AMPA-RECEPTOR MEDIATED PLASTICITY WITHIN THE RAT SPINAL CORD

A Thesis

by

KEVIN CORCORAN HOY JR.

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 2008

Major Subject: Psychology

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Approved by:

Chair of Committee, James Grau
Committee Members, Barry Setlow
Rajesh Miranda
Head of Department, Les Morey

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ABSTRACT

AMPA-Receptor Mediated Plasticity within the Rat Spinal Cord.

(August 2008)

Kevin Corcoran Hoy Jr., B.A., Kent State University

Chair of Advisory Committee: Dr. James W. Grau

Previous research from our laboratory has demonstrated that the spinal cord is capable of a simple form of instrumental learning. In this instrumental learning paradigm, rats typically receive a complete spinal transection at the second thoracic vertebra, and are tested 24 hours after surgery. Subjects that receive shock to a hind leg quickly learn to maintain the leg in a flexed position, reducing net shock exposure whenever that leg is extended (controllable shock). Subjects that receive shock that is independent of leg position do not exhibit an increase in flexion duration (uncontrollable shock). This behavioral deficit can be induced with shock to the leg or tail and as little as 6 minutes of uncontrollable shock impairs learning for up to 48 hours.

The present thesis explores how the related α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid-receptor (AMPAR) ionotropic glutamate receptor affects spinal instrumental learning. Experiment 1 showed that inactivation of the AMPAR by administration of an antagonist blocks the acquisition of instrumental learning in a dose dependant fashion. Experiment 2 demonstrated that blocking the AMPAR after the acquisition of the instrumental response subsequently blocked the maintenance of that

response. Experiment 3 revealed that antagonizing the AMPAR during uncontrollable shock blocked the acquisition of the learning deficit. Experiments 4-6 demonstrated that the activation of the AMPAR at high levels could acutely block the acquisition spinal instrumental learning. Understanding how the AMPAR influences learning in the spinal cord will lead us to develop therapeutic strategies for recovery of function after spinal cord injury.

DEDICATION

The author would like to dedicate this thesis to the Hoy family: Kevin Sr., Lisa, Keri, and Thomas. This thesis would not have been possible without their love and constant encouragement.

ACKNOWLEDGEMENTS

The author would like to thank Dr. James W. Grau for his patience and support during all phases of this work. The author also wishes to thank Drs. Barry Setlow and Rajesh Miranda for serving as committee members. The author thanks Dr. Kyle Baumbauer, Dr. Michelle Hook, Dr. Stephanie Washburn, Russell Huie, Denise Puga, and Sarah Woller for their comments on an earlier version of this thesis.

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INTRODUCTION

Neurons within the spinal cord are plastic and can support a range of behavioral phenomena. Using traditional learning tasks, the isolated spinal cord has been found to support single stimulus learning (Groves & Thompson 1970), Pavlovian conditioning (Patterson et al., 1973; Joynes & Grau, 1996), and instrumental learning (Grau et al., 1998). Recent studies suggest that understanding how the isolated spinal cord can be trained and behaviorally modified has important implications for the recovery of function after spinal cord injury (SCI) (Edgerton et al., 2006).

To study spinally mediated instrumental learning, our laboratory utilizes a modified master-yoke paradigm to assess plasticity within the spinal cord. Subjects are transected at the second thoracic vertebra (T2) and restrained in tubes that allow their hind limbs to hang freely. Subjects in the Master condition receive shock to the tibialis anterior muscle of one leg whenever that leg is extended, yielding an increase in flexion duration (Grau et al., 1998). Over time, these subjects learn to maintain the shocked leg in a flexed position that reduces net shock exposure (contingent shock). Yoked animals receive shocks concurrently with the master animals, independent of hind limb position (noncontingent shock) (Grau et al., 1998). When both sets of subjects are later tested with response contingent shock, the master animals quickly relearn to maintain the shocked leg in a flexed position that minimizes shock exposure (savings effect), while yoked animals fail to learn (learning deficit). Just six minutes of uncontrollable tail-shock is sufficient to induce a learning deficit that lasts 48 hours (Crown et al. 2002).

_____

This thesis follows the style of *Behavioral Neuroscience*.

Both the acquisition of spinal instrumental learning and the learning deficit depend on glutamatergic neurons (Joynes et al., 2004; Ferguson et al., 2006). The N-methyl-D-aspartic acid-receptor (NMDAR) and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid-receptor (AMPAR) are part of the same family of ionotropic glutamate receptors (Palmer et al., 2005). Engaging the AMPAR, through the binding of glutamate, results in rapid depolarization of the cell and slower activation of the NMDAR (Watt et al., 2004). Activation of the NMDAR allows Ca^{++} ions to flow freely into the cell (Bliss & Lomo 1973; Watkins & Jane 2006). A strong Ca^{++} influx initiates intracellular mechanisms that modify synaptic communication, altering components thought to contribute to learning and memory (Yang et al., 2004; Blair et al., 2001). This Ca^{++} influx alters synaptic function by modifying the open probability of NMDARs, activating AMPARs and AMPAR trafficking at the synaptic cleft (Palmer et al., 2005, Lau & Zukin, 2007). In the case of prolonged intense stimulation, Ca^{++} influx can bring about an NMDAR-dependant enhancement of synaptic function (long-term potentiation, LTP) that has been linked to an up-regulation of AMPARs (Yang et al., 2004; Palmer et al., 2005). Conversely, stimulation parameters that lead to an overall reduction in neural excitability (long-term depression, LTD) produce a reduction of AMPAR function (Palmer et al., 2005). A model of these neurochemical processes is presented in the figures 1 and 2 below.

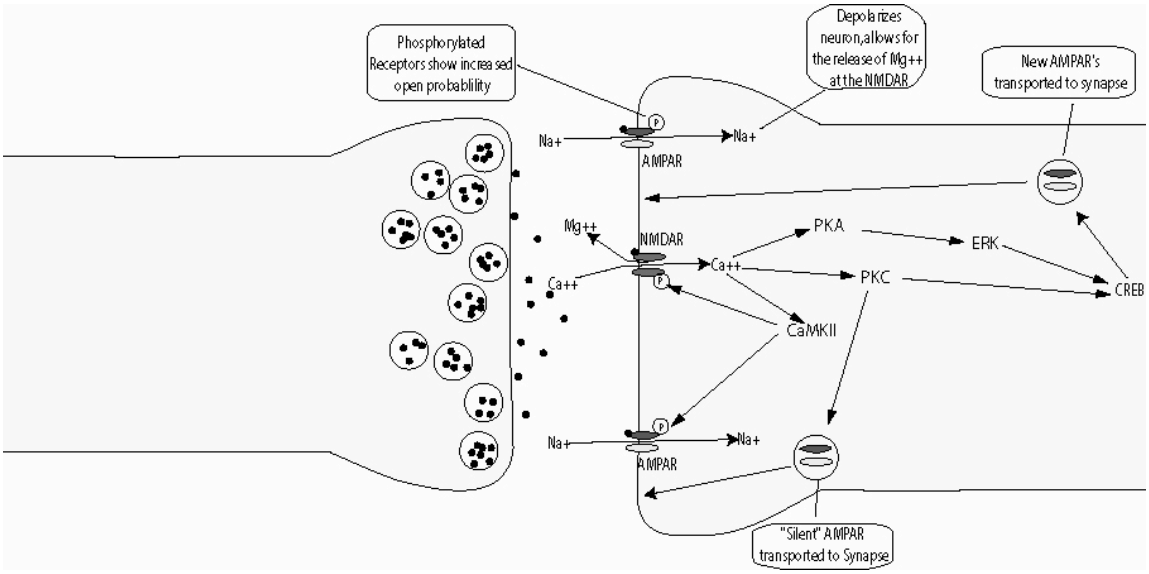

Figure 1. Theoretical model of LTP, showing the NMDAR-dependant Ca^{++} influx, and its impact on key intracellular signals (e.g. CaMKII, PKC) that impact AMPAR and NMDAR function. ● = Glutamate

