ROLE OF THE OPIOID SYSTEM IN THE BEHAVIORAL DEFICIT OBSERVED AFTER UNCONTROLLABLE SHOCK

A Thesis

by

STEPHANIE NICOLE WASHBURN

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

May 2005

Major Subject: Psychology

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Approved as to style and content by:

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James Grau (Chair of Committee)	Mary Meagher (Member)
_____	_____
Rajesh Miranda (Member)	Steve Rholes (Head of Department)

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ABSTRACT

Role of the Opioid System in the Behavioral Deficit Observed after Uncontrollable Shock. (May 2005)

Stephanie Nicole Washburn, B.A., Texas A&M University

Chair of Advisory Committee: Dr. James W. Grau

Spinal cord neurons can support a simple form of instrumental learning that can be used to assess behavioral potential (plasticity) within this system. In this paradigm, subjects completely transected at the second thoracic vertebra learn to minimize shock exposure by maintaining a hindlimb in a flexed position. Preexposure to uncontrollable shock (shock independent of leg position) disrupts this learning.

Activation of opioid receptors seems to contribute to the expression of the behavioral deficit observed after uncontrollable shock. Intrathecal application of naltrexone, a nonselective opioid receptor antagonist, blocked the expression, but not the induction, of the deficit. Treatment with nor-BNI, a kappa receptor antagonist, prior to testing had a similar effect, whereas mu (CTOP) and delta (naltrindole) receptor antagonists did not block the deficit. These findings suggest that prior exposure to uncontrollable shock induces a kappa opioid mediated event that inhibits learning. The current study examined the role of the kappa receptor in the behavioral deficit. Only GR89696, a selective kappa-2 receptor agonist, inhibited learning. This impairment was dose-dependent and, at the highest dose (30 nmol), inhibited learning for 96 hours.

However, GR89696 and uncontrollable shock did not interact in an additive fashion. Instead, an intermediate dose attenuated the induction of the deficit. These findings suggest that activation of kappa receptors, specifically the kappa-2 subtype, inhibit instrumental learning and block the induction of the learning deficit. Both effects may be linked to the inhibition of NMDA-mediated plasticity.

DEDICATION

The author would like to dedicate this thesis to her paternal grandparents, Mr. and Mrs. Raymond and Nellie Washburn, who passed away during its preparation.

ACKNOWLEDGEMENTS

The author would like to thank Dr. James W. Grau for his guidance, advice, and support during all phases of this work. Without his empirical expertise and patience this project would not have been possible. The author also wishes to thank Drs. Mary W. Meagher and Rajesh Miranda for serving as committee members. Additional thanks are due to Drs. Adam R. Ferguson and Michelle A. Hook for their unrelenting encouragement and advice. The author thanks Russell Huie, Denise Puga, Marissa Maultsby, Cynthia Lin, and Christine Petrich for their comments on an earlier version of this thesis. Finally, the author would like to thank her family, friends, and significant other for their understanding, encouragement, and guidance throughout the course of her studies.

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INTRODUCTION

Researchers have challenged the claim that the spinal cord is immutable by demonstrating that it can support simple forms of learning, including habituation, sensitization, and even Pavlovian and instrumental conditioning (Beggs, Steinmetz, Romano, & Patterson, 1983; Beggs, Steinmetz, & Patterson, 1985; Buerger & Fennessy, 1970; Chopin & Buerger, 1976; Fitzgerald & Thompson, 1967; Grau, Barstow, & Joynes, 1998; Patterson, Cegavske, & Thompson, 1973; Thompson & Spencer, 1966). Our laboratory uses an instrumental learning paradigm to assess spinal cord plasticity and to study the underlying neurobiological mechanisms of learning (Grau et al., 1998). In this paradigm, subjects undergo complete transection of the spinal cord and are placed into a tube that allows the hind limbs to hang freely. Whenever one hind limb falls below a set criterion, a shock is delivered to the tibialis anterior muscle, causing a reflexive upward flexion of the limb, which terminates the shock (controllable shock). Over the 30-minute training period, rats gradually learn to maintain the hind limb in a flexed position, effectively minimizing shock exposure. Animals exposed to uncontrollable shock (shock independent of leg position) fail to learn the relationship between limb position and shock onset when later tested with controllable shock. This behavioral deficit lasts up to 24 hours (Crown, Joynes, Ferguson, & Grau, 2002).

Previous studies have shown that both instrumental learning and the development of the behavioral deficit require NMDA-dependent processes (Ferguson, Crown, Patton, & Grau, 2002; Ferguson, Hook, & Grau, 2004; Joynes, Janjua, & Grau, 2004). NMDA receptor antagonists block both the acquisition and maintenance of the instrumental

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This thesis follows the format of *Behavioral Neuroscience*.

response when given prior to and during testing, respectively. This finding is not surprising in light of the fact that most forms of long-term potentiation (LTP), a phenomenon regarded as the biological substrate of learning and memory (Bliss & Lomo, 1973; Lynch & Baudry, 1984; Madison, Malenka, & Nicoll, 1991), require NMDA receptor function (Morris, Anderson, Lynch, & Baudry, 1986). Central sensitization, which is thought to underlie the development of hyperalgesia (enhanced pain) and allodynia (a previously nonpainful stimulus becomes painful), depends on many of the same molecular events as LTP, including NMDA receptor activation (Coderre, 1993; Dickenson, 1990, 1996; Dray, Urban, & Dickenson, 1994; Ji, Kohno, Moore, & Woolf, 2003; Wall & Woolf, 1984; Willis, Sluka, Rees, & Westlund, 1996; Woolf, 1983, 1984). A number of studies have shown that NMDA receptor antagonists are antihyperalgesic (Ren, Hylden, Williams, Ruda, & Dubner, 1992a; Ren, Williams, Hylden, Ruda, & Dubner, 1992b; Ren & Dubner, 1993). These same antagonists can prevent the development of the behavioral deficit if given prior to uncontrollable shock, suggesting that it also is dependent upon NMDA receptor activation (Ferguson et al., 2002, 2004). Interestingly, inflammatory agents routinely used in experimental models of central sensitization (e.g., carrageenan and formalin) mimic the effects of uncontrollable shock (Ferguson, Crown, Washburn, Miranda, & Grau, 2001; Huie, Ferguson, & Grau, 2004). Furthermore, a tactile allodynia accompanies the development of the deficit after uncontrollable shock (Ferguson, Crown, Dhruv, Washburn, & Grau, 2000), suggesting that the deficit may be linked to central sensitization. Based on these findings, we have argued that instrumental learning

requires a selective, NMDA-dependent modification in a particular response pathway (Ferguson et al., 2002, 2004; Grau et al., 1998; Joynes et al., 2004) and that uncontrollable shock disrupts this learning by inducing a form of overexcitation that diffusely saturates NMDA-mediated plasticity (Moser, Krobert, Moser, & Morris, 1998; Moser & Moser, 1999).

Opioid receptors, specifically the kappa subtype, seem to play a role in the expression of the behavioral deficit induced by uncontrollable shock. For example, intrathecal administration of naltrexone blocked the expression, but not the induction, of the behavioral deficit (Joynes & Grau, 2004). Subsequent studies revealed that the kappa antagonist nor-BNI had a similar effect. Mu (CTOP) and delta (naltrindole) receptor antagonists did not prevent the behavioral deficit, indicating that only kappa receptors are involved. Collectively, these results suggest that uncontrollable shock initiates a kappa receptor-mediated event that inhibits learning. Interestingly, dynorphin, an endogenous opioid peptide highly selective for the kappa receptor subtype, has been implicated in the pathophysiology of a variety of conditions, including Alzheimer's disease, traumatic brain injury, and spinal cord trauma (Caudle & Isaac, 1988; Faden, 1996). Studies have shown that opioid antagonists protect against the neuropathology observed in several experimental injury models (for a review see Faden, 1993). Using naloxone, Faden and colleagues (1981a, 1981b) showed a significant reduction in posttraumatic ischemia and improved behavioral recovery after cervical spinal cord injury in the cat. Additional studies identified the kappa receptor as the primary mediator of the pathophysiological actions of dynorphin (Faden, Takemori, &

Portoghese, 1987). High doses of intrathecal dynorphin results in maladaptive changes in the spinal cord, including hind limb paralysis, an irreversible loss of the tail-flick reflex, and release of excitatory amino acids (Bakshi, Newman, & Faden, 1990c; Caudle & Isaac, 1987; Faden & Jacobs, 1983). These effects can be blocked or reversed using NMDA antagonists, suggesting that the neurotoxic actions of dynorphin result from potentiation of NMDA receptor activity (Bakshi & Faden, 1990a, 1990b; Bakshi, Ni, & Faden, 1992; Caudle & Isaac, 1988). Modulation of excitatory amino acids may underlie this potentiation and the resulting neurotoxic consequences (Caudle & Isaac, 1988; Skilling, Sun, Kurtz, & Larson, 1992). Presumably, uncontrollable shock would cause the release of dynorphin. Based on our theory that the behavioral deficit results from a form of NMDA-mediated overexcitation within the spinal cord, this release may mediate the consequences of uncontrollable shock.

Although the activation of kappa receptors is thought to underlie the pathology observed after injury, a number of other studies have shown that some kappa agonists are protective in these same injury models (Birch et al., 1991; Hall, Wolf, Althaus, & Von Voigtlander, 1987). Treatment with selective kappa-2 agonists, both before and after cerebral ischemia, reduced neuronal cell loss in the CA_1 region of the hippocampus. Others have shown that GR89696, a kappa-2 agonist, prevents and reverses hyperalgesia and allodynia in animal models of inflammation, neuritis, and neuropathy (Eliav, Herzberg, & Caudle, 1999; Ho, Mannes, Dubner, & Caudle, 1997). These neuroprotective effects can be reversed with opioid receptor antagonists, indicating that opioid receptors mediate these effects (Birch et al., 1991; Eliav et al., 1999).

