 1976; Chance, 1980; Lewis, Cannon, & Liebeskind, 1980; Lewis, Sherman, & Liebeskind, 1982; Watkins & Mayer, 1982b). For example, under some conditions opioid antagonists (e.g. naloxone or naltrexone) and morphine tolerance undermine stressor-induced antinociception (Akil et al., 1976; Grau, Hyson, Maier, Madden, & Barchas, 1981; Lewis, Cannon, & Liebeskind, 1980; Maier et al., 1980). While under different conditions the antinociceptive response appears to be nonopioid in nature and is not affected by either of these manipulations (Grau et al., 1981; Lewis, Cannon, & Liebeskind, 1980; Watkins & Mayer, 1982b).

In addition, the controllability an animal exerts over the onset and termination of aversive stimuli is crucial to the behavioral and physiological changes that result from exposure to a stressor. Rats treated with 80 intermittent inescapable shocks show a behavioral deficit as assessed by shuttlebox escape acquisition (Maier et al., 1983);

This thesis follows the style and format of *Behavioral Neuroscience*.

whereas, an equal amount of controllable shock does not yield the same results. Further, exposure to intermittent stimulation induces a short-term analgesic reaction that can be re-aroused 24 hours later with a brief shock (Maier et al., 1979). This short and long-term analgesic effect of intermittent shock is naltrexone reversible (Maier et al., 1980), and reflects an up-regulation of opioid sensitive systems (Grau et al., 1981). These findings suggest that initial intermittent shock exposure sensitizes the opioid analgesic system, and enhances the antinociceptive impact of moderate shock and systemic morphine (Grau et al., 1981, Maier et al., 1980). Researchers have found that the opioid induced antinociception that results from 80 inescapable intermittent shocks is modulated by the pituitary-adrenal axis (MacLennan et al., 1982). This indicates that it is crucial for the animal to identify the stressor as uncontrollable to induce a hormonal-mediated opioid analgesia (MacLennan et al., 1982; Watkins & Mayer, 1982b). Subsequent research has linked these behavioral changes to brain-mediated mechanisms. Exposure to uncontrollable aversive stimulation produces a strong activation of serotonin (5-HT) cells in the caudal dorsal raphe nucleus (DRN) during inescapable shock and at later behavioral testing (Grahn et al, 1999; Maier et al., 1995), and depletion of serotonin has been shown to attenuate stimulation-produced analgesia (Akil & Mayer, 1972).

In contrast to intermittent shock, exposure to 3 min of continuous stimulation does not lead to poor escape learning in a different test where escape is possible (Maier et al., 1983). Research by Terman and colleagues (1984) showed that continuous shock applied at brief durations or at low intensities induces an opioid antinociception that does

not depend on the HPA-axis, while longer duration or higher intensity shocks cause a naltrexone-insensitive antinociception. Interestingly, Meagher et al. (1993) found that nociceptive signals can directly impact intraspinal circuits, and induce an antinociception that is not brain mediated. Long (25-s) intense (1.5mA) shocks applied to the tail induce a robust antinociception in spinally transected rats. Also, just as increasing shock intensity in intact rats engages a nonopioid analgesia, stronger shocks (3.0 mA) produce a naltrexone-insensitive antinociception in spinalized rats. These findings suggest that nociceptive input can activate both opioid and nonopioid analgesic systems in the isolated spinal cord.

More surprisingly, recent work has found that the spinal cord can support a simple instrumental response (Buerger & Fennessy, 1970; Chopin & Buerger, 1976; Grau, Barstow & Joynes, 1998). Exposure to 6 minutes of brief (80 ms) intermittent shock attenuates spinal instrumental learning (Grau, Barstow, & Joynes, 1998). An equal amount of continuous shock (14.4 or 360 s) does not produce this deficit. In fact, co-administration of continuous shock has a protective effect that opposes the induction of the learning deficit (Crown et al., 2002). The effect of intermittent shock on spinal learning lasts up to 48 hours and is blocked by the opioid antagonist naltrexone (Crown et al., 2002; Joynes & Grau, 2004). In addition, prior exposure to controllable shock prevents the learning deficit, and instrumental training combined with naltrexone reverses the expression of the behavioral deficit (Crown & Grau, 2001). What is not know is whether intermittent shock has a similar effect on nociceptive processing in the isolated spinal cord, as compared to intact animals. Evidence suggests that it does not;

instead, intermittent shock enhances mechanical reactivity in spinally transected rats (Ferguson, Crown, & Grau, 2006).

Brain and spinal mechanisms appear to respond in very different ways to nociceptive information. The current experiments explored this issue by evaluating the impact of intermittent shock on nociceptive processing in the isolated spinal cord. Withdrawal from noxious thermal stimulation (the tail-flick test) was used as a common dependent variable in all experiments. Experiments 1-3 assessed the impact of intermittent shock on long (25-s) intense (1.5 mA) continuous shock-induced antinociception. Experiment 4 measured the antinociceptive consequence of intermittent and continuous shock on systemic morphine in spinalized rats. Experiment 5 identified the opioid receptors activated by continuous shock, and Experiment 6 examined the effect of intermittent shock on a pharmacologically induced antinociception.

GENERAL METHOD

Subjects

Male, Sprague-Dawley rats obtained from Harlan (Houston, TX) were utilized as subjects for these experiments. Animals were approximately 100-120 days old and weighed between 310 and 410 grams. Subjects were individually housed with water and food available *ad libitum*, and maintained on a 12 hour light-dark schedule. Behavioral testing was conducted during the light portion of the cycle.

Surgery

Surgeries consisted of a complete transection of the spinal cord at the second thoracic vertebra (T2). Animals were anesthetized with pentobarbital (50mg/kg, i.p.), and the area surrounding the shoulders was shaved and sterilized with iodine. An anterior-posterior incision approximately 1.5 cm in length was made over the second thoracic vertebra, and the tissue immediately anterior to T2 cleared to expose the spinal cord. The exposed cord was transected with cauterization, and the ensuing space was filled with Gelfoam (Harvard Apparatus, Holliston, MA). Thereafter, a cannula (25 cm of polyethylene tubing) fitted with a stainless steel wire (0.09 mm diameter) was inserted into the subarachanoid space on the dorsal surface of the cord. The cannula was inserted 9 cm down the ventral column, and the exposed end of the tubing was secured with the use of an adhesive to the skin. The incision was closed with Michel Clips (Fine Science Tools, Foster City, CA), and immediately thereafter, the animals received an injection of 0.9% saline (2.5 ml, i.p.) to maintain hydration.