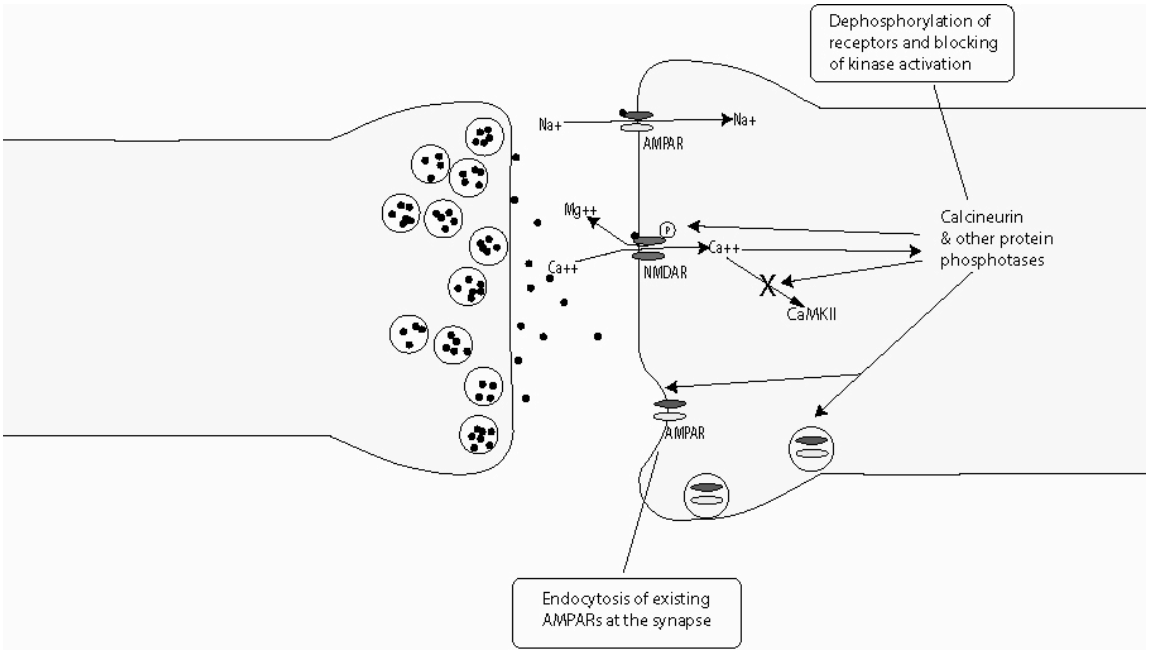

Figure 2. Theoretical model of LTD, showing how the activation of phosphotases can down-regulate AMPAR function. ● = Glutamate

Much is known regarding the role of the NMDAR in synaptic plasticity. Over the past 20 years, hundred of studies have used pharmacological agents to explore the role of the NMDAR in brain-mediated plasticity (Kopp et al., 2007; Lau & Zukin, 2007). In the brain, injection of NMDAR antagonists into the amygdala blocks the acquisition of fear conditioning and injections into the hippocampus disrupt spatial learning (Robbins & Murphy 2006). In hippocampal slice preparations, blocking the activation of the NMDAR disrupts the induction of LTP/LTD (Morris et al., 1986; Robbins & Murphy 2006). The NMDAR also plays a key role within the spinal cord. As in the brain, the NMDAR is involved in spinal LTP/LTD (You & Arendt-Nielsen, 2005; Ikeda et al., 2006). The NMDAR has also been linked to the wind-up and sensitization of nociceptive neurons (Wang et al., 2005). In spinal instrumental learning, pretreatment with a NMDAR antagonist disrupts both the acquisition and maintenance of the instrumental response (Joynes et al., 2004; Ferguson et al., 2006).

Relatively few studies have used pharmacological techniques to explore the role of the AMPAR. The lack of study is surprising given that changes in AMPAR function are thought to mediate changes in synaptic efficiency. A literature search revealed just a handful of studies examining the impact of AMPAR agonists/antagonists on brain function. Injections into the hippocampus of an AMPAR antagonist caused an impairment in a one-trial place memory task (Bast et al., 2005). Injections of an AMPAR agonist into the lateral ventricle, ventral tegmental area, zona incerta, or lateral preoptic area have been shown to cause an increase in locomotor activity and, at high doses, induce seizures (Turski et al., 1981; Shreve & Uretsky, 1989; Supko et al., 1991;

Dunn et al., 2005). Others have shown that an AMPAR agonist within the supramammillary or posterior hypothalamic nuclei increases response rate in a brain-dependant instrumental learning task (Ikemoto et al., 2004).

At the level of the spinal cord, pharmacological treatments that affect the AMPAR have been shown to impact nociceptive reactivity (Imamachi et al., 1999; Gorman et al., 2001; Yezierksi, 2005; You et al., 2005). Administering an AMPAR antagonist has been shown to increase tail-flick latencies and hind paw withdrawal from noxious stimuli, supporting the idea that the AMPAR carries part of the spinally mediated pain signal (Kong & Yu, 2006; Imamachi et al., 1999). Paradoxically, high doses of AMPA, an AMPAR agonist, have also been linked to increased tail-flick latencies (Advokat et al., 1994). Chronic administration of AMPAR agonists in the spinal cord induces a lasting effect (excitotoxicity) that results in tissue damage and a loss of plasticity (Nakamura et al., 1994; Yezierski, 2005). No studies have examined the role of AMPAR's in spinal learning.

The present experiments will explore the role of the AMPAR in instrumental learning in the isolated spinal cord. Experiments 1 through 3 assess the impact of the AMPAR antagonist CNQX, on the acquisition of an instrumental response (Experiment 1), the maintenance of instrumental responding (Experiment 2), and the induction of the learning deficit (Experiment 3). The next set of experiments examines the effects of the AMPAR agonist AMPA. Experiment 4 explores the impact of an agonist on instrumental learning, Experiment 5 investigates the long-term effects of an agonist, and Experiment 6 examined whether AMPAR activation fosters instrumental learning.

GENERAL METHOD

Subjects

All subjects, male Sprague-Dawley rats (100-120 days old 300-450 g), were obtained from Harlan Laboratories (Houston, TX). Subjects were individually housed with water and food *ad libitum*, and maintained on a 12-hour light dark cycle. Behavioral testing and surgeries were performed during the light portion of the cycle.