Electrophysiological studies have shown that kappa-2 receptor activation inhibits current flow through the NMDA receptor (Caudle, Chavkin, & Dubner, 1994), and kappa agonists have been shown to presynaptically inhibit glutamate release (Werz & McDonald, 1983). This inhibition of synaptic current and transmitter release has been demonstrated in both the brain (Caudle et al., 1994; Wagner, Caudle, & Chavkin, 1992; Wagner, Terman, & Chavkin, 1993) and spinal cord (Randic, Cheng, & Kojic, 1995; Werz & McDonald, 1985). These NMDA inhibitory actions are thought to underlie the neuroprotection provided by kappa agonists (Ho et al., 1997; Birch et al., 1991). If this is true, then GR89696 may prevent the induction of the deficit if given prior to uncontrollable shock. However, it may also prevent learning if given immediately before testing due to its inhibition of NMDA receptor function.

The two divergent models of the effects of kappa agonists on NMDA receptor function (potentiation vs. inhibition) lead to very different predictions in the investigation of their role in the behavioral deficit. Table 1 depicts these differential predictions. If kappa agonists induce NMDA-mediated overexcitation of spinal cord neurons, as suggested in model A, then they should substitute for uncontrollable shock, which is also thought to induce a form of overexcitation (Joynes et al., 2004). Acquisition of the instrumental response is disrupted when this overexcitation reaches a level that saturates NMDA-mediated plasticity. High doses of kappa agonists that excite spinal cord neurons beyond this level should impair instrumental learning and may even cause cell loss that permanently abolishes the capacity for learning (Caudle & Isaac, 1987). Low doses that cause excitation not exceeding this threshold may actually

facilitate instrumental learning and should have no long-term impact. However, according to model A, if combined with uncontrollable shock, these same doses should exacerbate the behavioral deficit. Overall, the predictions made by model A suggest that kappa agonists, at least at high concentrations, are detrimental to spinal cord function.

Table 1. Table illustrating the predictions made by the theory of NMDA receptor facilitation (model A) versus those made by the theory of NMDA receptor inhibition (model B). Both models predict that kappa agonists will impair instrumental learning. However, model A predicts that this impairment will be dose-dependent, whereas model B predicts impairment at all effective dosages. Long-term effects are predicted by model A, but not by model B. The potentiation of NMDA receptors by kappa agonists suggests that these agents will exacerbate the consequences of uncontrollable shock. Model B makes the opposite prediction: that kappa agonists will protect against uncontrollable shock.

	Impact on Instrumental Learning	**Long-Term Effect**	**Interaction with Shock**
Biochemical Model A: NMDA Facilitation	Facilitation at low doses Disruption at high doses	At high Doses	Additive
Biochemical Model B: NMDA Inhibition	Disruption at high and low doses	Not Anticipated	Protective

Model B, which suggests that kappa agonists inhibit this receptor system, also predicts that these agonists will impair instrumental learning if given immediately before testing. This prediction is based on the finding that NMDA receptor antagonists disrupt instrumental learning when active in the spinal cord (Joynes et al., 2004). However, in contrast to model A, model B suggests that this impairment will occur independent of dose. Any dose that sufficiently inhibits NMDA receptor activation should induce a behavioral deficit when active in the system. Unlike model A, model B predicts that neither high nor low doses of a kappa agonist will have a long-term impact and that once

the drug has cleared the system, the capacity for instrumental learning should return. Although kappa agonists may impair learning when given immediately before testing, model B predicts that the inhibitory actions of these compounds will prevent the development of the behavioral deficit if they are given prior to uncontrollable shock. These predictions made by model B suggest that the NMDA inhibitory nature of kappa agonists undermine plasticity under some conditions but can be used to prevent overexcitation in others.

The current experiment examines the impact of opioid agonists on instrumental learning to test the predictions made by these contradictory models. Experiment 1, which consisted of three separate experiments, examined the impact of kappa (Experiment 1A), mu (Experiment 1B), and delta (Experiment 1C) receptor agonists on instrumental learning 10 minutes after drug treatment. Experiments 2 and 3 examined the dose-response relationship (Experiment 2) and timecourse (Experiment 3) of the kappa-2 receptor agonist, GR89696. Finally, Experiment 4 investigated the interaction between GR89696 and uncontrollable shock.

GENERAL METHOD

Subjects

Male Sprague-Dawley rats obtained from Harlan (Houston, TX) served as subjects. Animals were approximately 100-120 days old and weighed between 360 and 460 g. Subjects were maintained on a 12-hr light-dark schedule and housed individually. Food and water was available *ad libitum*, and behavioral testing was performed during the light portion of the cycle.

Surgery

Subjects were anesthetized with pentobarbital (50 mg/kg, i.p.), and the area surrounding the shoulders was shaved and sterilized with iodine. An anterior-posterior incision, approximately 1.5 cm long, was made over the 2nd thoracic vertebra (T2). The tissue immediately anterior to T2 was then cleared, and the exposed spinal cord was transected using cauterization. The resulting space was filled with Gelfoam (Harvard Apparatus, Holliston, MA), and a cannula consisting of 25 cm of polyethylene tubing (PE-10, VWR International, Bristol, CT) fitted with a stainless steel wire (0.09 mm diameter) (Small Parts Inc., Miami Lakes, FL) was inserted into the subarachanoid space on the dorsal surface of the cord. The cannula was inserted 9 cm down the vertebral column, and the exposed end of the tubing was secured externally to the skin with cyanoacrylate, as described by Yaksh and Rudy (1976). The wound caudal to the exposed tubing was closed with Michel Clips (Fine Science Tools, Foster City, CA), and the stainless steel wire was carefully removed.

Rats were injected with 0.9% saline (2.5 ml, i.p.) immediately following surgery, and the hindlimbs were secured in a natural flexed position with a piece of porous tape (Ortholetic 1.3 cm width) wrapped once around the body and legs to prevent muscular damage due to unnatural extension during recovery. Subjects were allowed to recover in a temperature-controlled room (26.7°C) with food and water available *ad libitum*. Bladder expression took place twice a day and immediately before any behavioral procedures were conducted. At the end of testing, animals were euthanized with pentobarbital (100 mg/kg).

Transections were confirmed by a) visually inspecting the cord during surgery, b) observing behavior after recovery to ensure complete paralysis below the forelimbs and no vocalization when exposed to leg shock, and c) examining the cord *post mortem* in a randomly selected subset of subjects.

Apparatus

Uncontrollable shock was administered while rats were loosely restrained in opaque black Plexiglas tubes that were 22 cm in length and 6.8 cm in diameter. A flat floor constructed from a sheet of black Plexiglas 5.5 cm wide was attached 5.3 cm below the top of the tube, and chamber fans provided approximately 60 dB of background noise. Tailshock was delivered using an electrode constructed from a modified fuse clip. The electrode was coated with electrocardiogram (ECG) gel (Harvard Apparatus, Holliston, MA) and secured with porous tape approximately 6 cm behind the base of the tail. Constant-current 1.5-mA shock was delivered using a 660-V transformer. A Macintosh computer controlled the onset and offset of shock.

Instrumental testing was conducted while rats were loosely restrained in tubes (23.5 cm [length] x 8 cm [internal diameter]); see Grau et al., 1998, Figure 1). Two slots in the tube, (5.6 cm [length] x 1.8 cm [width]), 4 cm apart, 1.5 cm from the end of the tube), allowed both hind legs to hang freely. Shock was delivered using a BRS/LVE (Laurel, MD) shock generator (Model SG-903). Two electrodes, one inserted into the skin (approximately 1.5 cm above the tarsals) and another inserted into the tibialis anterior muscle (approximately 1.7 cm above the first electrode), were connected to a computer-controlled relay that regulated the application of leg shock.

Leg position was monitored during testing using a contact electrode constructed from a 7.0 cm long, 0.46 mm diameter stainless steel rod taped to the foot. The last 2.5 cm of the electrode was insulated from the foot with heat-shrink tubing. A fine wire (0.01 sq mm [36 AWG], [l] x 20 cm) attached to the end of the rod extended from the rear of the foot and was connected to a digital input monitored by a Macintosh computer. A plastic rectangular dish (11.5 cm [w] x 19 cm [l] x 5 cm [d]) containing a NaCl solution was placed approximately 7.5 cm below the restraining tube. A drop of soap was added to the solution to reduce surface tension. A ground wire was then connected to a 1.0 mm wide stainless steel rod, which was placed in the solution. When the contact electrode attached to the rat's paw touched the solution (indicating an extended ankle joint), it completed the circuit monitored by the computer and delivered a shock to the tibialis anterior. The state of this circuit was sampled at a rate of 30 times/s.

Flexion force was measured prior to testing by attaching a monofilament plastic line ("4 lb test" Stren, Dupont, Wilmington DE) to the rat's foot immediately behind the

plantar protuberance. The 40 cm length of line was passed through an eyelet attached to the apparatus directly under the paw, 16 cm beneath the base of the tube. The end of the line was attached to a strain gauge (Fort-1000, World Precision Instruments, New Haven, CT) fastened to a ring stand. After the line was connected to the rat's paw, the ring stand was positioned so that the line was taut, just barely registering on the gauge. The strain gauge was calibrated by determining the relationship between voltage and force in Newtons.

Instrumental Learning Testing Procedure

All subjects were allowed to recover for 24 hours following surgery, and the hindlimbs were shaved and marked for electrode placement prior to testing. A wire electrode was then inserted through the skin over the distal portion of the tibialis anterior (1.5 cm from the plantar surface of the foot), and one lead from the generator was attached to this wire. A contact electrode was secured to the foot between the second and third digits with a piece of porous tape. The shock generator was set to deliver a 0.4 mA shock, and the proximal portion of the tibialis anterior (approximately 1.7 cm proximal to the wire electrode) was probed with a 2.5-cm stainless steel pin attached to a shock lead to find a robust flexion response. The pin was then inserted 0.4 cm into the muscle. A strain gauge was utilized to verify that a single, intense (1.6 mA, 0.3 s), test shock elicited at least a 0.8 N flexion force. Next, shock intensity was set at a level that elicited a flexion force of 0.4 N.