During recovery, animals were maintained in a temperature-controlled environment (25.5 °C) with food and water available at *ad libitum*. Bladders were expressed at least twice a day, and immediately before performing any behavioral procedures. To confirm full transection of the cord: a) a visual inspection was performed during surgery, b) animals were monitored to ensure complete paralysis below the forelimbs and a lack of vocalization during shock exposure, and c) cords were examined in a randomly selected subset of post-mortem subjects.

Apparatus

During tailshock delivery, rats were loosely restrained in opaque black Plexiglas tubes (22 cm [length] and 6.8 cm [diameter]). A 660-V transformer was used to generate tailshock. AC shock was administered through electrodes constructed from a modified fused clip covered in electrode paste, and taped to the rat's tail approximately 7.5 cm from the tip. A computer was used to control the onset and offset of tailshock. AC shock was delivered 80 ms in duration and occurred at a variable time schedule with a mean of 2 s (range 0.2-3.8 s) for intermittent shock delivery, or three long (25-s) continuous 1.5 mA tailshocks.

Nociceptive reactivity to radiant heat was accessed with an automated tail-flick device. Heat was provided by a 375-W movie light that was focused onto the rat's tail by means of a condenser lens positioned 8 cm below the light source. The light source was illuminated approximately 2 cm of the rat's tail. Light intensity was controlled by an AC potentiometer (#6681-W, Leviton, Little Neck, NY), and the rat's tail was rested on a 0.5cm deep groove embedded on an aluminum block positioned 4.7 cm below the

condenser lens. When the subjects failed to respond, the test trial was terminated after 8 s of heat exposure to avoid tissue damage.

Statistics

All data were analyzed using an analysis of variance (ANOVA), with an *a priori* alpha value of .05. Group differences were further evaluated using Duncan's New Multiple Range *post hoc* tests.

EXPERIMENT 1

In intact animals, intermittent shock up-regulates an opioid sensitive system. For example, exposure to 80 intermittent shocks enhances the opioid antinociception produced by moderate shock (Maier et al., 1979), and increases the analgesic effect of systemic morphine (Grau et al., 1981). However, current research has shown that intermittent shock has a divergent effect on nociceptive processing in the isolated spinal cord. For example, Crown et al. (2002) found that intermittent shock does not induce antinociception in spinally transected rats. Instead, intermittent shock enhances mechanical reactivity in spinalized rats (Ferguson, Crown, & Grau, 2006). The current experiment assesses the impact of intermittent shock on continuous shock-induced antinociception in the spinal cord. The continuous shock schedule utilized in this experiment, and subsequent others, have been shown by Meagher and colleagues (1993) to induce a naltrexone-reversible antinociception.

Procedure

Twenty-four hours after spinal transection, subjects were placed in restraining tubes and given three baseline tail-flick tests, each separated by a 2-minute interval. Immediately after baseline testing, subjects were counterbalanced across groups (n = 8 in each group), and shock electrodes were attached to the rats' tail with porous tape. Subjects first received either 6 minutes of intermittent brief (80 m sec) shock or nothing, and immediately thereafter, half the subjects in each of the groups received long (25 s) continuous shock or remained unshocked. Finally, tail electrodes were removed and tail-flick latencies were assessed 5 times at 2-minute intervals.

Results

Mean tail flick latencies are depicted in Figure 1. Baseline tail-flick latencies are presented on the left side of each panel. An analysis of variance (ANOVA) verified that baseline tail-flick latencies did not differ across groups, $F(3, 28) < 1.0$, $p > .05$.

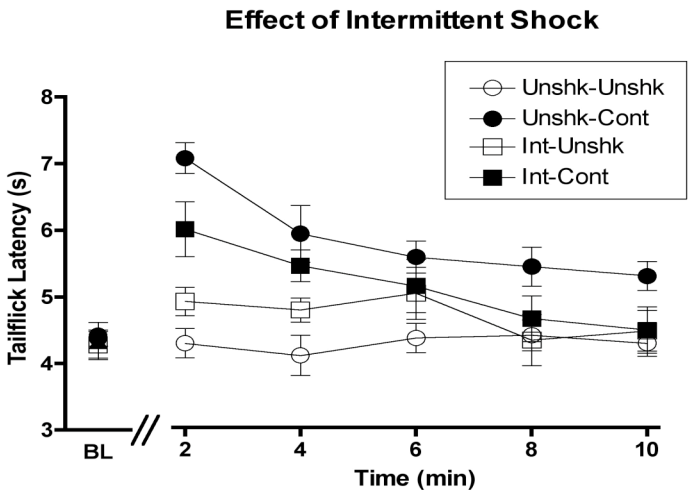

Figure 1. Tailflick latencies significantly increased from baseline after 3, 25-s continuous tailshocks. Intermittent shock, or an equal period of restraint, did not significantly increase tail-flick latencies across the 10-minute test trial. Pre-exposure to intermittent shock decreased continuous shock-induced increases in tail-flick latencies.

The mean tail-flick latencies observed after shock, or after an equivalent period of restraint, are presented to the right of the baseline scores. As in previous studies, exposure to intermittent shock alone, or an equivalent period of restraint, had little effect on tail-flick latencies. In contrast, exposure to continuous shock induced an antinociception that was attenuated by prior exposure to intermittent shock. An analysis

of covariance (ANCOVA), controlling for baseline scores, revealed a significant main effects of shock, $F(3,27) = 13.20$, $p < .001$ and trials, $F(4, 108) = 5.038$, $p < .001$. A significant Trials X Shock interaction was also found, $F(12, 108) = 2.66$, $p < .01$.

Post hoc comparisons of the group means showed that subjects that received just continuous shock exhibited significantly longer tail-flick latencies from groups that received 6 minutes of intermittent shock or an equivalent period of restraint, and subjects that were administered intermittent shock prior to continuous shock, $p < .05$. In addition, subjects exposed to intermittent shock prior to continuous shock displayed increased tail-flick latencies relative to unshocked control subjects, $p < .05$. No other differences were significant, $p > .05$.