Surgery

The surgical procedure consisted of a complete transection of the second thoracic vertebra (T2). Anesthesia was induced using a concentration of 5% Isoflurane and maintained at a 2% concentration during surgery. The T2 vertebra was located and an incision was made rostral-caudal to the vertebra. A laminectomy was performed to expose the cord rostral of T2. Heat cautery was used to transect the exposed cord and the cavity formed was filled with gelfoam (Harvard Apparatus, Holliston, MA). A 25 cm catheter (PE-10, VWR International Bristol, CT), held rigid with a 0.9 mm stainless steel wire (Small Parts Inc. Miami Lakes, FL), was inserted 9 cm into the subarachnoid space on the dorsal surface of the spinal cord (Yaksh & Rudy, 1976). Following insertion, the wire was gently removed and the exposed tubing adhered to the skin externally using cyanoacrylate. The incision was closed using Michel Clips (Fine Science Tools, Foster City, CA). All subjects received injections of 0.9% saline (2.5 ml i.p.) immediately following surgery, and the subject's legs were taped using a piece of porous tape (Ortholetic 1.3 cm width) in a secure natural position. Subjects were then allowed to recover for 18-24 hours before testing in a temperature-controlled room

(25.5° C) with free access to food and water. Bladders were expressed twice daily and immediately before any behavioral procedures were conducted. When behavioral testing was complete, all animals were euthanized with a lethal dose of pentobarbital (100 mg/kg).

The surgical transections were verified by 1) observing behavior during the recovery period to confirm complete paralysis and a lack of vocalization to leg shock, 2) visual inspection of the transection site during surgery, and 3) post-mortem cord examination in a random sample of subjects.

Apparatus

Uncontrollable shock was administered while the subjects were loosely restrained in black Plexiglas tubes (22 cm [l] X 6.8 cm [w]) with holes drilled in them for ventilation. A flat floor was attached 5.3 cm below the top of the tube that is 5.5 cm wide. Tailshock was delivered using a modified fuse clip, coated with ECG gel (Harvard Apparatus, Holliston, MA) and secured with porous tape approximately 6 cm behind the base of the tail. A constant current 1.5 mA shock was delivered using a 660-V transformer.

Procedure

Instrumental testing was conducted while rats were loosely restrained in tubes (23.5 cm X 8 cm). Two slots (5.6 cm X 1.8 cm) 4 cm apart allowed both hind legs to hang freely. Shock was delivered using a BRS/LVE (Laurel, MD) shock generator (Model SG-903). Electrodes were placed over the tibialis anterior muscle and connected to a computer-controlled relay that regulated the application of leg shock.

To monitor leg position during testing a contact electrode made of a 7-cm piece of stainless steel wire 0.46 mm in diameter (Small Parts Inc. Miami Lakes, FL) was taped to the plantar surface of the foot. A fine wire (0.26 mm [diameter]; 20 cm [length]) was attached to the end of the foot electrode and connected to a digital input monitored by a Macintosh computer. A rectangular plastic dish (11.5 cm [w] X 19 cm [l] X 5 cm [d]) containing a solution of NaCl was placed approximately 7.5 cm below the restraining tube. Soap was added to the solution to reduce the surface tension of the water in the dish. A stainless steel electrode (1 mm diameter) was connected to a ground wire and placed into solution. When the ankle joint was extended, the contact electrode touched the saline solution completing a circuit monitored by the computer.

Flexion force is set prior to testing to a force of 0.4 N. A monofilament plastic line (4 lb. test Stren, Dupont, Wilmington DE) was tied behind the plantar protuberance of the foot. The 40 cm line was run underneath a bar below the subject to extend the joint of the leg. The end of the line was connected to a strain gauge (Fort-1000, World Precision Instruments, New Haven, CT). After the line was connected to the subject's paw, the strain gauge was positioned so that the line was taut, just barely registering on the gauge. The strain gauge was calibrated by determining the relationship between a change in voltage and force in Newtons.

Instrumental learning behavioral procedure

The subjects were given 18-24 hours to recover after surgery. Prior to instrumental testing, the hind limbs were shaved and marked for electrode placement. To minimize lateral leg movements, a piece of porous tape (Ortholetic, 1.3 cm) was

wrapped around the leg above the tarsus and attached under the front panel of the restraining tube. A wire electrode was inserted into the skin distal to the tibialis anterior (1.5 cm from the plantar surface of the foot) and a lead from the generator was then attached to the electrode. A second wire electrode (0.26 mm [d]) was inserted 0.4 cm into the tibialis anterior muscle 1.7 cm above the other electrode. The monofilament line was tied around the subject's hind paw and connected to the strain gauge. A single intense shock was used to verify the amount of shock needed to attain a 0.4 N flexion force. After applying three 0.15-s leg-shocks to establish a resting position of the leg, the level of saline solution was adjusted to 4 mm (8 mm for higher criterion testing) above the tip of the contact electrode. Rats were exposed to 30 minutes of response-contingent shock during instrumental testing. When the rat's paw was extended, and the contact electrode was in solution, the circuit was completed and a shock was applied to the tibialis anterior muscle. When the hindlimb was in the flexed position the circuit was open and the shock was terminated. Leg position was monitored at a sampling rate of 30 Hz by a Macintosh computer.

Behavioral measures

Three measures were assessed during the 30-minute instrumental training session: time in solution, response number, and response duration. The session was divided into 30 one-minute bins to measure performance over time. When the contact electrode left the solution, response number increased by 1. The computer also recorded net time in solution. Response duration was derived from time in solution and response

number using the following equation: Response Duration$_i$ = (60 s- time in solution$_i$) / (Response Number$_i$ + 1), where *i* is the current time bin.

Throughout this document both response number and response duration will be reported. As described in the previous section, when a subjects' contact electrode leaves the solution, their response number increases by 1. A subject that has its leg in a flexed position for the entire length of a time bin would therefore have a response number of zero. Likewise, a subject that stopped responding would also have a response number of zero. As a result a low response number could indicate either successful learning or a failure to learn. My primary measure response duration, avoids this problem and yields a monotonic increase as function of learning.

Statistics

All data were analyzed using repeated measure analysis of variance (ANOVA). Alpha values of .05 or below were considered statistically significant.

EXPERIMENT 1

Prior work indicates that pretreatment with an AMPAR antagonist blocks the induction of windup and increases tail withdrawal latencies to electrical stimuli in spinal rats (You et al., 2005). The present experiment examined whether the AMPAR antagonist CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) impacts instrumental learning in spinal rats. If AMPAR activation is necessary for instrumental learning, then blocking the action of the AMPAR with an antagonist (CNQX) should inhibit instrumental learning.

Procedure

Eighteen spinally transected rats were used in Experiment 1 (n=6). The rats were placed in the instrumental learning apparatus and the catheter was threaded through a hole in the tube to administer the drug. The animals received 10 μl of drug or vehicle followed by 20 μl saline flush. Subjects received CNQX (40 nmol or 80 nmol) or its vehicle (DMSO). Twenty minutes after drug injection, the instrumental testing session was initiated. All subjects received 30 minutes of controllable shock.

Results

To ensure drug treatment did not affect baseline reactivity, the shock intensity needed to induce a 0.4 N change in flexion force after drug administration was analyzed. Mean shock intensities ranged from 0.60 ($\pm$ 0.02) to 0.70 ($\pm$ 0.03) mA. An ANOVA revealed no significant differences in baseline reactivity, $F(2,15)= 1.00$, $p > .05$. Initial flexion responses were similarly measured and analyzed. Mean initial responses ranged

from 0.15 (± 0.01) to 0.16 (± 0.02) seconds and were not significantly different, $F(2,15) < 1.00$, $p > .05$.