To minimize lateral leg movements, a 20 cm piece of porous tape was wrapped around the leg and was attached to a bar extending across the apparatus directly under

the front panel of the restraining tube. The tape was adjusted so that it was taut enough to slightly extend the knee. Finally, three short (0.15 s) shock pulses were applied, and the level of the salt solution was adjusted so that the tip of the contact electrode (attached to the rat's foot) was submerged 4.0 mm below the surface of the water. The rat's capacity to perform the instrumental response was then tested with exposure to 30 min of controllable shock. Whenever the rat's leg fell below the level of the salt solution, the electrodes delivered a shock to the tibialis anterior muscle, causing the ankle to flex.

Behavioral Measures

Three behavioral measures—response number, response duration and time in solution—were used to assess a subject's capacity to perform the instrumental response (see Grau et al., 1998). Performance was measured over time in 30 1-min time bins. The computer monitoring leg position recorded an increase in response number whenever the contact electrode left the salt solution. Response duration was derived from time in solution and response number using the following equation: $\text{Response Duration}_i = (60 \text{ s} - \text{time in solution}_i)/(\text{Response Number}_i + 1)$, where i is the current time bin.

Statistical Analysis

The effects of experimental treatment over time were analyzed using a repeated measures analysis of variance (ANOVA). Group differences were further evaluated using Duncan's New Multiple Range post hoc tests.

EXPERIMENT 1

We have previously shown that only kappa antagonists given immediately before testing eliminate the behavioral deficit induced by uncontrollable shock (Joynes & Grau, 2004). If the deficit results from activation of kappa receptors, then an agonist for this receptor should substitute for uncontrollable shock and produce the behavioral deficit. Furthermore, we would not expect a delta or mu opioid receptor agonist to cause a deficit. The current experiment tests this hypothesis.

The kappa opioid receptor is thought to exist in at least two forms, kappa-1 and kappa-2, both of which exist within the spinal cord (Caudle et al., 1994, 1998; Zukin, Eghbali, Olive, Unterwald, & Tempel, 1988). The prior study by Joynes and Grau (2004) implicated the kappa receptor but did not differentiate between receptor subtypes. The present study addressed this issue by using two different kappa opioid receptor agonists, each selective for either the kappa-1 or kappa-2 subtype.

Experiment 1 consisted of three independent experiments. Experiment 1A examined the issue of kappa opioid receptor specificity using a kappa-1 (U69593) or kappa-2 (GR89696) opioid receptor agonist. Experiment 1B assessed the impact of a mu opioid receptor agonist (DAMGO) on instrumental learning. Finally, Experiment 1C tested whether the delta opioid receptor agonist, DPDPE, induced a behavioral deficit. All drugs were administered intrathecally (i.t.) at equal molar (30 nmol) concentrations. This dose was chosen based on previous work by Ho et al. (1997) that examined the impact of these opioid agonists on hyperalgesia.

Method

In all experiments, leg electrodes were implanted, and rats were placed in the instrumental testing apparatus (as described in the General Method section) with their cannula threaded through an airhole in the tube for administration of drug, 24 hours following surgery. A baseline flexion force was taken before subjects received any pharmacological agents, and shock intensity was adjusted to yield a flexion response of 0.4 N. After an initial flexion force was obtained, subjects received intrathecal administration of 10 μl of drug (30nmol) or saline followed by a 10 μl saline flush using a Hamilton syringe. Flexion force was re-recorded 10 minutes after drug treatment to ensure that the drug did not disrupt the flexion response, and shock intensity was re-adjusted accordingly. Subjects were then tested with 30 minutes of controllable shock to either the right or left hindlimb in a counterbalanced fashion.

Results

Experiment 1A: Impact of kappa agonists. To ensure that the drug treatment did not affect baseline behavioral reactivity, the shock intensity required to produce a 0.4 N change in flexion force both before and after drug administration were analyzed. Mean shock intensity needed to elicit a 0.4 N change in flexion force before drug treatment ranged from 0.51 ($\pm$ 0.05) to 0.58 ($\pm$ 0.06) mA and after drug administration ranged from 0.51 ($\pm$ 0.06) to 0.66 ($\pm$ 0.07) mA. An ANOVA failed to yield any significant effects of U69593 or GR89696 on shock intensity either before or after drug delivery, all Fs < 1.42, $p > .05$.

The effect of U69593 and GR89696 on our primary measure of learning, response duration, is depicted in Figure 1A. As in prior studies, saline-treated animals exhibited increased response durations over time. This learning was blocked by GR89696, whereas U69593 had no effect. A repeated measures ANOVA on response duration revealed significant main effects of drug, $F(2, 21) = 4.68$, $p < .05$ and time, $F(29, 609) = 3.06$, $p < .01$. A significant Drug X Time interaction was also found, $F(58, 609) = 1.76$, $p < .01$. Post hoc comparisons of the group means, displayed in Figure 1B, revealed that only subjects that received GR89696 prior to testing differed from the other groups, $p < .05$. No other group differences were significant, $p > .05$.

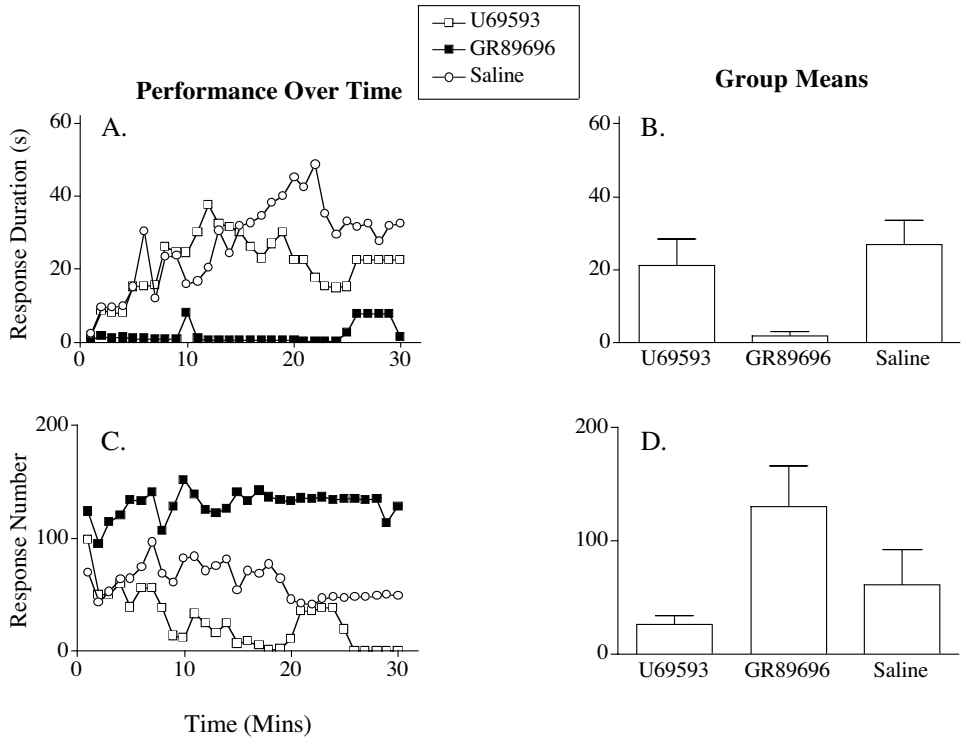

Figure 1. The impact of U69593 and GR89696 administered 10 minutes prior to instrumental testing on response duration (A) and response number (C) over time. The right panels (B & D) represent group means (±SE) for response duration and response number, respectively.

Figure 1C summarizes the impact of U69593 and GR89696 on response number. All subjects, except those given GR89696 prior to testing, displayed a decrease in response number as they acquired the instrumental response. Rats given GR89696 failed to learn and, consequently, exhibited a high level of responding during testing. However, these group differences did not reach statistical significance, all Fs < 3.18, p > .05.

Experiment 1B: Impact of a mu agonist. Mean shock intensity before drug administration ranged from 0.53 (± 0.06) to 0.56 (± 0.06) mA and after drug treatment ranged from 0.57 (± 0.06) to 0.61 (± 0.07) mA. No significant group differences were found for shock intensity either before or after treatment with DAMGO, all Fs < 1.0, p > .05.

Figure 2A depicts the effect of DAMGO on response duration across time. Saline-treated animals exhibited an increase in response durations over the 30-minute testing period. DAMGO had no impact on this learning. An ANOVA revealed a main effect of time, $F(29, 406) = 2.66$, $p <$ .001. Neither the main effect of drug nor the Drug X Time interaction approached significance, both Fs < 1.0, p > .05.

Figure 2C shows the effect of DAMGO on response number. As saline-treated subjects learned the instrumental response they showed a progressive decrease in the number of responses made over the thirty-minute testing period. DAMGO-treated subjects displayed a similar pattern of results. An ANOVA on this measure revealed only a significant main effect of time, $F(29, 406) = 1.93$, $p < .01$. Neither the main

effect of drug nor the Drug X Time interaction reached significance, both *F*s < 1.0, *p* > .05.