Discussion

In summary, long (25-s) intense (1.5 mA) continuous shocks induced an increase in tail-flick latencies; while, 6 minutes of brief (80 ms) intermittent shocks, or an equal period of restraint, failed to induce a change in nociceptive reactivity. Of interest, prior exposure to intermittent shock attenuated the antinociceptive consequences of continuous shock.

EXPERIMENT 2

The results of Experiment 1 revealed that intermittent shock blocks the antinociceptive consequence of continuous shock in the spinal cord. The current experiment assessed if intermittent shock, given after continuous shock, also attenuated the antinociceptive effect of continuous shock.

Procedure

Twenty-four hours after spinal transection, subjects were placed in restraining tubes and baseline scores were collected as previously described. Next, all subjects were exposed to continuous shock, and then, half the subjects received either 6 minutes of intermittent shock (n =8) or an equivalent period of restraint (n = 8). Lastly, test tail-flicks were collected.

Results

Mean tail flick latencies are depicted in Figure 2. Baseline tail-flick latencies are presented on the left side of each panel. An ANOVA verified that baselines did not differ prior to shock treatment, $F(1, 14) = 1.45$, $p > .05$.

The mean tail-flick latencies are displayed to the right of the baseline scores. Continuous shock induced antinociception that was not attenuated by subsequent exposure to intermittent shock. An ANCOVA, controlling for baseline scores, failed to reveal any significant effects, all Fs < 1.0, $p > .05$.

Discussion

Experiment 2 revealed that intermittent shock given after continuous shock failed to attenuate continuous shock-induced antinociception. The current results, and those of

Experiment 1, suggest that the interaction between intermittent and continuous shock depend on the temporal order of stimulation. The impact of intermittent shock after continuous shock stands in contrast to Grau et al. (1990). There we showed that a weak shock distractor caused the antinociception to decay more rapidly. Different results may have been obtained because the distractor used in the earlier paper was similar to the inducing shock (neither was intermittent). Work in the memory literature suggests that the magnitude of a distractor effect depends on stimuli similarity – the more similar the items are, the bigger the effect. Continuous and intermittent shock seem to engage independent systems, and hence, are dissimilar (providing a potential explanation as to why a distractor effect was not observed).

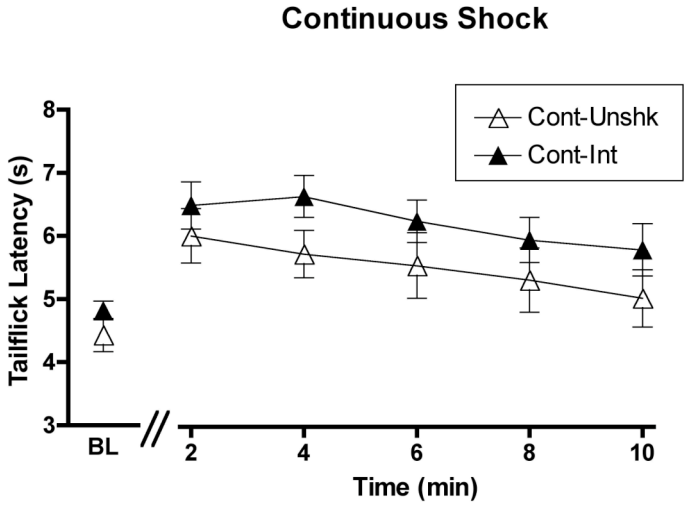

Figure 2. Intermittent shock failed to attenuate increases in test tail-flick latencies when intermittent shock was given after continuous shock.

EXPERIMENT 3

In intact rats, intermittent shock induces a pain modulatory effect that last 24 hours (Maier et al., 1979). In the isolated spinal cord, intermittent shock induces a learning deficit that lasts up to 48 hours (Crown et al, 2002). The results of Experiment 1 revealed that intermittent shock attenuates the antinociceptive consequences of continuous shock. The current experiment assessed if the impact of intermittent shock on continuous shock–induced antinociception lasts 24 hours.

Procedure

Experiment 3 utilized the same design as Experiment 1; however, a 24-hour interval was introduced between the offset of intermittent shock and the onset of continuous shock. Briefly, 24 transected rats were restrained in Plexiglas tubes and given three baseline tail-flick tests, each two minutes apart. After electrode placement, rats were exposed to either 6 minutes of brief intermittent shock (n = 12) or remained unshocked (n =12). Next, all rats were returned to their home cage. Twenty-four hours later, rats were returned to the testing room, where half the rats in each condition (Intermittent and Unshocked) received continuous shock. Finally, electrodes were removed and all rats received 5 tail-flick tests at 2-minute intervals.

Results

The results are depicted in Figure 3. The mean baseline tail-flick latencies are shown on the left side of the graph. An ANOVA indicated that no differences existed among groups prior to shock, all Fs < 1.28, $p > .05$.

The tail-flick latencies observed after shock are presented to the right of the baseline data. As expected, subjects that were exposed to just 6 minutes of intermittent shock, and unshocked controls, did not show a significant change in tail-flick latencies. When a 24-hour interval was introduced between intermittent and continuous stimulation, intermittent shock failed to attenuate continuous shock-induced antinociception. An ANCOVA, controlling for baseline scores, revealed a significant main effect of continuous shock treatment, $F(1, 27) = 39.163$, $p < .001$, and a significant Trials X Continuous shock interaction, $F(4, 108) = 5.81$, $p < .001$. No other term approached significance, all Fs < 1.0, $p > .05$. *Post hoc* comparisons of the group means

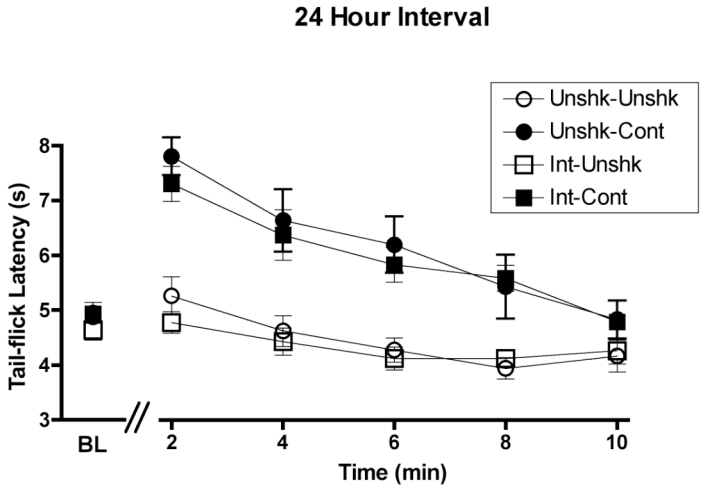

Figure 3. Exposure to six minutes of intermittent shock, or an equivalent period of restraint, did not induce an increase in tail-flick latencies. Three, 25-s continuous tailshocks induced a robust antinociception. Introducing a 24-hour interval between intermittent and continuous shock blocked the effect of intermittent shock on continuous shock-induced antinociception.

showed that the groups that received continuous shock on Day 2 had significantly higher tail-flick latencies than subjects that received only six minutes of intermittent shock and unshocked controls, $p < .05$.