The effect of CNQX on instrumental learning is depicted in Figure 3. Subjects that received the vehicle (DMSO) and the lowest dose (40 nmol) of CNQX were able to maintain a flexion response to reduce net shock exposure over the 30 minute testing session. Rats pretreated with the highest dose (80 nmol) of CNQX failed to learn. A one-way repeated measures ANOVA for response duration revealed a significant main effect of drug treatment, $F(2,15)= 6.47$, $p < .01$, and a Trials X Drug interaction $F(29,58)= 1.85$, $p < .01$. *Post hoc* comparisons showed that subjects that received the highest dose of CNQX (80 nmol) differed from the groups that received 40 nmol of CNQX or its vehicle (DMSO) ($p < .05$).

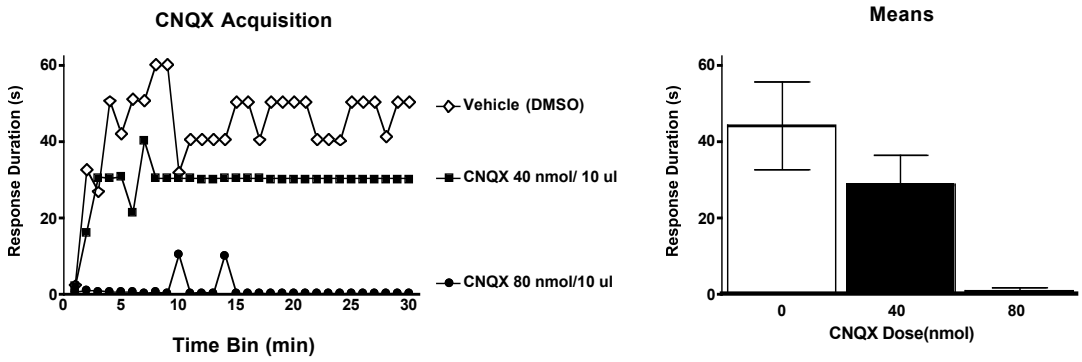

Figure 3. Response duration over the 30-min testing session (left) and group means (right). The error bars represent standard error of the mean (SEM).

The rate of responding across the 30-min of testing is illustrated in Figure 4. As usual, subjects that failed to learn exhibited the highest rate of responding. A one-way ANOVA revealed a significant main effect of drug treatment $F(2,15)= 22.68$, $p < .01$.

The Trials X Drug interaction was also significant $F(29,58)= 5.49$, $p < .01$. *Post hoc* comparisons showed that subjects that received the highest dose of CNQX (80 nmol) differed from the groups given the lower CNQX dose (40 nmol) or its vehicle (DMSO) ($p < .01$).

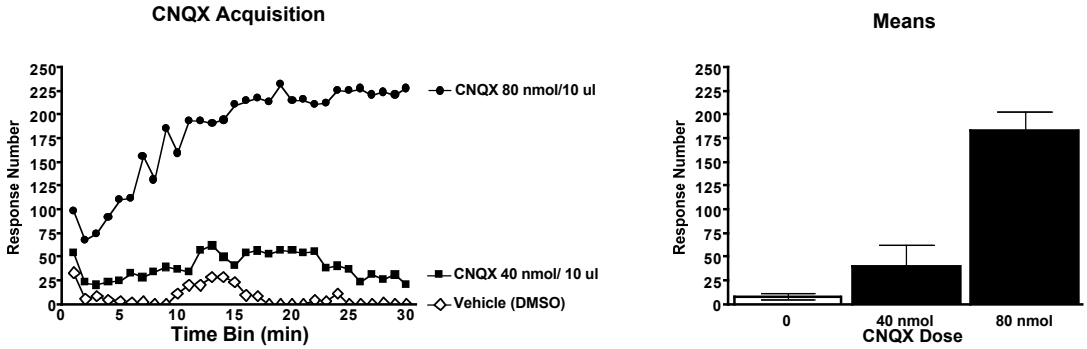

Figure 4. Response number across the 30-min testing session (left) and corresponding group means (right). The error bars represent standard error of the mean (SEM).

Discussion

Vehicle treated animals were able to acquire the instrumental response. CNQX disrupted instrumental learning in a dose dependent fashion. These data indicate that the AMPAR plays a critical role in spinally mediated instrumental learning.

EXPERIMENT 2

Prior research has shown that administering an NMDAR antagonist *after* the instrumental response has been acquired disrupts the *maintenance* of the acquired response (Joynes et al., 2004). Experiment 2 examined whether an AMPAR antagonist also disrupts the maintenance of instrumental responding.

Procedure

A 2x2 factorial design was used for this experiment (n=8). After subjects were setup for instrumental testing, half the subjects received 30 minutes of training with response contingent shock (pretrained). Twenty-five minutes into the session, half of the subjects in each condition received CNQX (80 nmol) while the remaining subjects received the vehicle (DMSO). Five minutes later, both the pretrained and untrained subjects were tested for 30 minutes with response-contingent shock. For comparison, a fifth group was pretrained and administered MK-801 (10 nmol) prior to testing (Ferguson et al., 2006).

Results

To ensure that baseline reactivity was not different across groups, the shock intensity needed to elicit a 0.4 N change in flexion force was analyzed. Mean shock intensities ranged from 0.58 ($\pm$ 0.03) to 0.69 ($\pm$ 0.04) mA. An ANOVA revealed no significant differences in baseline reactivity, $F(4,35)= 1.10$, $p > .05$. Initial flexion responses were similarly measured and analyzed. Initial flexion response means ranged

from 0.14 ($\pm$ 0.01) to 0.23 ($\pm$ 0.07) and these differences were not significant, $F(4,35) < 1.00$, $p > .05$.

As shown in Figure 5, the 3 pretrained groups exhibited a progressive increase in response duration and did not differ prior to drug treatment, $F < 1.0$, $p > .05$. During the second 30 minutes of instrumental testing, pretrained subjects given CNQX exhibited a decline in response duration. As in Experiment 1, CNQX blocked acquisition in untrained subjects. To focus on the maintenance of learning we statistically analyzed the last 30 minutes of testing. A two-way repeated measures ANOVA of the final 30 minutes of the testing session revealed a significant main effect of drug treatment $F(1,28)=21.96$, $p < .01$ and training condition $F(1,28)=10.26$, $p < .01$, with no other significant relationships. Additional analyses were performed to determine whether CNQX and MK-801 have comparable effects on the maintenance of the instrumental response. Inspection of Figure 5 suggests that both drugs had a similar effect. Supporting this, a one-way repeated measures ANOVA yielded a significant effect of treatment condition, $F(4,29)=7.41$, $p < .01$. *Post hoc* comparisons showed that the CNQX-untrained group was significantly different from all other groups ($p < .05$), verifying that CNQX blocks the acquisition of learning (Experiment 1). The CNQX-pretrained group was significantly different from the vehicle-pretrained group ($p < .05$), showing that CNQX also disrupts the maintenance of learning. Furthermore, the MK-801 comparison group was significantly different from the vehicle-pretrained group ($p < .05$), but not significantly different from the CNQX-pretrained group. These results indicate that MK-801 treatment blocks the maintenance of the instrumental response in a

similar fashion to CNQX. The vehicle-untrained group was significantly different from the vehicle-pretrained group ($p < .05$) showing that pretraining has a significant effect on learning.