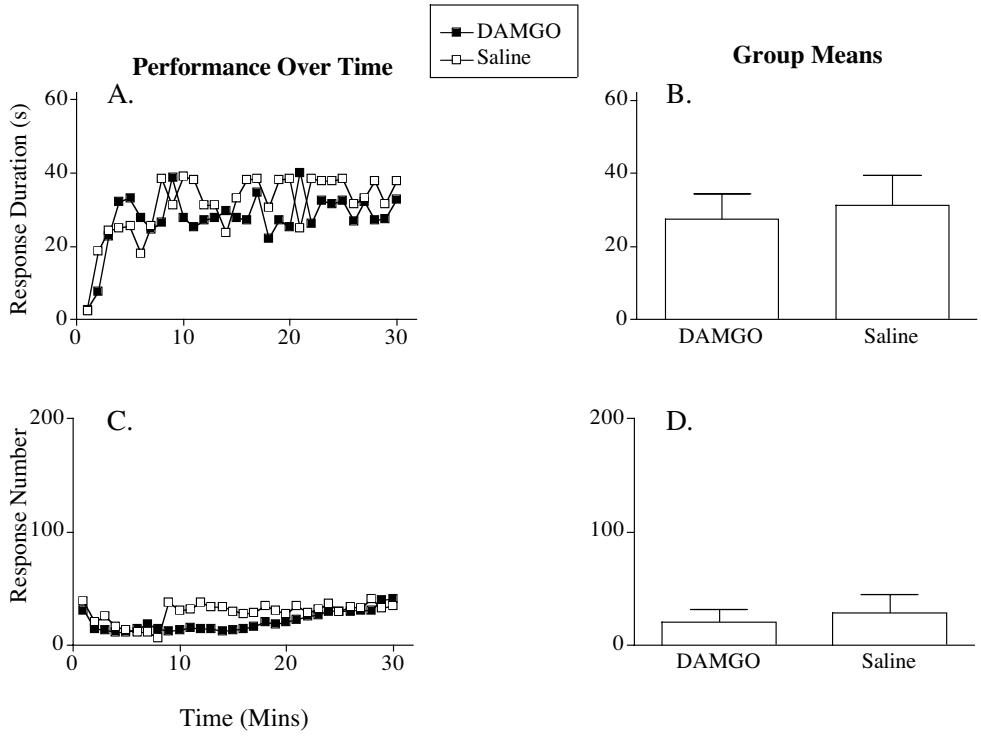

Figure 2. Response duration (A) and number of responses (C) made by subjects that received DAMGO 10 minutes prior to instrumental testing across time. The right panels (B & D) display the group means (±SE) for response duration and response number, respectively.

Experiment 1C: Impact of a delta agonist. Mean shock intensity needed to induce a 0.4 N change in flexion force before drug treatment ranged from 0.64 (± 0.09) to 0.67 (± 0.07) and after treatment ranged from 0.67 (± 0.06) to 0.69 (± 0.09). One-way ANOVAs on shock intensity both before and after administration of DPDPE revealed no significant effects of the drug, all *F*s < 1.0, *p* > .05.

The effect of DPDPE on response duration is depicted in Figure 3A. Subjects that received saline prior to testing exhibited an increase in response duration over the testing period. DPDPE treatment had no effect on this learning. An ANOVA yielded only a significant main effect of time, $F(29, 464) = 3.42$, $p < .001$. Neither the main effect of drug nor the Drug X Time interaction reached significance, both Fs < 1.0, $p > .05$.

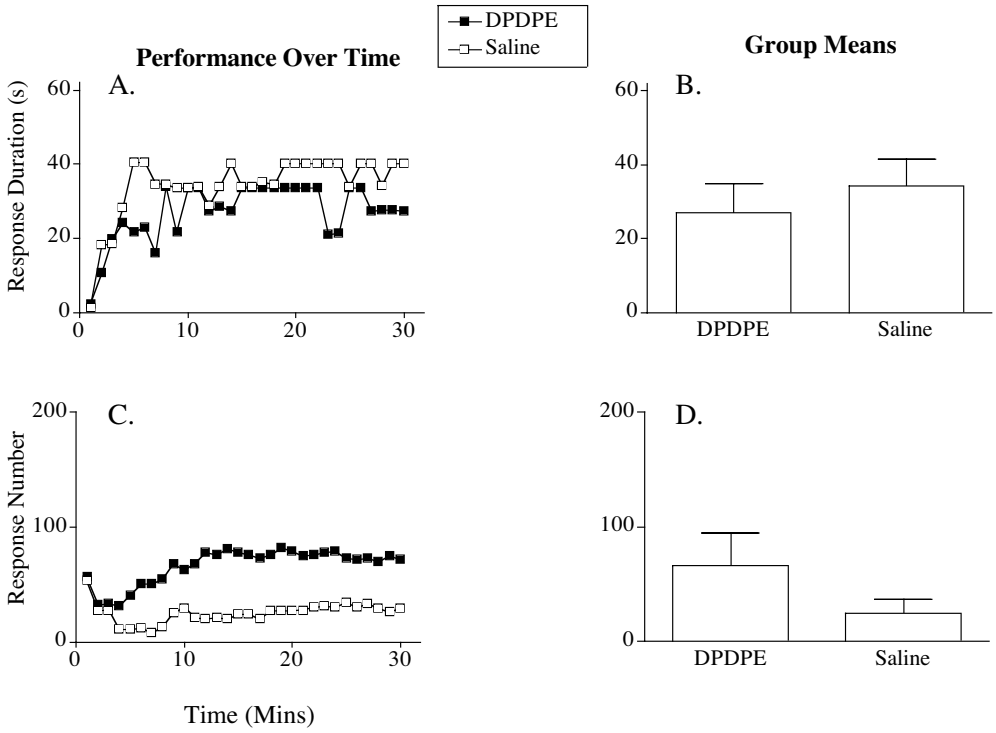

Figure 3. The effect of DPDPE on response duration (A) and response number (C) in subjects tested 10 minutes after drug treatment. The group means (±SE) for response duration and response number are presented in the right panels (B & D), respectively.

The effect of DPDPE on response number is depicted in Figure 3C. Saline-treated subjects displayed a decrease in response number as they acquired the instrumental response.

Although DPDPE-treated animals exhibited a higher level of responding across the test period, there was not a significant effect of drug treatment on response number, nor a Drug X Time interaction, both Fs < 1.56, $p > .05$. Only the main effect of time reached significance, $F(29, 464) = 1.96$, $p < .01$.

In summary, Experiment 1A determined that when subjects were tested 10 minutes following drug delivery, only the selective kappa-2 opioid receptor agonist, GR89696, disrupted learning. The selective kappa-1 opioid receptor agonist, U69593, had no such impact on learning. Experiments 1B and 1C verified that mu (DAMGO) and delta (DPDPE) opioid receptor agonists did not produce a deficit when subjects were tested 10 minutes after administration. These findings suggest that only the kappa-2 opioid receptor subtype plays a role in the behavioral deficit observed after uncontrollable shock.

EXPERIMENT 2

Experiment 1 found that only the selective kappa-2 opioid receptor agonist, GR89696, disrupted instrumental learning. Experiment 2 establishes the effective dose by assessing the impact of GR89696 across three molar concentrations.

Method

Twenty-four hours following spinal transection, subjects received intrathecal administration of 10 μl of GR89696 at one of four doses (0, 1.2, 6, or 30 nmol) followed by a 10 μl saline flush. The cannula was removed ten minutes later, and the animals were immediately tested for instrumental learning (as described in the General Methods section).

Results

Experiment 1 determined that GR89696 had no impact on the shock intensity necessary to produce a 0.4 N change in flexion force. The mean shock intensity after drug treatment ranged from 0.38 ($\pm$ 0.05) to 0.47 ($\pm$ 0.03) mA and were not significant, $F(3, 20) < 1.0$, $p > .05$.

The effect of GR89696 on response duration is depicted in panel A of Figure 4. Subjects that received saline exhibited an increase in response duration over the 30-minute testing period. GR89696 disrupted this learning in a dose-dependent fashion. A repeated measures ANOVA revealed significant main effects of dose and time, Fs > 3.37, $p < .05$. The Time X Dose interaction failed to reach significance, $F(87, 580) = 1.08$, $p > .05$. Post hoc comparisons of the group means, depicted in Figure 4B, determined that subjects that received saline or the lowest dose of GR89696 (1.2 nmol)

differed from the group that received the highest dose (30 nmol) of the drug, $p < .05$. No other group differences were significant, $p > .05$.

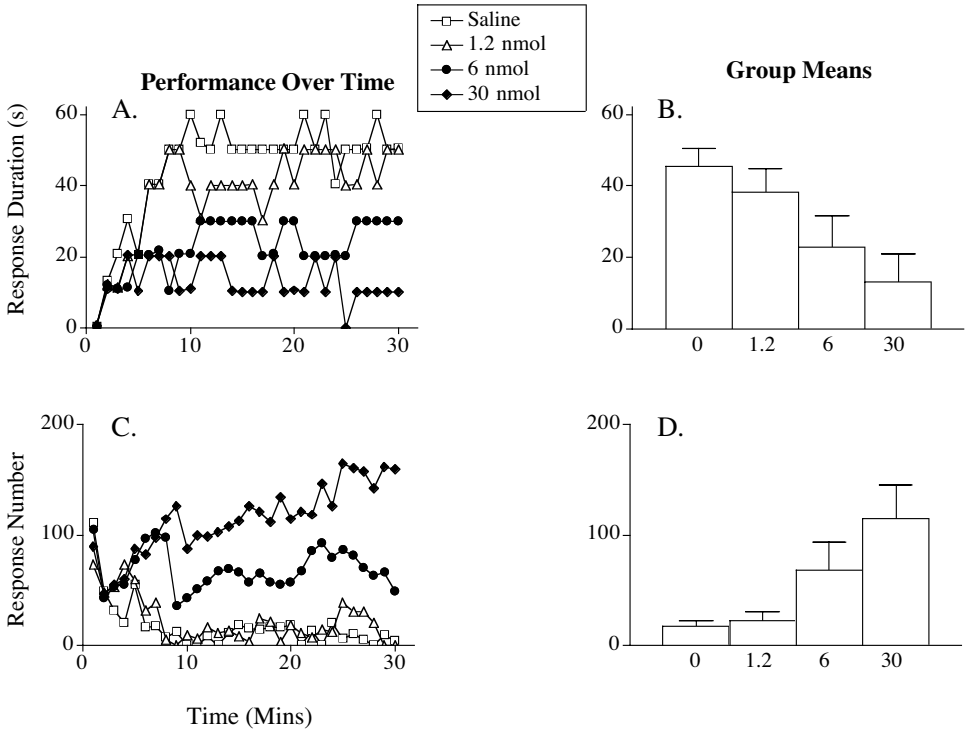

Figure 4. The impact of three doses of GR89696 on response duration (A) and response number (C) across time in subjects tested 10 minutes following drug delivery. The right panels (B & D) portray the group means (±SE) for response duration and response number, respectively.