The tail-flick latencies observed after shock are presented to the right of the baseline data. As expected, subjects that were exposed to just 6 minutes of intermittent shock, and unshocked controls, did not show a significant change in tail-flick latencies. When a 24-hour interval was introduced between intermittent and continuous stimulation, intermittent shock failed to attenuate continuous shock-induced antinociception. An ANCOVA, controlling for baseline scores, revealed a significant main effect of continuous shock treatment, $F(1, 27) = 39.163$, $p < .001$, and a significant Trials X Continuous shock interaction, $F(4, 108) = 5.81$, $p < .001$. No other term approached significance, all Fs < 1.0, $p > .05$. *Post hoc* comparisons of the group means showed that the groups that received continuous shock on Day 2 had significantly higher tail-flick latencies than subjects that received only six minutes of intermittent shock and unshocked controls, $p < .05$.

Discussion

The results indicated that the effect of intermittent shock on continuous shock-induced antinociception decayed after a 24-hour interval. This finding contrasts the long-term analgesic effect of intermittent shock in intact animals (Maier et al., 1979).

EXPERIMENT 4

Intermittent shock in intact rats affects nociceptive reactivity by up-regulating a morphine-sensitive system, and thus, enhances the antinociceptive impact of systemic morphine (Grau et al., 1981). The current experiment assessed if intermittent and/or continuous shock enhanced the antinociceptive consequence of systemic morphine in spinally transected rats.

Procedure

Twenty-four hours after surgery, rats (N= 48) were randomly given an intraperitoneal injection of either saline or morphine (5 mg/kg). After 30 minutes, baseline scores were collected as previously described. Immediately thereafter, rats received one of three shock treatments: intermittent, continuous, or remained unshocked. Thus, Experiment 4 consisted of a 2 (morphine or saline) X 3 (intermittent, continuous or unshocked) factorial design. After shock exposure, tail-flick latencies were assessed again 5 times at 2-minute intervals for all subjects.

Results

The results are presented in Figures 4. The mean baseline tail-flick latencies are shown on the left side of the graph. An ANOVA indicated that no differences existed among groups prior to shock, all Fs < 1.0, $p > .05$.

The mean test tail-flick latencies are presented to the right of the baseline scores. Saline and morphine unshocked groups, and the saline intermittent group, did not show significant increases in tail-flick latencies. Saline and morphine rats showed a robust increase in tail-flick latencies after continuous shock. Morphine rats showed a significant