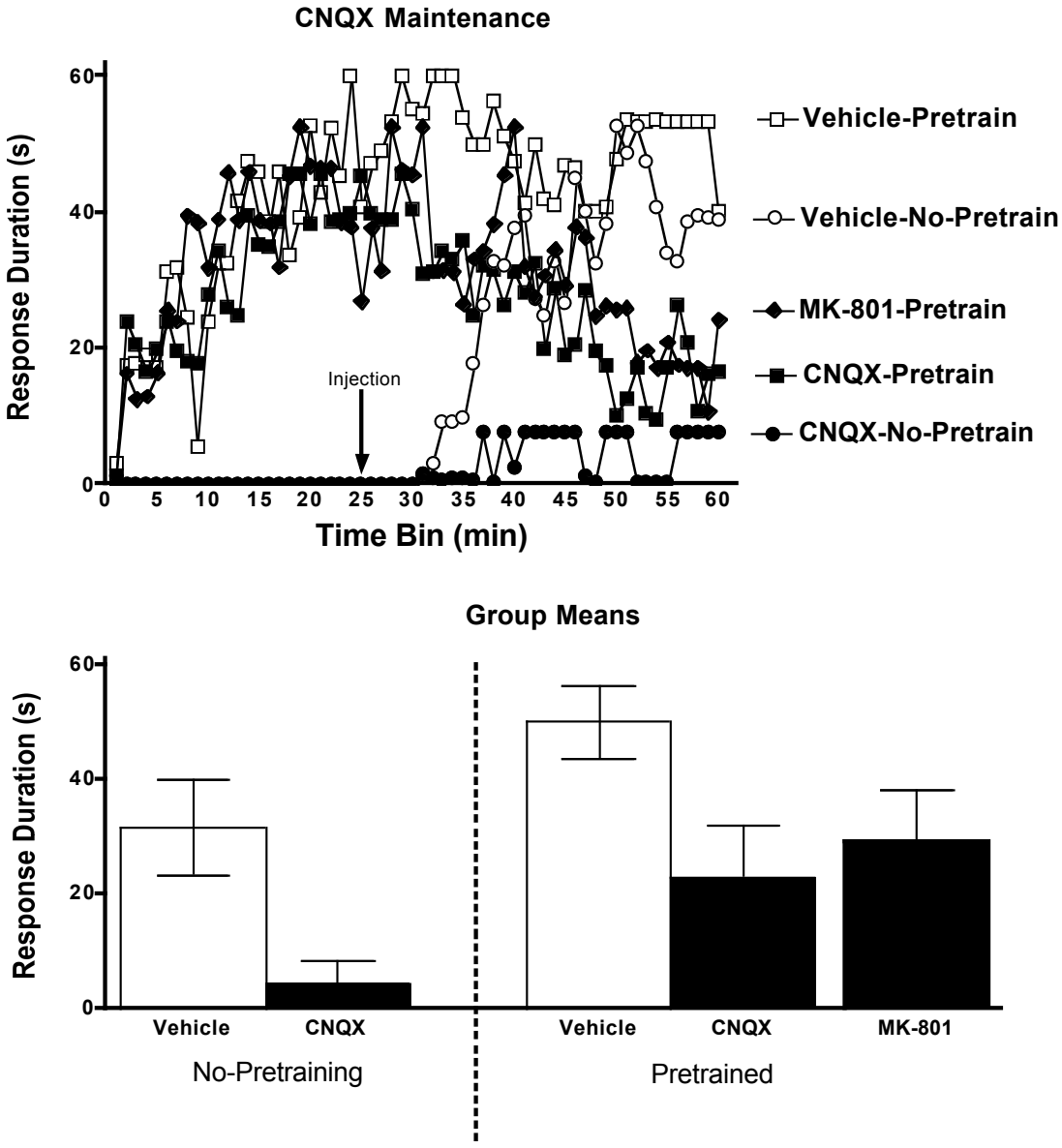

Figure 5. Response durations across the 60-min testing/training session (top), with group means for the final 30-min (bottom), error bars represent standard error of the mean (SEM).

As usual subjects that exhibited an increase in flexion duration made fewer instrumental responses (Figure 6). Subjects that were untrained responded at a high rate indicative of a failure to learn. Subjects given CNQX or MK-801 after pretraining exhibited a decline in flexion duration, but this loss of learning was not accompanied by a proportional increase in response number. For statistical analyses we only evaluated the data from the 30-min testing session, when subjects were tested under common conditions with controllable shock. A two-way repeated measures ANOVA revealed a significant main effect of drug treatment $F(1,28)=18.91$, $p < .01$, training condition $F(1,28)=30.47$, $p < .01$, and Drug treatment X Training condition interaction $F(1,28)=13.70$, $p < .01$. As expected, vehicle pre-trained rats that maintained the instrumental response exhibited few responses during testing. To compare differences across all groups, a one-way repeated measures ANOVA was also used to analyze the data yielding a significant affect of drug/training condition $F(4,29)= 12.51$, $p > .01$. Untrained vehicle treated rat exhibited fewer responses as they acquired the instrumental response. As observed in Experiment 1, untrained CNQX treated rats that failed to learn exhibited a high rate of responding. Surprisingly, pre-trained rats given CNQX or MK-801, who exhibited a decline in response duration did not exhibit a proportional increase in response number. *Post hoc* comparisons confirmed that the CNQX-untrained group was significantly different from all other groups, ($p < .05$). No other comparisons were significant.