Figure 4C illustrates the impact of GR89696 on response number. As vehicle-treated subjects learned the instrumental response, they showed a progressive decrease in the number of responses made over the 30-minute testing period. GR89696 dose-dependently prevented the acquisition of the flexion response, resulting in a steady increase in the number of responses made. An ANOVA revealed a significant main

effect of dose, $F(3, 580) = 4.20$, $p < .05$. The main effect of time did not reach significance, nor did the Time X Dose interaction, Fs < 1.02, $p > .05$. Post hoc comparisons of the group means, displayed in Figure 4D, determined that subjects that received the highest dose of GR89696 (30 nmol) differed from those that received either saline or the lowest dose of the drug (1.2 nmol), $p < .05$. No other group differences were significant, $p > .05$.

EXPERIMENT 3

Experiments 1 and 2 demonstrated that the kappa-2 opioid receptor agonist, GR89696, impairs learning when subjects are tested 10 minutes after drug treatment. The deficit observed after uncontrollable shock lasts at least 24 hours and wanes within 96 hours (Crown et al., 2002). Experiment 3 tests whether the deficit induced by GR89696 follows a similar time course. Because the long-term consequences of drug treatment likely vary as a function of dose, 2 doses were tested (6 and 30 nmols). Both of these doses disrupted learning/performance when subjects were tested 10 minutes after drug delivery. Experiment 3 tests whether these doses affect performance when testing is delayed by 24 or 96 hours.

Method

Twenty-four hours following surgery, subjects were brought to the instrumental testing room and received intrathecal delivery of GR89696 (10 μl) at one of three doses (0, 6, or 30 nmol) followed by a 10 μl saline flush. The cannula was removed ten minutes later, and all animals were returned to the recovery room, where they remained until instrumental testing either 24 or 96 hours later, yielding a 3 (0, 6, or 30 nmol) X 2 (24 or 96 hr) factorial design. Subjects received daily injections of saline (2.5 ml, i.p.) to prevent dehydration. On the day of testing, rats were brought to the instrumental testing room and tested for learning with 30 minutes of controllable shock.

Results

Mean ($\pm$ SEM) shock intensity ranged from 0.43 ($\pm$ 0.04) to 0.55 ($\pm$ 0.04) mA. No significant group differences were observed on this measure, all $Fs < 1.35$, $p > .05$.

Figure 5A depicts the impact of GR89696 on response duration when subjects are tested 24 and 96 hours after drug delivery. All saline-treated rats, independent of time point, exhibited increased response durations over the 30-minute testing period. The lowest dose of GR89696 (6 nmol) had no effect on this learning at either time point. However, the highest dose (30 nmol) of the drug disrupted learning at both the 24- and 96-hour time points. A repeated measures ANOVA revealed significant main effects of dose and time, Fs > 3.69, $p < .05$. The main effect of test time did not reach significance, nor did the Dose X Delay or the Dose X Delay X Time interactions, all Fs < 1.05, $p > .05$. Post hoc comparisons of the group means (Figure 5B) revealed that all subjects in the 6 nmol group tested at either the 24- or 96-hour timepoint differed from those in the 30 nmol group that were tested 24 hours after drug treatment, $p < .05$. Subjects that received vehicle 24 hours prior to testing also differed from this group, $p < .05$. No other group differences were significant, $p > .05$.

All subjects, except those given the highest dose of GR89696 prior to testing, displayed a decrease in response number as they acquired the instrumental response (Figure 5C). Rats that received the highest dose of drug 24 or 96 hours prior to testing failed to learn and, consequently, exhibited a high level of responding during testing. In the latter portion of the test session, these subjects exhibited some habituation, which led to a decrease in response number. However, an ANOVA failed to reveal any significant effects, all Fs < 2.04, $p > .05$.

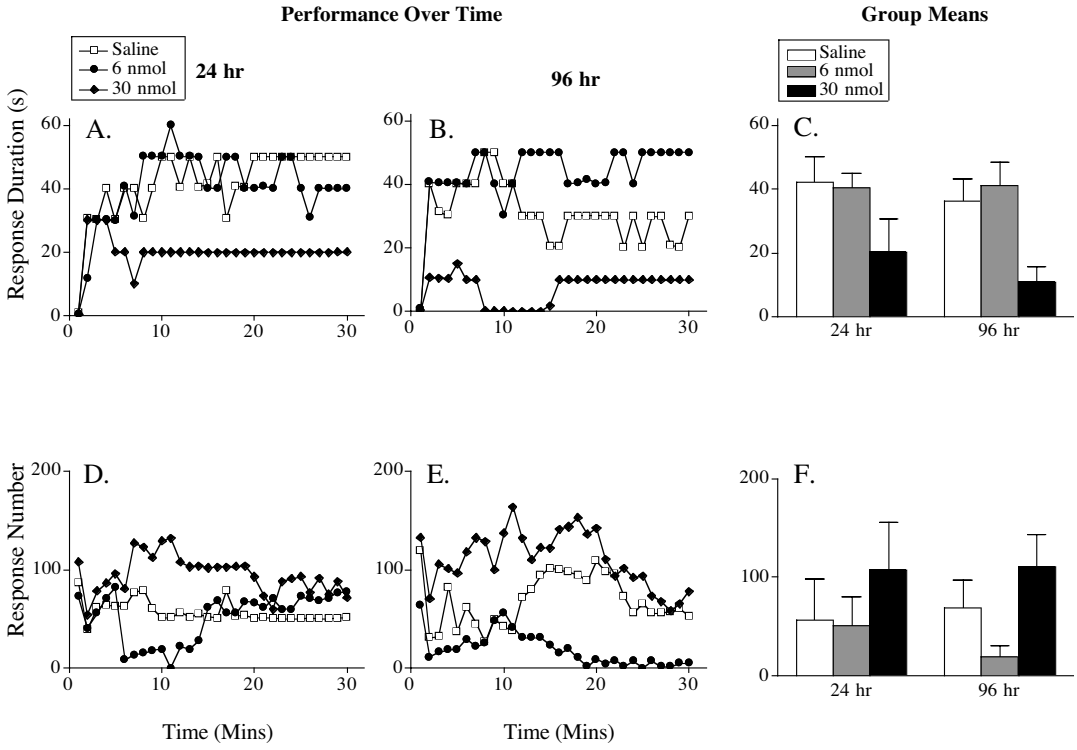

Figure 5. The effect of GR89696 on response duration (panels A&B) and response number (panels D&E) across time in subjects tested 24 and 96 hours after drug treatment. The group means (±SE) for response duration and response number appear in the right panels (C&F), respectively.

To summarize, animals that received the highest dose of GR89696 failed to learn when tested either 24 or 96 hours after treatment. However, the lowest dose of the drug did not impair learning at either time point. This finding suggests that the deficit induced by the high dose of GR89696 does not follow the same timecourse as uncontrollable shock. The drug may have a longer lasting effect possibly because the highest dose induces a degenerative process that permanently alters the capacity for learning.

EXPERIMENT 4

If uncontrollable shock and GR89696 produce a long-term deficit because they engage similar biochemical systems, as suggested by model A, then these two treatments should summate to produce a more robust effect. Alternatively, GR89696 may prevent the induction of the behavioral deficit if it inhibits NMDA receptors during uncontrollable shock as suggested in model B. This experiment tests these predictions.

Method

Twenty-four hours following surgery, subjects received intrathecal administration of either saline or GR89696 (6 nmol) followed by uncontrollable tailshock or nothing, yielding a 2 (saline or GR89696) X 2 (shock or nothing) design. Ten minutes later the cannula was removed, and the animals were placed into opaque back Plexiglas tubes (as described above). Three pieces of porous tape were extended across the tube to loosely restrain the subject, and an electrode coated with ECG gel was secured approximately 6 cm behind the base of the tail with a piece of porous tape. Rats then received 6 minutes of uncontrollable tailshock (1.5 mA, intermittent) or an equal amount of tube restraint (no shock). After shock treatment, subjects were returned to the recovery room until they were tested for instrumental learning 24 hours later.

Results

Mean ($\pm$ SEM) shock intensity ranged from 0.40 ($\pm$ 0.05) to 0.45 ($\pm$ 0.02) mA. No significant group differences were observed on this measure, all $Fs < 0.69$, $p > .05$.

The effect of GR89696 administered prior to uncontrollable shock on response duration is presented in Figure 6A. Unshocked saline controls displayed increased

response durations over the 30-minute testing period. Uncontrollable shock disrupted this learning, an effect that was attenuated by GR89696. A two-way, repeated measures ANOVA revealed a significant main effect of time, $F(29, 580) = 4.03$, $p < .05$, and a significant Drug X Shock X Time three-way interaction, $F(29, 580) = 2.62$, $p < .05$. To further characterize this interaction, one-way ANOVAs were used to evaluate the impact of GR89696 in shocked and unshocked subjects, independently. In shocked rats, drug treatment significantly improved performance over time, $F(29, 290) = 1.82$, $p < .05$. Drug treatment did not have a significant effect in the unshocked subjects, $F(29, 290) = 4.45$, $p < .05$.

Figure 6C illustrates the impact of GR89696 treatment given prior to uncontrollable shock on response number. Saline-treated subjects that received uncontrollable shock prior to testing displayed a higher rate of responding than any other group as they failed to acquire the instrumental response. The impact of shock treatment was attenuated by GR89696. An ANOVA uncovered a significant Drug X Shock interaction, $F(1, 20) = 4.70$, $p < .05$. No other significant effects were found, $p > .05$.

In summary, there was no indication that the long-term effect of the kappa agonist and shock showed additivity to produce a more robust long-term deficit. Instead, GR89696 attenuated the induction of the behavioral deficit when delivered prior to uncontrollable shock. This protective effect may be mediated through its action on the NMDA receptor.