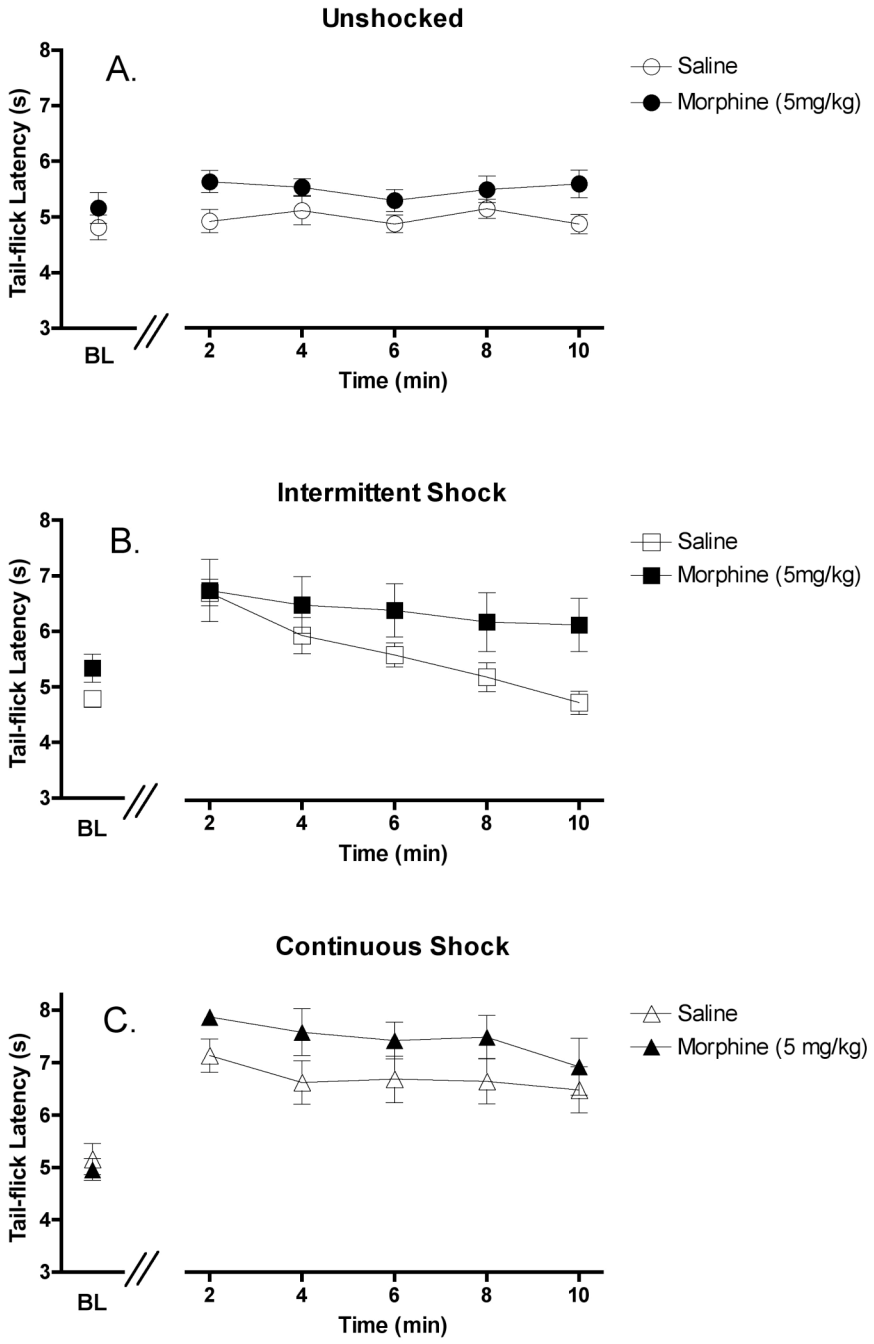

Figure 4. Morphine (5mg/kg) and saline unshocked rats did not show an increase in tail-flick latencies (A). Morphine rats showed a moderate increase in tail-flick latencies after intermittent shock, as compared to saline-intermittent rats (B). Saline rats showed antinociception after continuous shock. Morphine rats exposed to continuous shock showed a robust antinociception (C).

increase in tail-flick latencies after intermittent shock. An ANCOVA, controlling for baseline scores, revealed that there was a significant main effect of drug, $F(1,41) = 9.31$, $p < .005$ and shock $F(2, 41) = 27.47$, $p < .001$. The Trials X Shock interaction was also significant, $F(8, 164) = 3.66$, $p < .001$. The main effect of trials was not significant, $F(4, 164) < 1.0$, $p > .05$, nor was the Trials X Drug interaction significant, $F(4, 164) < 1.0$, $p > .05$.

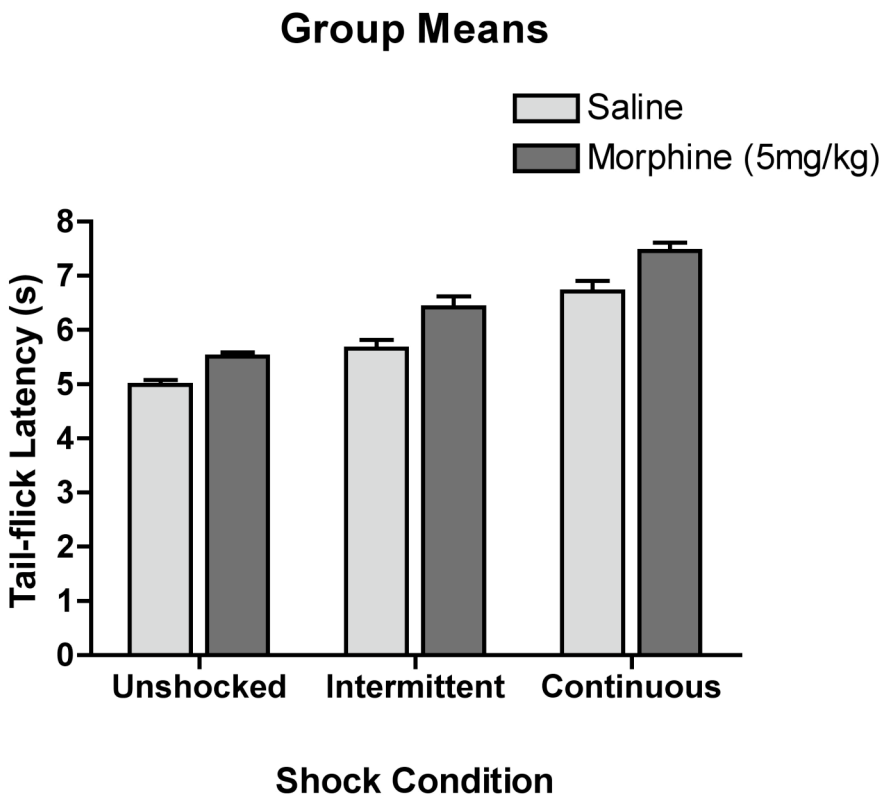

Figure 5. Mean tail-flick latencies for all groups.

Post hoc comparisons of the group means (group means are presented in Figure 4B) revealed that the saline-continuous shocked group had significantly higher tail-flick latencies than both the morphine and saline unshocked groups, and the saline-intermittent shocked group. The morphine-intermittent shocked group had significantly higher tail-flick latencies than the saline-intermittent shocked group, and the morphine and saline unshocked groups. Lastly, the morphine-continuous shocked group had significantly higher tail-flick latencies than all other groups, $p < .05$.

Discussion

In summary, the morphine dose (5 mg/kg) utilized in this experiment was not sufficient to induce an antinociceptive response, per se. As expected, exposure to intermittent shock alone did not significantly impact nociceptive reactivity, while continuous shock induced an antinociceptive response. Interestingly, intermittent shock enhanced the antinociceptive consequence of systemic morphine; and, morphine rats exposed to continuous shock showed a significant increase in mean tail-flick latencies as compared to all other groups. Thus, both intermittent and continuous shock enhanced the antinociceptive consequences of systemic morphine.

EXPERIMENT 5

The spinal cord has three distinct classes of opioid receptors: μ, δ, and κ. Previous research has shown that the antinociceptive effect of continuous shock can be blocked by the non-selective opioid antagonist, naltrexone (Meagher et al., 1993). The current experiment was designed to identify the opioid receptor that underlines continuous shock-induced antinociception using selective opioid antagonists directed at the μ (CTOP), δ (naltrindole), and κ (nor-BNI) opioid receptors.

Procedure

Twenty-four hours after spinal transection and cannulization, rats were moved to the testing room (26.5 °C) and placed in restraining tubes. Rats were given three baseline tail-flick tests, each separated by a 2-minute interval. Next, rats were randomly assigned to one of four drug conditions (n = 10 in each group): vehicle, CTOP, naltrindole, or nor-BNI (all drugs at a dose of 10 nmol). The drug was administered (i.t.) in a 10 µl volume using a Hamilton syringe into the exposed end of the cannula, followed by a 20 µl saline flush over a period of 3 minutes. Thereafter, subjects in each drug condition received 3, 25s tailshocks. Lastly, tail-flick latencies were assessed again 5 times at 2-minute intervals in all subjects.