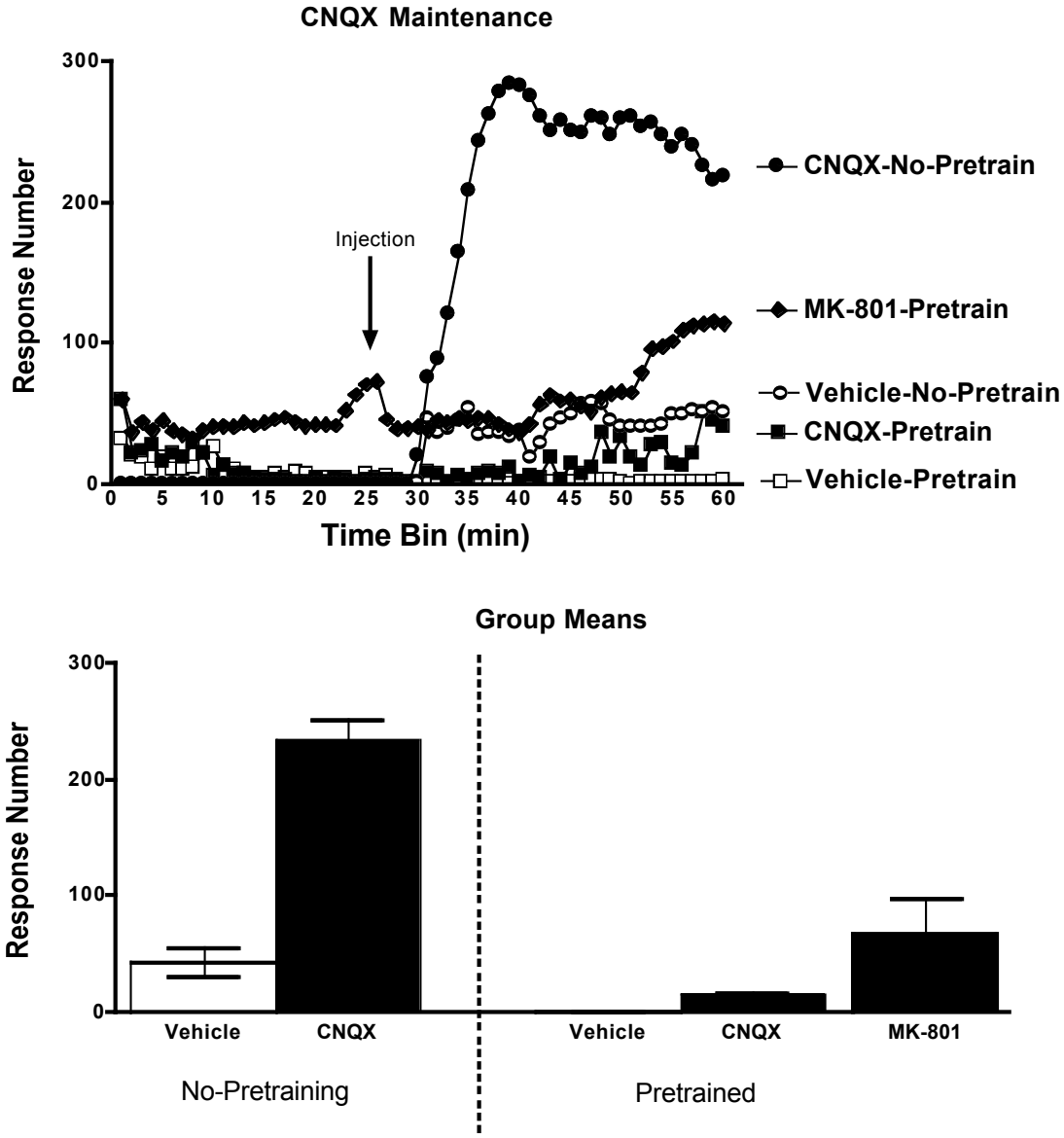

Figure 6. Response number for the entire testing/training session (top) and group means for the final 30-mins of testing (bottom). Error bars represent standard error of the mean (SEM).

Discussion

Vehicle treated rats acquired an increase in flexion duration and maintained it throughout the testing session. The maintenance of this instrumental response was

disrupted by administration of an AMPAR antagonist. The MK-801 group replicated prior work (Joynes et al. 2004) and showed that CNQX had a similar disruptive effect. While drug treatment had a detrimental effect on the maintenance of the learned response, the disruption in learning was not accompanied by the expected increase in rate of responding. Based on these results, we can conclude that the AMPAR is essential both to the acquisition and maintenance of spinally mediated instrumental learning. However, blocking AMPAR activation did not completely reverse the effects of pretraining.

EXPERIMENT 3

Spinal rats that have previously received leg-shock independent of leg position (uncontrollable shock) fail to learn when subsequently tested with response contingent shock (Crown et al., 2002). Experiment 3 examined whether CNQX would block the induction of the behavioral deficit when administered prior to uncontrollable shock. The highest concentration (80 nmol/10 μl) was used in this experiment. If the AMPAR is required for the induction of the learning deficit, then blocking the action of the AMPAR with CNQX should eliminate the learning deficit when subjects are tested with response-contingent shock 24 hours later.

Procedure

A 2x2 factorial design was used in this experiment (n=10). Subjects were pretreated with CNQX or its vehicle, DMSO. Thirty-minutes later, subjects were placed in Plexiglass tubes and the tail electrodes were attached. Half the subjects in each drug condition received 6 minutes of uncontrollable shock, while the other half remained unshocked. The animals were returned to the recovery room and, 24 hrs later, tested for 30-min with response-contingent shock.

Results

The shock intensity needed to elicit a 0.4N response prior to testing was assessed to rule out any baseline differences in reactivity. Mean shock intensities ranged from 0.65 (+/- 0.03) to 0.71 (+/- 0.04) mA. An ANOVA revealed no significant differences, $F(3,36)= 1.16$, $p > .05$. Mean initial flexion duration ranged from 0.14 (+/- 0.01) to 0.24 (+/- 0.05), and these differences were not statistically significant $F(3,36)= 2.07$, $p > .05$.

Unshocked subjects, regardless of drug condition, exhibited a progressive increase in response duration during testing (Figure 7). Vehicle-treated subjects exposed to uncontrollable shock failed to learn. This learning deficit was blocked by pretreatment with CNQX. An ANOVA revealed a significant effect of drug treatment $F(1,36)=8.21$, $p < .01$, shock condition $F(1,36)=9.07$, $p < .01$, a Drug X Shock condition interaction $F(1,36)=4.99$, $p < .05$, and a Trials X Drug interaction $F(29,1044)=1.77$, $p < .01$. *Post hoc* comparisons showed that the vehicle treated-shocked group differed from the other three ($p < .05$).

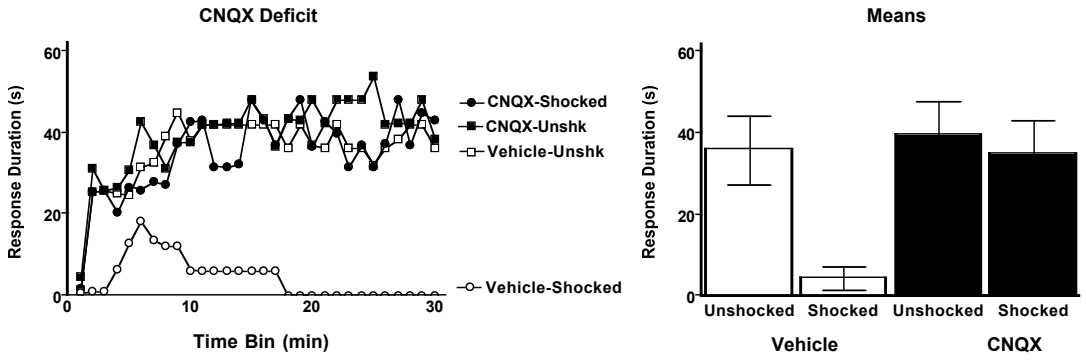

Figure 7. Experiment 3, response duration across all time bins (left) and group means (right). Error bars represent standard error of the mean (SEM).

Rate of responding was also analyzed (Figure 8) and yielded almost identical statistical results. As expected, subjects that were given uncontrollable shock 24 hours before testing responded at a high rate, indicative of a failure to learn. Subjects that received CNQX treatment prior to uncontrollable shock displayed a low rate of responding similar to the Vehicle treated and CNQX treated unshocked groups. An ANOVA revealed a significant effect of drug treatment $F(1,36)=10.75$, $p < .01$, shock

condition $F(1,36)=14.85$, $p < .01$, a Drug X Shock condition interaction $F(1,36)=10.65$, $p < .01$, a Trials X Drug interaction $F(29,1044)=3.68$, $p < .01$, a Trials X Shock condition interaction $F(29,1044)=4.50$, $p < .01$, and a Trials X Drug X Shock condition interaction $F(29,1044)=3.39$, $p < .01$. *Post hoc* comparisons showed significant differences between the vehicle-shocked group and all other groups ($p < .05$).