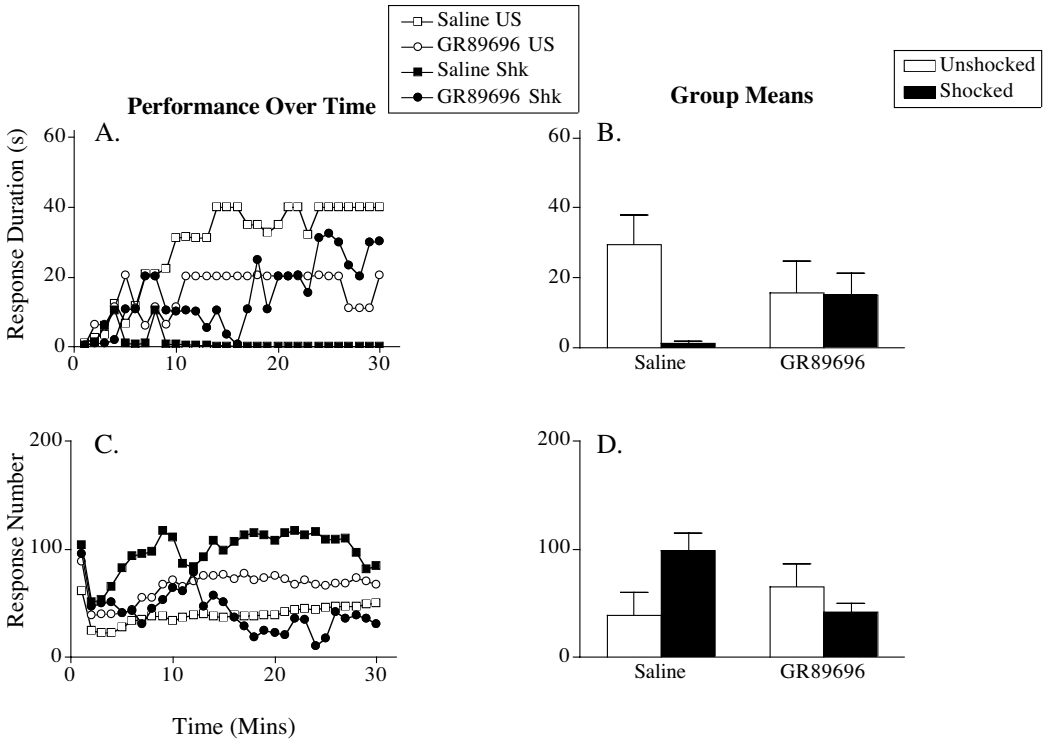

Figure 6. The impact of GR89696 administered prior to uncontrollable shock on response duration (A) and response number (C) across time. Subjects were tested 24 hours after shock exposure. The right panels (B & D) represent the group means (±SE) for response duration and response number, respectively. US=unshocked; Shk=shocked.

DISCUSSION AND SUMMARY

The findings from Experiment 1 support the hypothesis that the behavioral deficit induced by uncontrollable shock results from activation of the kappa opioid receptor system, specifically the kappa-2 subtype. This finding complements previous studies demonstrating that nor-BNI, a kappa receptor antagonist, prevents the deficit if given immediately before testing (Joynes & Grau, 2004). Because nor-BNI has a higher affinity for the kappa-1 receptor (Randic et al., 1995), the earlier data appeared to implicate this form of the kappa receptor. However, nor-BNI exhibits cross reactivity with the kappa-2 receptor (Ni et al., 1995). Further, the kappa-2 receptor is the predominant form within the lumbosacral region of the spinal cord (Gouarderes, Audigier, & Cros, 1982; Gouarderes, Attali, Audigier, & Cros, 1983, Caudle et al., 1998), where instrumental learning appears to occur (Liu, Crown, Miranda, & Grau, 2002). These observations, in conjunction with the relative effectiveness of the kappa-1 and kappa-2 agonists, suggest that instrumental learning is modulated by the kappa-2 receptor.

Both of the biochemical models of NMDA modulation by kappa agonists predict that higher doses of GR89696 will disrupt learning. Experiment 1 only examined the impact of a relatively high dose of the drug; therefore, we examined the dose response function in Experiment 2. We found that GR89696 disrupts learning in a dose-dependent fashion when administered 10 minutes prior to instrumental testing. This result supports biochemical model B, which predicts that both high and low doses will impair learning through NMDA inhibition. Model A predicts that low, non-toxic doses

of the drug could facilitate learning by enhancing NMDA function. Within the dose range tested, there was no evidence that GR89696 facilitated learning.

Joynes and Grau (2004) previously showed that a kappa opioid antagonist inhibits the expression of the deficit, but not its induction. The latter suggests that kappa opioid release per se does not underlie the long-term consequences of shock treatment. Experiment 3 addressed this issue by testing whether GR89696 impaired learning when subjects were tested 24 and 96 hours after treatment. We found that only the highest dose (30 nmol) of GR89696 produced a long-lasting impairment that outlasted the typical effect of uncontrollable shock. This finding supports the prediction made by model A that high doses of a kappa agonist could have long-term effects. However, the result that a lower dose (6 nmol), which had a short-term effect, did not produce a long-term learning deficit supports models A and B, which both predict that low doses will not produce any long-term consequences. This outcome was not surprising given that there is no evidence suggesting that low doses of GR89696 induce long-lasting changes to the NMDA receptor system. Therefore, it should not have any impact once it has cleared the system.

The impairment produced by the highest dose of GR89696 may persist longer than 96 hours because it produces a more robust, longer-lasting deficit than that seen after uncontrollable shock. Extending the time between drug treatment and testing beyond 96 hours would answer this question. If learning does not return after an extended period of time, an alternative explanation, such as cell death, may be more feasible. Supporting this, research has shown that intrathecal infusion of high doses of

dynorphin into lower regions of the spinal cord causes motor impairment accompanied by neuronal cell loss (Bakshi et al., 1992; Caudle & Isaac, 1987). This cell loss was evident 24 hours after injection (Gaumann, Grabow, Yaksh, Casey, & Rodriguez, 1990) and appears to involve activation of caspase-3 and mitochondrial release of cytochrome c (Singh et al., 2003). Interestingly, we observed cell death in the lower lumbar regions of the spinal cord of subjects exposed to uncontrollable stimulation that peaked 24 hours after exposure (Liu et al., 2003). This evidence supports the prediction made by model A that high doses of a kappa agonist exacerbate NMDA-mediated overexcitation to produce permanent cell loss and a long-term disruption of learning.

Experiment 4 found that GR89696 did not worsen the consequences of uncontrollable shock as model A predicted. Instead, GR89696 protected spinal cord neurons from the consequences of uncontrollable stimulation. This protective effect may be mediated by the inhibition of NMDA receptor function, proposed in model B, during uncontrollable stimulation. This finding has important clinical implications for both maladaptive (central sensitization) and adaptive (instrumental learning) plasticity in the spinal cord. Indeed, others have shown certain kappa agonists can be used to prevent and reverse hyperalgesia and allodynia in animal models of neuropathic pain (Eliav et al., 1999; Ho et al., 1997). Our work suggests that kappa agonists can be used to preserve adaptive forms of plasticity within the spinal cord.

Overall, the results from this study suggest that kappa agonists have both beneficial and detrimental effects on instrumental learning. They produce a long-lasting behavioral deficit at high doses and cause a short-term behavioral deficit when lower

concentrations are present in the system during instrumental testing. However, kappa agonists can also protect spinal cord neurons against the consequences of uncontrollable stimulation. These contradictory findings can be rationalized in the following way: Kappa agonists exert both antinociceptive and pronociceptive effects mediated through kappa and NMDA receptors, respectively. From an evolutionary perspective, this system may have developed both antinociceptive and pronociceptive properties to allow escape from danger (antinociceptive) and protection of the damaged tissue (hyperalgesia and allodynia) once the threat has passed (Caudle & Mannes, 2000). Lai and colleagues (2001) suggest that these nociceptive effects are regulated by the amount of endogenous kappa opioids released into the system after noxious input, with low concentrations resulting in inhibition of neuronal activity (analgesia) and higher concentrations (from sustained release) resulting in excitation of spinal cord neurons (pain and/or loss of function). Based on this idea, excessive amounts of endogenous kappa opioids would accumulate following CNS injury and could lead to the development of central sensitization (neuropathic pain) and loss of plasticity, both of which could hinder recovery of function. This outcome is predicted by model A and supported by our finding that high doses of a kappa agonist produce a long-lasting behavioral deficit. Treatment strategies that prevent this accumulation of endogenous kappa opioids after injury, like kappa opioid antagonists, would indeed foster recovery of function.

In addition to the amount of kappa opioids in the cord, other factors, such as the type of noxious input, may influence the effects of these peptides. For example, we have shown that continuous uncontrollable shock produces antinociception in the tail-flick

test and does not impair learning (Crown et al., 2002). An equivalent amount of uncontrollable intermittent shock, on the other hand, does not induce antinociception in the tail-flick test, but rather induces a tactile allodynia (Ferguson et al., 2000) and impairs learning for up to 24 hours (Crown et al., 2002). One interpretation of these findings is that continuous shock elicits an endogenous kappa opioid release that protects the cord, whereas intermittent shock engages a maladaptive release that disrupts spinal cord plasticity.