Results

The results are presented in Figure 6. The mean baseline tail-flick latencies are shown on the left side of the graph. Subjects in the CTOP condition displayed slightly lower baseline scores than the other groups. An ANOVA revealed that baselines differed among groups prior to testing, $F(3, 36) = 2.97$, $p < .05$. *Post hoc* comparisons of the

group means showed that subjects in the CTOP condition had significantly different tail-flick latencies than all other groups, $p < .05$.

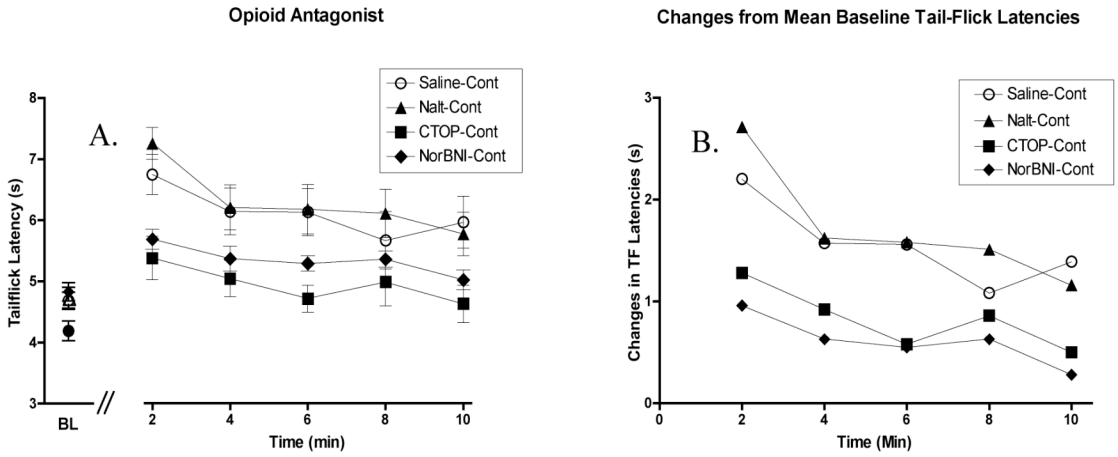

Figure 6. Saline rats exposed to 3, 25-s continuous tailshocks showed an increase in tail-flick latencies. C-TOP and Nor-BNI significantly decreased tail-flick latencies across the 10-min test trial, but Naltrindole (Nalt) did not (A). Changes in mean tail-flick latencies from baseline were compared at each test trials, across conditions. CTOP and Nor-BNI rats showed a decrease in mean tail-flick latencies from baseline scores at all test trials.

The mean test tail-flick latencies are presented to the right of the baseline scores. Saline subjects exhibited an increase in tail-flick latencies after continuous shock. Groups treated with CTOP and Nor-BNI showed lower tail-flick latencies than subjects treated with either saline or naltrindole. An ANCOVA, controlling for baseline scores, revealed that there was a significant main effect of drug, $F(3,35) = 4.73$, $p < .01$. The main effect of trials was not significant, $F(4, 140) = 1.95$, $p > .05$, nor was the Trials X

Drug interaction significant, $F(12, 140) = 1.36$, $p > .05$. *Post hoc* comparisons of the group means showed that subjects treated with CTOP and nor-BNI had significantly lower tail-flick latencies, after continuous shock exposure, than subjects treated with saline and naltrindole, $p < .05$. No other differences were significant, $p > .05$.

A further analysis was conduced to insure that the differences in baseline scores did not affect the results of the experiment. Changes in tail-flick latencies from the mean baseline scores were analyzed for all conditions, across trials. The results are presented in Figure 5. This analysis yielded the same results as those already presented. Subjects treated with CTOP and Nor-BNI showed significantly lower changes in tail-flick latencies from baseline scores, in contrast to groups treated with saline and naltrindole, $p < .05$.

Discussion

The antinociceptive effect of continuous shock (3, 25-s continuous tailshocks) was blocked by the μ (CTOP) and κ (nor-BNI) opioid antagonists, while the δ (naltrindole) antagonist had no effect. The results suggest that both the μ and κ opioid receptors are involved in continuous shock-induced antinociception. These results were independent of differences found in baseline scores prior to testing. Of interests, Watkins et al., (1982) have shown that blocking the μ and δ or μ and κ opioid receptors (i.t.) abolishes the naltrexone-insensitive analgesic effects of 5-40 inescapable tailshocks, and significantly reduces the analgesic effects of footshock in intact animals. These findings

suggest that brain and spinal mechanisms can induce an antinociceptive response that is dependent on opioid receptor interaction in the spinal cord.

EXPERIMENT 6

In the previous experiment, blocking the μ and κ opioid receptors attenuated the antinociceptive consequence of continuous shock treatment. This finding suggests that activation of the μ and κ opioid receptors underlie continuous-shock induced antinociception. The current experiment assessed if intermittent shock could oppose the antinociceptive effect of DAMGO (μ opioid agonist) or Dynorphin A (κ opioid agonist).

Procedure

Twenty-four hours after spinal transection and cannulization, 36 rats were placed in restraining tubes and baseline scores were collected. Next, electrodes were attached to the rat's tail and half the subjects (n = 18) received 6 minutes of intermittent tail shock, while the other half of the subjects remained unshocked (n = 18). Thereafter, one third of the rats in each shock condition were randomly assigned to one of three drug conditions: saline, DAMGO or DYN A. Subjects received an intrathecal administration of 10 µl of saline or drug (at a dose of .005 nmol for DAMGO or 10 nmol for DYN A), followed by a 10 µl saline flush with a Hamilton syringe during the span of three min. Pilot data were collected to verify that the drug doses utilized for this experiment induced an antinociceptive response that was comparable to that of continuous shock treatment. Thus, Experiment 4 consisted of a 2 (Intermittent or unshocked) X 3 (DAMGO, DYN A or Saline) factorial design. Lastly, tail-flick latencies were collected 5 times at 2-minute intervals to assess any changes in nociceptive reactivity.