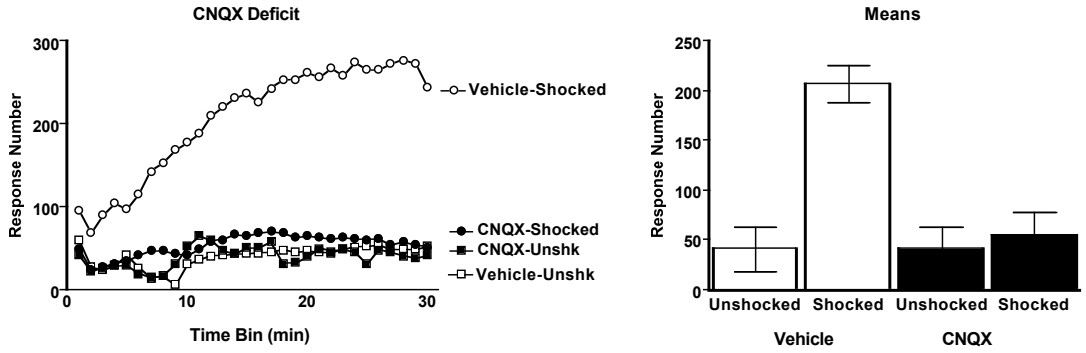

Figure 8. Experiment 3, response number across all time bins (left) and group means (right). Error bars represent standard error of the mean (SEM).

Discussion

The learning deficit previously shown by Crown (2002) was replicated in this experiment, verifying that 6-minutes of uncontrollable shock produces a robust deficit 24-hours later. Pretreatment with CNQX blocked the induction of this behavioral deficit. These results indicate that blocking the action or the AMPAR prior to uncontrollable shock protects against its detrimental effects. Furthermore, CNQX administration 24-hours prior to instrumental testing (CNQX-unshocked group) showed no long-term effect on instrumental learning.

EXPERIMENT 4

In Experiments 1-3 I blocked the action of the AMPAR and observed its affects in our instrumental learning paradigm. In Experiment 4, I examined how activation of the AMPAR affected spinal instrumental learning. Others have shown that activation of AMPAR using self-administered AMPA in the supramammillary or posterior hypothalamic nuclei caused an increase in response rate of instrumental learning and an overall increase in dopamine levels (Ikemoto et al., 2004). Furthermore, those effects were attenuated in the presence of CNQX. Here I examined how administration of AMPA to the isolated spinal cord would affect instrumental learning. I hypothesized that activation of the AMPAR by the agonist AMPA could, at a low dose, foster an LTP-like enhancement in learning. At a high dose, AMPAR activation could produce a diffuse over-excitation (saturation) that disrupts learning (Moser et al., 1998).

Procedure

To evaluate the effect of AMPA administration across a range of doses, subjects were given either AMPA: 0.125 nmol, 0.50 nmol, and 2.0 nmol, or Vehicle (n=10). Subjects received the drug treatment 30 minutes prior to testing. Following drug treatment the subjects were tested for 30 minutes in the learning paradigm as described previously.

Results

To ensure drug treatment did not affect baseline reactivity, the shock intensity needed to induce a 0.4 N change in flexion force after drug administration was analyzed. Mean shock intensities ranged from 0.60 ($\pm$ 0.03) to 0.70 ($\pm$ 0.03) mA. An ANOVA

revealed no significant differences in baseline reactivity, $F(3,36)= 1.139$, $p > .05$. Mean initial flexion durations ranged from 0.11 (± 0.01) to 0.14 (± 0.01), and these differences were not significant, $F(3,36) < 1.00$, $p > .05$.

The effect of AMPA administration prior to instrumental learning is depicted in Figure 9. Subjects that received the highest dose of AMPA (2.0 nmol) prior to instrumental learning showed a significant deficit compared to the other doses of AMPA or its vehicle. An ANOVA yielded a significant main effect of drug treatment, $F(3,36)=6.22$, $p < .01$. A trend analysis of drug treatment yielded a significant linear, $F(1,36)=6.75$, $p < .05$, and a quadratic contrast, $F(1,36)=9.11$, $p < .01$. *Post hoc* comparisons showed that subjects that received the highest dose of AMPA (2.0nmol) were significantly different from the groups that received lower doses (0.125 nmol & 0.25 nmol) or the vehicle, $p < .05$.

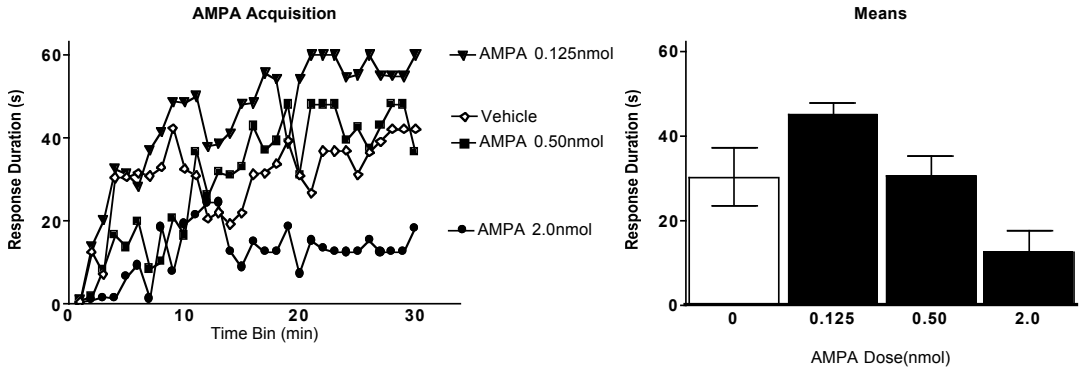

Figure 9. Experiment 4, response duration across all time bins (left) and group means (right). Error bars represent standard error of the mean (SEM).

The impact of pretreatment with AMPA on the rate of responding is illustrated in Figure 10. An ANOVA revealed a significant effect of drug treatment, $F(3,36)=2.81$, $p =$

.05. Neither the linear or quadratic contrasts revealed significant results, all F's > 2.60, p > .05. *Post hoc* comparisons showed a significant difference between the highest dose of AMPA (2.0 nmol) and the lowest dose of AMPA (0.125 nmol), $p < .05$.

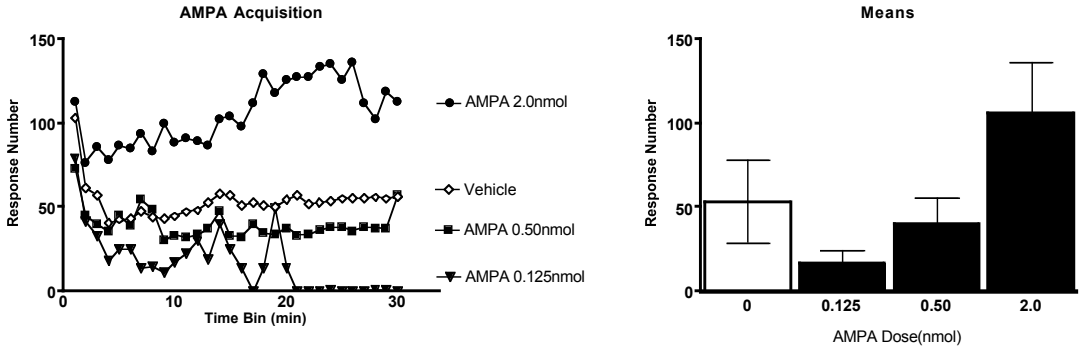

Figure 10. Experiment 4, response number across all time bins (left) and group means (right). Error bars represent standard error of the mean (SEM).