The finding that kappa agonists inhibit instrumental learning within the spinal cord mirrors results from earlier studies examining the role of opioids in learning and memory. The impact of opioids in areas of the brain associated with reward has been extensively investigated (Beluzzi & Stein, 1977; Mucha & Herz, 1985); however, this discussion will only focus on forms of learning and memory associated with the hippocampus. The simplicity of the hippocampus has made it an ideal structure for studying the neurobiological mechanisms of learning and memory. Three major pathways exhibit LTP in the hippocampus: the perforant-path, the Schaffer collateral, and the mossy fibers (Morris & Johnston, 1995). NMDA receptor activation induces LTP within the perforant-path and the Schaffer collaterals but not in the mossy fibers. The coexistence of glutamate and opioids within the mossy fibers led researchers to speculate that opioids modulate plasticity at these synapses (Morris & Johnston, 1995). Further investigation revealed that, in general, mu and delta receptor activation enhances synaptic plasticity in the hippocampus, whereas activation of kappa receptors inhibits it (Terman, Wagner, & Chavkin, 1994; Wagner et al., 1993; Weisskopf, Zalutsky, &

Nicoll, 1993). Behavioral studies demonstrating that opioids affect spatial memory extend these findings. Doses of naloxone that block kappa receptors improve spatial memory in the radial maze task (Gallagher, King, & Young, 1983; Collier & Routtenberg, 1984), and microinjection of dynorphin into the hippocampus impairs spatial learning in the Morris water maze (Sandin et al., 1998). The results from these hippocampal studies support the idea put forth by model B that kappa agonists inhibit NMDA receptor function and that any form of learning that depends on this receptor system will be impaired by kappa receptor activation. Collectively, these findings indicate that opioids play a pivotal role in learning and memory processes in the hippocampus. Our work suggests that these effects may be conserved across the nervous system, including the spinal cord.

REFERENCES

Bakshi, R., & Faden, A. I. (1990a). Blockade of the glycine modulatory site of NMDA receptors modifies dynorphin-induced behavioral effects. *Neuroscience Letters*, *110*, 113-117.

Bakshi, R., & Faden, A. I. (1990b). Competitive and non-competitive NMDA antagonists limit dynorphin A-induced rat hindlimb paralysis. *Brain Research*, *507*, 1-5.

Bakshi, R., Newman, A. H., & Faden, A. I. (1990c). Dynorphin A-(1-17) induces alterations in free fatty acids, excitatory amino acids, and motor function through an opiate-receptor-mediated mechanism. *Journal of Neuroscience*, *10*, 3793-3800.

Bakshi, R., Ni, R. X., & Faden, A. I. (1992). N-methyl-D-aspartate (NMDA) and opioid receptors mediate dynorphin-induced spinal cord injury: behavioral and histological studies. *Brain Research*, *580*, 255-264.

Beggs, A. L., Steinmetz, J. E., & Patterson, M. M. (1985). Classical conditioning of a flexor nerve response in spinal cats: Effects of tibial nerve CS and a differential conditioning paradigm. *Behavioral Neuroscience, 99*, 496-508.

Beggs, A. L., Steinmetz, J. E., Romano, A. G., & Patterson, M. M. (1983). Extinction and retention of a classically conditioned flexor nerve response in acute spinal cat. *Behavioral Neuroscience, 97*, 530-540.

Belluzzi, J. D., & Stein, L. (1977). Enkephaline may mediate euphoria and drive-reduction reward. *Nature*, *266*, 556-558.

Birch, P. J., Rogers, H., Hayes, A. G., Hayward, N. J., Tyers, M. B., Scopes, D. I.,

Naylor, A., & Judd, D. B. (1991). Neuroprotective actions of GR89696, a highly potent and selective kappa-opioid receptor agonist. *British Journal of Pharmacology*, *103*, 1819-1823.

Bliss, T. V., & Lomo, T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *Journal of Physiology*, *232*, 331-356.

Buerger, A. A., & Fennessy, A. (1970). Long-term alteration of leg position due to shock avoidance by spinal rats. *Nature, 225*, 751-752.

Caudle, R. M., Chavkin, C., & Dubner, R. (1994). Kappa 2 opioid receptors inhibit NMDA receptor-mediated synaptic currents in guinea pig CA3 pyramidal cells. *Journal of Neuroscience*, *14*, 5580-5589.

Caudle, R. M., Finegold, A. A., Mannes, A. J., Tobias, M. D., Kenshalo, D. R., Jr., & Iadarola, M. J. (1998). Spinal kappa1 and kappa2 opioid binding sites in rats, guinea pigs, monkeys and humans. *Neuroreport*, *9*, 2523-2525.

Caudle, R. M., & Isaac, L. (1987). Intrathecal dynorphin(1-13) results in an irreversible loss of the tail-flick reflex in rats. *Brain Research*, *435*, 1-6.

Caudle, R. M., & Isaac, L. (1988). A novel interaction between dynorphin(1-13) and an N-methyl-D-aspartate site. *Brain Research*, *443*, 329-332.

Caudle, R. M., & Mannes, A. J. (2000). Dynorphin: friend or foe? *Pain*, *87*, 235-239.

Chopin, S. F., & Buerger, A. A. (1976). Instrumental avoidance conditioning in the spinal rat. *Brain Research Bulletin, 1*, 177-183.

Coderre, T. J. (1993). The role of excitatory amino acid receptors and intracellular messengers in persistent nociception after tissue injury in rats. *Molecular Neurobiology, 7*, 229-246.

Collier, T. J., & Routtenberg, A. (1984). Selective impairment of declarative memory following stimulation of dentate gyrus granule cells: a naloxone-sensitive effect. *Brain Research*, *310*, 384-387.

Crown, E. D., Joynes, R. L., Ferguson, A. R., & Grau, J. W. (2002). Instrumental learning within the spinal cord: IV. Induction and retention of the behavioral deficit observed after noncontingent shock. *Behavioral Neuroscience, 116*, 1032-1051.

Dickenson, A.H. (1990). A cure for wind up: NMDA receptor antagonists as potential analgesics. *Trends in Pharmacological Sciences, 11*, 307-309.

Dickenson, A. H. (1996). Balances between excitatory and inhibitory events in the spinal cord and chronic pain. *Progress in Brain Research, 110*, 225-231.

Dray, A., Urban, L., & Dickenson, A. (1994). Pharmacology of chronic pain. *Trends in Pharmacological Sciences, 15*, 190-197.

Eliav, E., Herzberg, U., & Caudle, R. M. (1999). The kappa opioid agonist GR89,696 blocks hyperalgesia and allodynia in rat models of peripheral neuritis and neuropathy. *Pain*, *79*, 255-264.

Faden, A.I. (1993). Role of opioids in central nervous system injury. In A. Hertz, H. Akil & E.J. Simon (Eds.), *Handbook of experimental pharmacology* (pp. 325-341). Berlin: Springer-Verlag.

Faden, A. I. (1996). Neurotoxic versus neuroprotective actions of endogenous opioid

peptides: implications for treatment of CNS injury. *NIDA Research Monograph*, *163*, 318-330.

Faden, A. I., & Jacobs, T. P. (1983). Dynorphin induces partially reversible paraplegia in the rat. *European Journal of Pharmacology*, *91*, 321-324.

Faden, A. I., Jacobs, T. P., & Holaday, J. W. (1981a). Opiate antagonist improves neurologic recovery after spinal injury. *Science*, *211*, 493-494.

Faden, A. I., Jacobs, T. P., Mougey, E., & Holaday, J. W. (1981b). Endorphins in experimental spinal injury: therapeutic effect of naloxone. *Annals of Neurology*, *10*, 326-332.

Faden, A. I., Takemori, A. E., & Portoghese, P. S. (1987). Kappa-selective opiate antagonist nor-binaltorphimine improves outcome after traumatic spinal cord injury in rats. *Central Nervous System Trauma*, *4*, 227-237.

Ferguson, A.R., Crown, E. D., Dhruv, S., Washburn, S. N., & Grau, J. W. (2000). Instrumental learning in spinalized rats: The GABA antagonist bicuculline prevents the behavioral deficit observed after noncontingent shock. *Society for Neuroscience Abstracts*, *26*, 2207.

Ferguson, A.R., Crown, E.D., Patton, B.C., & Grau, J.W. (2002, November). *Intrathecal administration of the NMDA antagonist MK-801 prevents the behavioral deficit observed after noncontingent shock in spinalized rats* (Poster session presented at the annual meeting of the Society for Neuroscience, Orlando, FL, Program No. 166.7 [Abstract]). Retrieved December 29, 2004, from http://sfn.scholarone.com/itin/2002/

Ferguson, A. R., Crown, E. D., Washburn, S. N., Miranda, R. C., & Grau, J. W. (2001). Instrumental learning in spinalized rats: Peripheral inflammation undermines behavioral potential in the spinal cord. *Society for Neuroscience Abstracts*, *27*, 2202.

Ferguson, A.R., Hook, M.A., & Grau, J.W. (2004, October) *Uncontrollable stimulation inhibits plasticity within the rat spinal cord: Role of group I metabotropic glutamate receptors* (Poster session presented at the annual meeting of the Society for Neuroscience, San Diego, CA, Program No. 418.2 [Abstract]). Retrieved December 29, 2004, from http://sfn.scholarone.com/itin/2004/

Fitzgerald, L. A., & Thompson, R. F. (1967). Classical conditioning of the hindlimb flexion reflex in the acute spinal cat. *Psychonomic Science, 47*, 345-351.

Gallagher, M., King, R. A., & Young, N. B. (1983). Opiate antagonists improve spatial memory. *Science*, *221*, 975-976.

Gaumann, D. M., Grabow, T. S., Yaksh, T. L., Casey, S. J., & Rodriguez, M. (1990). Intrathecal somatostatin, somatostatin analogs, substance P analog and dynorphin A cause comparable neurotoxicity in rats. *Neuroscience*, *39*, 761-774.

Gouarderes, C., Attali, B., Audigier, Y., & Cros, J. (1983). Interaction of selective mu and delta ligands with the kappa 2 subtype of opiate binding sites. *Life Sciences*, *33 Suppl 1*, 175-178.

Gouarderes, C., Audigier, Y., & Cros, J. (1982). Benzomorphan binding sites in rat lumbo-sacral spinal cord. *European Journal of Pharmacology*, *78*, 483-486.

Grau, J. W., Barstow, D. G. & Joynes, R. L. (1998). Instrumental learning within the spinal cord: I. Behavioral properties. *Behavioral Neuroscience, 112,* 1366-1386.