Results

The results are shown in Figure 7. The baseline scores are depicted on the left

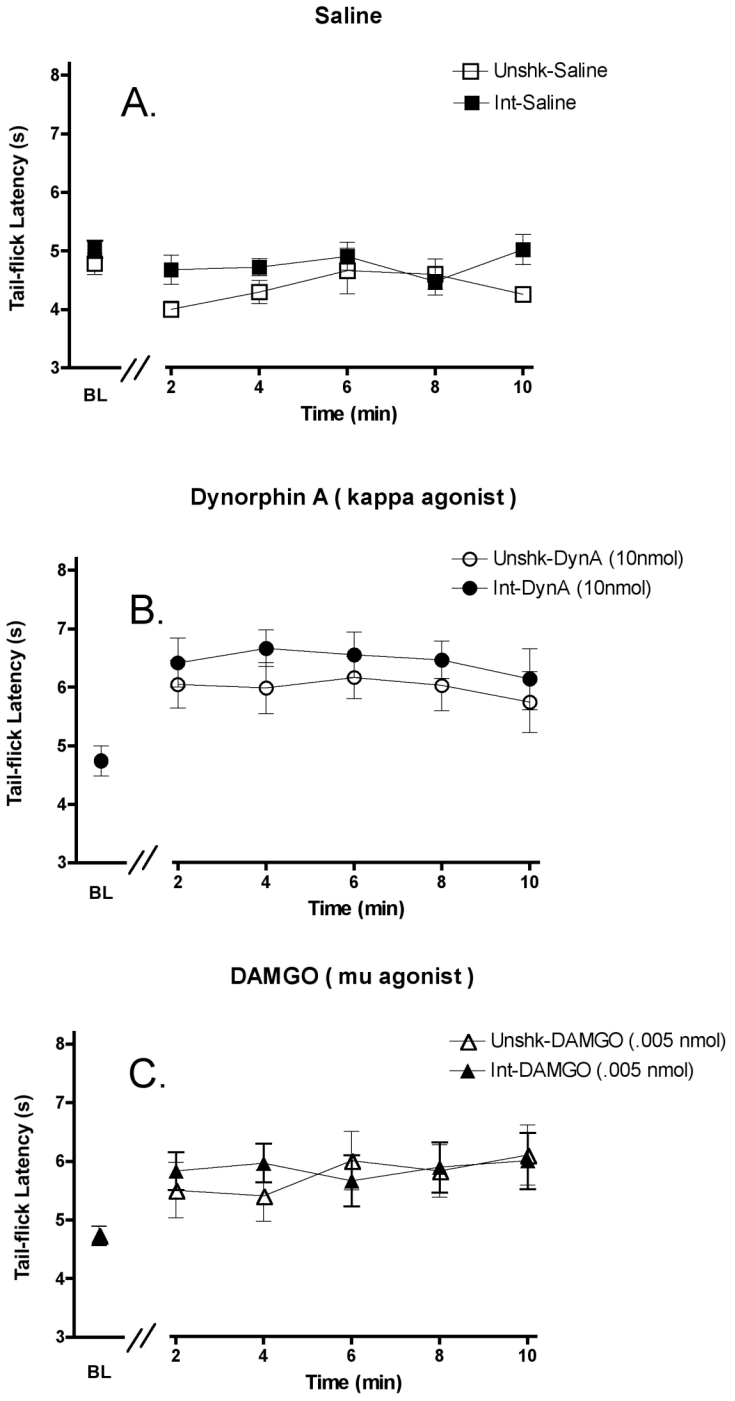

Figure 7. Intermittent shock did not produce an increase in tail-flick latencies (A). Intermittent shock failed to attenuate a drug-induced antinociception in DYN A (B) and DAMGO (C) treated rats.

side of each graph. An ANOVA revealed that there was no differences in mean baseline tail-flick latencies across groups, all *F*s < .1.0, $p > .05$. Test tail-flick scores are presented to the right of the baseline data. As expected, saline subjects did not exhibit a change in tail-flick latencies across the 10 minutes of testing. Unshocked groups that were exposed to either opioid agonist, DAMGO or DYN A, showed an increase in tail-flick latencies. This drug-induced antinociception was not attenuated by intermittent shock. An ANCOVA, controlling for baseline scores, revealed a significant main effect of drug treatment, $F(2, 29) = 10.07$, $p < .001$. The main effects of shock and trials did not reach significance, nor did the Shock X Drug or the Trials X Shock X Drug interactions, all *F*s < 1.0, $p > .05$.

Post hoc comparisons of the group means revealed that all groups that were treated with an opioid agonist, independent of shock condition, exhibited longer tail-flick latencies than the unshocked saline group, $p < .05$. Both groups treated with DYN A showed significantly higher tail-flick latencies than the saline-intermittent shock group, $p < .05$. No other group differences were significant, $p > .05$.

Discussion

Intermittent shock has been found to attenuate the antinociceptive effect of continuous shock (Experiment 1). Further, evidence indicates that continuous shock activates the μ and κ opioid receptors (Experiment 5). In this experiment, however, intermittent shock failed to reverse the antinociceptive effect of DAMGO (μ opioid agonist) or Dynorphin A (κ opioid agonist).

CONCLUSIONS

A substantial body of work exists to suggest that brain and spinal mechanisms react differently to nociceptive information. The current experiments were designed to identify parallels and differences in the way the spinal cord processes nociceptive information, as compared to intact animals. Through the course of this investigation, it became apparent that intermittent and continuous stimulation have an opponent relationship on nociceptive processing in the isolated spinal cord. A common dependent variable was used in all experiments to assess changes in nociceptive reactivity, the tail-flick test. In addition, pharmacological manipulations were employed to identify the opioid receptors activated by continuous shock, and to decipher at what synaptic level (e.g. pre or post synaptically) intermittent shock affects the release of endogenous opioids.

Intact animals exposed to 80 intermittent shocks show a decrease in pain reactivity (Maier et al, 1979). However, current research has shown that intermittent shock fails to induce antinociception in the isolated spinal cord (Crown et al., 2002). These findings suggest that intermittent stimulation has a divergent effect on brain and spinal nociceptive processing. Indeed, Experiment 1 revealed that 6 minutes of brief (80 ms) intermittent shock did not induce an antinociceptive response. In fact, prior exposure to intermittent shock attenuated the antinociception induced by long (25-s) intense (1.5 mA) continuous shocks. Research with intact animals has shown that intermittent shock produces an opioid analgesia that is dependent on the HPA-axis. Assuming that shock exposure does not engage an HPA-mediated stress response in transected rats, it is not

surprising that intermittent shock fails to induce antinociception in spinally transected animals. In addition, Experiment 1 revealed that intermittent and continuous shock have divergent effects on nociceptive reactivity in the spinal cord. Long (25-s) intense (1.5 mA) continuous shocks induced antinociception, while 6 minutes of brief (80 ms) intermittent shock did not. Lastly, it was found that intermittent shock given prior to continuous shock attenuated continuous shock-induced antinociception.

Experiment 2 assessed if the impact of intermittent shock on continuous-shock induced antinociception was dependent on the order of stimuli presentation. The results of Experiment 2 revealed that indeed it was. When intermittent shock was given after continuous shock, intermittent shock failed to block the antinociceptive consequences of continuous shock. Research with intact animals has shown that the effects of intermittent shock on pain reactivity and learning lasts up to 24 hours (Maier et al., 1979). In the spinal cord, the detrimental effects of intermittent shock on instrumental learning last up to 48 hours (Crown et al, 2002). Experiment 3 examined if the effects of intermittent shock on continuous shock-induced antinociception also last 24 hours. The results revealed that intermittent shock did not have a lasting effect on continuous shock-induced antinociception.

Research with intact animals has shown that intermittent shock enhances the analgesic impact of systemic morphine (Grau et al, 1981). However, it has been shown that intermittent shock induces an increase in mechanical reactivity in spinalized rats (Ferguson, Crown, & Grau, 2006). It seems a paradox to suggest that intermittent shock, which induces allodynia, would also enhance morphine antinociception. Nevertheless,

the results of Experiment 4 suggest that this is the case. The dose of morphine (5 mg/kg) used in this experiment did not induce antinociception, nor did intermittent shock. Surprisingly, rats exposed to both morphine and intermittent shock showed a significant antinociceptive response. The effect of continuous shock was less surprising. It induced a moderate antinociception, when combined with systemic morphine a more robust antinociception was observed. Previous research has shown that both continuous and intermittent shock activate opioid systems within the spinal cord (Joynes & Grau, 2004; Meagher et al., 1993). Though these spinal effects have divergent consequences on spinal learning and nociceptive reactivity, both shock schedules enhanced the antinociceptive effects of morphine.

Meagher et al. (1993) found that long (25-s) intense (1.25 mA) continuous shocks produce a naltrexone-reversible antinociception. The results of Experiment 5 showed that continuous shock activates both the μ and κ opioid receptors. Equal molar concentrations of either the μ (CTOP) or κ (nor-BNI) opioid receptor antagonist blocked continuous shock-induced antinociception. Experiment 6 assessed if prior exposure to intermittent shock attenuated a pharmacologically induced antinociception. Results revealed that intermittent shock failed to attenuate the antinociception induced by DAMGO (μ opioid agonist) or Dynorphin A (κ opioid agonist). This finding suggests that intermittent shock may influence the release of an endogenous opioid rather than the post release effects; if the action was post release, intermittent shock should have also affected the antinociception induced by a systemic opioid (Grau et al., 1981). Indeed, intermittent shock attenuated continuous-shock induced antinociception only when

intermittent shock was given prior to continuous shock (Experiment 1). When intermittent shock was given after continuous shock (and theoretically, after the release of endogenous opioids), intermittent shock failed to attenuate continuous shock-induced antinociception (Experiment 2). These findings are similar to the work of Watkins and Mayer (1982a). They found that 90s of inescapable shock applied to the front paw induces an analgesic response that is blocked by an intrathecal dose of naloxone. Of particular interest, the efficacy of naltrexone to attenuate footshock-induced analgesia (FSIA) is order-dependent. Naloxone attenuates FSIA when given prior to shock, but fails to block FSIA when given immediately after shock (Watkins & Mayer, 1982a).

Clinical Implications

In intact rats, intermittent shock induces a hormonal-mediated opioid analgesia (MacLennan et al., 1982). Not surprisingly, spinal transection prevents the expression of intermittent shock-induced antinociception. What is surprising is that intermittent shock causes a learning deficit in the isolated spinal cord, similar to what is seen in intact animals (Grau, Barstow, & Joynes, 1998; Crown et al., 2002; Joynes & Grau, 2004; Ferguson, Crown, & Grau, 2006). The spinal cord can support a simple instrumental response (Buerger & Fennessy, 1970; Chopin & Buerger, 1976; Grau, Barstow & Joynes, 1998), and prior exposure to uncontrollable shock attenuates this spinal learning. Conversely, exposure to continuous shock (14.4-360 s) induces antinociception, but not the learning deficit. In fact, simultaneous administration of continuous shock and intermittent shock prevents the expression of the learning deficit. These results suggest

that intermittent and continuous stimulation have divergent effects on nociceptive reactivity and learning in the isolated spinal cord.

This fundamental difference in the way the spinal cord responds to intermittent and continuous stimulation is a clinically relevant issue. Intermittent shock has been shown to induce tactile hyperreactivity and to undermine spinal instrumental learning (Ferguson, Crown, & Grau, 2006). These results are similar to the effects of carrageenan-induced inflammation (Ferguson, Crown, & Grau, 2006). In addition, just 6 minutes of brief (80 ms) intermittent shock can significantly hinder recovery of locomotor function after spinal cord injury (Grau et al., 2004). Conversely, continuous stimulation has been shown to cause analgesia in intact animals and antinociception in spinalized rats (Crown et al, 2002; Meagher, Grau, & King, 1990). More importantly, continuous shock can be seen as a model of transcutaneous electrical nerve stimulation (TENS). TENS is defined by the American Physical Association as the application of electrical stimulation to the skin for pain control. Several theories support the use of TENS to produce pain relief, including the gate control theory and release of endogenous opioids (for review see Sluka & Walsh, 2003). In rats, TENS has been shown to reduce hyperalgesia after carrageenan administration (Ainsworth et al., 2006), and to decrease the release of the excitatory neurotransmitters glutamate and aspartate in animals with joint inflammation (Sluka, Vance, & Lisi, 2005). In instances when inhibitory supraspinal systems become compromised, such as after a spinal cord injury, therapeutic electrical stimulation might be useful in lessening the detrimental effects of nociceptive insult to the spinal cord. Ideally, if continuous shock inhibits the adverse

effects of intermittent stimulation, TENS might be used in humans to attenuate over-excitation and cell death after spinal cord injury.

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