Hall, E. D., Wolf, D. L., Althaus, J. S., & Von Voigtlander, P. F. (1987). Beneficial effects of the kappa opioid receptor agonist U-50488H in experimental acute brain and spinal cord injury. *Brain Research*, *435*, 174-180.

Ho, J., Mannes, A. J., Dubner, R., & Caudle, R. M. (1997). Putative kappa-2 opioid agonists are antihyperalgesic in a rat model of inflammation. *Journal of Pharmacology and Experimental Therapeutics*, *281*, 136-141.

Huie, J.R., Ferguson, A.R., & Grau, J.W. (2004, October) *Instrumental learning in spinalized rats: neuropathic pain inhibits learning in the spinal cord* (Poster session presented at the annual meeting of the Society for Neuroscience, San Diego, CA, Program No. 418.2 [Abstract]). Retrieved December 29, 2004, from http://sfn.scholarone.com/itin/2004/

Ji, R. R., Kohno, T., Moore, K. A., & Woolf, C. J. (2003). Central sensitization and LTP: do pain and memory share similar mechanisms? *Trends in Neurosciences*, *26*, 696-705.

Joynes, R. L., & Grau, J. W. (2004). Instrumental learning within the spinal cord: III. Prior exposure to noncontingent shock induces a behavioral deficit that is blocked by an opioid antagonist. *Neurobiology of Learning and Memory*, *82*, 35-51.

Joynes, R. L., Janjua, K., & Grau, J. W. (2004). Instrumental learning within the spinal cord: VI. The NMDA receptor antagonist, AP5, disrupts acquisition and maintenance of an acquired flexion response. *Behavioural Brain Research*, *154*, 431-438.

Lai, J., Ossipov, M. H., Vanderah, T. W., Malan, T. P., Jr., & Porreca, F. (2001). Neuropathic pain: the paradox of dynorphin. *Molecular Interventions*, *1*, 160-167.

Liu, G .T., Crown, E.R., Miranda, R.C., Grau, J.W. (2002, November). *Instrumental learning within the rat spinal cord: Localization of the essential neural circuit* (Poster session presented at the annual meeting of the Society for Neuroscience, Orlando, FL, Program No. 167.17 [Abstract]). Retrieved December 29, 2004, from http://sfn.scholarone.com/itin/2002/

Liu, G.T., Miranda, R. C., Wilkie, J. M., Washburn, S. N., Bolding, K. A., Hook, M.A., Crown, E. D., Ferguson, A. R., Grau, J. W. (2003, November) *Exposure to uncontrollable nociceptive stimulation increases vulnerability to apoptosis in spinally transected rats circuit* (Poster session presented at the annual meeting of the Society for Neuroscience, New Orleans, LA, Program No. 497.1 [Abstract]). Retrieved December 29, 2004, from http://sfn.scholarone.com/itin/2003/

Lynch, G., & Baudry, M. (1984). The biochemistry of memory: a new and specific hypothesis. *Science*, *224*, 1057-1063.

Madison, D. V., Malenka, R. C., & Nicoll, R. A. (1991). Mechanisms underlying long-term potentiation of synaptic transmission. *Annual Review of Neuroscience*, *14*, 379-397.

Morris, R. G., Anderson, E., Lynch, G. S., & Baudry, M. (1986). Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5. *Nature*, *319*, 774-776.

Morris, B. J., & Johnston, H. M. (1995). A role for hippocampal opioids in long-term functional plasticity. *Trends in Neurosciences*, *18*, 350-355.

Moser, E. I., Krobert, K. A., Moser, M. B., & Morris, R. G. (1998). Impaired spatial learning after saturation of long-term potentiation. *Science, 281,* 2038–2042.

Moser, E.I., & Moser, M-B. (1999). Is learning blocked by saturation of synaptic weights in the hippocampus? *Neuroscience Biobehavioral Reviews, 23*, 661-672.

Mucha, R. F., & Herz, A. (1985). Motivational properties of kappa and mu opioid receptor agonists studied with place and taste preference conditioning. *Psychopharmacology*, *86*, 274-280.

Ni, Q., Xu, H., Partilla, J. S., de Costa, B. R., Rice, K. C., Kayakiri, H., & Rothman, R. B. (1995). Opioid peptide receptor studies. 3. Interaction of opioid peptides and other drugs with four subtypes of the kappa 2 receptor in guinea pig brain. *Peptides*, *16*, 1083-1095.

Patterson, M.M., Cegavske, C.F., & Thompson, R.F. (1973). Effects of a classical conditioning paradigm on hind-limb flexion nerve response in the immobilized cat. *Journal of Comparative and Physiological Psychology, 84*, 88-97.

Randic, M., Cheng, G., & Kojic, L. (1995). kappa-opioid receptor agonists modulate excitatory transmission in substantia gelatinosa neurons of the rat spinal cord. *Journal of Neuroscience*, *15*, 6809-6826.

Ren, K., & Dubner, R. (1993). NMDA receptor antagonists attenuate mechanical hyperalgesia in rats with unilateral inflammation of the hindpaw. *Neuroscience Letters*, *163*, 22-26.

Ren, K., Hylden, J. L., Williams, G. M., Ruda, M. A., & Dubner, R. (1992a). The effects of a non-competitive NMDA receptor antagonist, MK-801, on behavioral hyperalgesia

and dorsal horn neuronal activity in rats with unilateral inflammation. *Pain*, *50*, 331-344.

Ren, K., Williams, G. M., Hylden, J. L., Ruda, M. A., & Dubner, R. (1992b). The intrathecal administration of excitatory amino acid receptor antagonists selectively attenuated carrageenan-induced behavioral hyperalgesia in rats. *European Journal of Pharmacology*, *219*, 235-243.

Sandin, J., Nylander, I., Georgieva, J., Schott, P. A., Ogren, S. O., & Terenius, L. (1998). Hippocampal dynorphin B injections impair spatial learning in rats: a kappa-opioid receptor-mediated effect. *Neuroscience*, *85*, 375-382.

Singh, I. N., Goody, R. J., Goebel, S. M., Martin, K. M., Knapp, P. E., Marinova, Z., Hirschberg, D., Yakovleva, T., Bergman, T., Bakalkin, G., & Hauser, K. F. (2003). Dynorphin A (1-17) induces apoptosis in striatal neurons in vitro through alpha-amino-3-hydroxy-5-methylisoxazole-4-propionate/kainate receptor-mediated cytochrome c release and caspase-3 activation. *Neuroscience*, *122*, 1013-1023.

Skilling, S. R., Sun, X., Kurtz, H. J., & Larson, A. A. (1992). Selective potentiation of NMDA-induced activity and release of excitatory amino acids by dynorphin: possible roles in paralysis and neurotoxicity. *Brain Research*, *575*, 272-278.

Terman, G. W., Wagner, J. J., & Chavkin, C. (1994). Kappa opioids inhibit induction of long-term potentiation in the dentate gyrus of the guinea pig hippocampus. *Journal of Neuroscience*, *14*, 4740-4747.

Thompson, R. F. & Spencer, W. A. (1966). A model phenomenon for the study of neuronal substrates of behavior. *Psychological Review, 73*, 16-43.

Wagner, J. J., Caudle, R. M., & Chavkin, C. (1992). Kappa-opioids decrease excitatory transmission in the dentate gyrus of the guinea pig hippocampus. *Journal of Neuroscience*, *12*, 132-141.

Wagner, J. J., Terman, G. W., & Chavkin, C. (1993). Endogenous dynorphins inhibit excitatory neurotransmission and block LTP induction in the hippocampus. *Nature*, *363*, 451-454.

Wall, P. D., & Woolf, C. J. (1984). Muscle but not cutaneous C-afferent input produces prolonged increases in the excitability of the flexion response in the rat. *Journal of Physiology, 356*, 443-458.

Weisskopf, M. G., Zalutsky, R. A., & Nicoll, R. A. (1993). The opioid peptide dynorphin mediates heterosynaptic depression of hippocampal mossy fibre synapses and modulates long-term potentiation. *Nature*, *362*, 423-427.

Werz, M. A., & MacDonald, R. L. (1983). Opioid peptides selective for mu- and delta-opiate receptors reduce calcium-dependent action potential duration by increasing potassium conductance. *Neuroscience Letters*, *42*, 173-178.

Werz, M. A., & Macdonald, R. L. (1985). Dynorphin and neoendorphin peptides decrease dorsal root ganglion neuron calcium-dependent action potential duration. *Journal of Pharmacology and Experimental Therapeutics*, *234*, 49-56.

Willis, W. D., Sluka, K. A., Rees, H., & Westlund, K. N. (1996). Cooperative mechanisms of neurotransmitter action in central nervous sensitization. *Progress in Brain Research, 110*, 151-166.

Woolf, C. J. (1983). Evidence for a central component of post-injury pain hypersensitivity. *Nature, 305*, 686-688.

Woolf, C. J. (1984). Long term alterations in the excitability of the flexion reflex produced by peripheral tissue injury in chronic decerebrate rat. *Pain, 18*, 325-343.

Yaksh, T. L., & Rudy, T. A. (1976). Chronic catheterization of the spinal subarachnoid space. *Physiology and Behavior*, *17*, 1031-1036.

Zukin, R. S., Eghbali, M., Olive, D., Unterwald, E. M., & Tempel, A. (1988). Characterization and visualization of rat and guinea pig brain kappa opioid receptors: evidence for kappa 1 and kappa 2 opioid receptors. *Proceedings of the National Academy of Sciences U S A*, *85*, 4061-4065.

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Grau, J.W., Washburn, S.N., Hook, M.A., Ferguson, A.R., Crown, E.D., Garcia, G., Bolding, K.A., Wilkie, J., Miranda, R.C. (2004). Uncontrollable stimulation undermines recovery after spinal cord injury. *Journal of Neurotrauma*, *21*, 1795-1817.

Hook, M.A., Ferguson, A.R., Garcia, G., Washburn, S.N., Koehly, L.M., & Grau, J.W. (2004). Monitoring recovery after injury: procedures for deriving the optimal test window. *Journal of Neurotrauma*, *21*, 109-18.