Discussion

I hypothesized that a low dose of AMPA could benefit instrumental performance, while a high dose could have a disruptive effect. This should yield a dose-response curve with a significant inflection. As expected, a significant quadratic (one inflection) relation was observed. The highest dose of AMPA (2.0 nmol) disrupted the acquisition of instrumental learning. This could reflect a saturation of plasticity or a neurotoxic effect. Experiment 5 will examine these possibilities. Experiment 6 will seek further evidence that a low dose of AMPA (0.125 nmol) facilitates instrumental learning/performance.

EXPERIMENT 5

A high dose of AMPA could disrupt learning in Experiment 4 because the resultant over-excitation has a non-reversible effect (e.g. neurotoxic) that permanently abolishes the capacity for instrumental learning (Gorman et al., 2001). Experiment 5 examined this possibility by testing whether a high dose of AMPA produces a lasting impairment.

Procedure

All subjects received a complete transection at T2, and were prepared with an intrathecal catheter. Twenty-four hours post-surgery subjects received drug treatment (AMPA 2.0 nmol or Saline) (n=10). A positive control group received acute AMPA (2.0nmol) administration 30 minutes prior to instrumental testing (n=10). Forty-eight hours post-surgery all subjects received instrumental testing.

Results

To ensure drug treatment did not affect baseline reactivity, the shock intensity needed to induce a 0.4 N change in flexion force after drug administration was analyzed. Mean shock intensities ranged from 0.60 ($\pm$ 0.05) to 0.70 ($\pm$ 0.05) mA. An ANOVA revealed no significant differences in baseline reactivity, $F(2,27) < 1.0$, $p > .05$. Mean initial flexion durations ranged from 0.14 ($\pm$ 0.01) to 0.13 ($\pm$ 0.01) and did not differ, $F(2,27) < 1.00$, $p > .05$.

Subjects that received AMPA treatment 24 hours prior to testing (delayed) displayed similar response durations to vehicle treated controls (Figure 11). Subjects that received acute AMPA treatment failed to learn this flexion response. A one-way

repeated measures ANOVA for response duration revealed a significant effect of Drug $F(2,27) = 6.23$, $p < .01$ and a Trials X Drug interaction F(2,58) =1.82, p < .01. *Post hoc* analysis showed significant differences between the AMPA-positive control group and both groups that received treatment 24 hours prior to instrumental testing ($p < .05$).

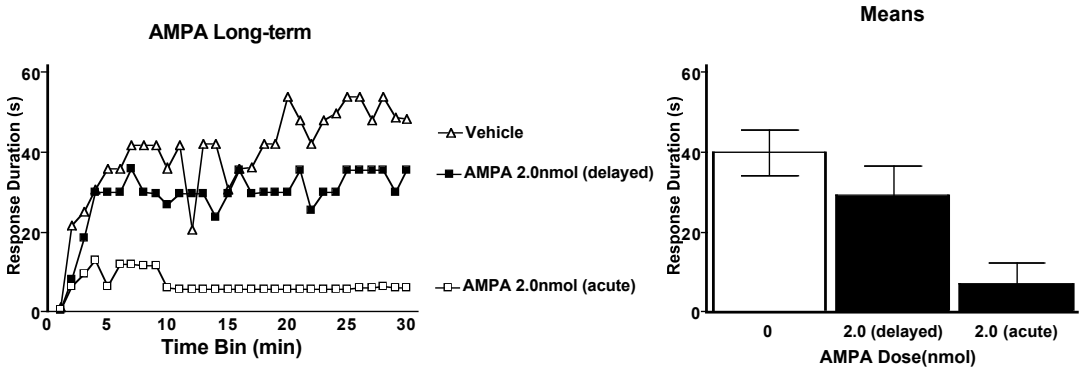

Figure 11. Experiment 5, response duration across all time bins (left) and group means (right). Error bars represent standard error of the mean (SEM).

Subjects that received acute AMPA treatment responded at a higher rate than subjects that received the vehicle treatment (Figure 12). A one-way repeated measures ANOVA on response number revealed a significant effect of Drug, $F(2,27)=3.76$, $p > .05$. *Post hoc* analysis showed a significant difference between the acute-AMPA group and the vehicle treated rats ($p < .05$), no other comparisons were significant.

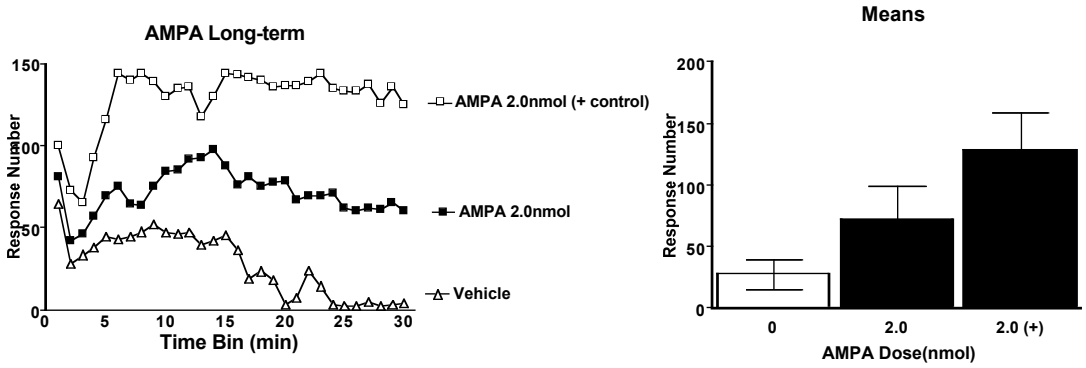

Figure 12. Experiment 5, response number across all time bins (left) and group means (right). Error bars represent standard error of the mean (SEM).

Discussion

Acute AMPA (2.0 nmol) administration blocked the acquisition of learning, replicating the results from Experiment 4. A high dose of AMPA (2.0 nmol), at a 24-hour delay, did not have a long-term effect on my primary measure of learning, response duration. Subjects given AMPA 24 hours prior to testing exhibited more responses than the vehicle treated controls, but this difference was not statistically significant.

EXPERIMENT 6

The results of Experiment 4 suggest that a low dose of AMPA fosters learning. While this yielded a significant quadratic relation between drug dose and instrumental performance, group comparisons did not reveal a significant difference between the lowest dose of AMPA and the vehicle controls. Because, the vehicle treated group rapidly acquired the task, a ceiling effect could have masked a drug-induced enhancement of learning. The present experiment addressed this issue by testing subjects using a higher response criterion. Under these conditions, vehicle treated subjects show poor performance, which could potentially unveil an AMPA-induced enhancement of learning.

Procedure

This experiment utilizes a 2 X 2 experimental design (n=14): Subjects received AMPA (0.125 nmol) or its vehicle (saline), and were tested at either the normal (4 mm) foot electrode depth or at a higher criterion (8 mm).

Results

The shock intensity needed to induce a 0.4 N change in flexion force after drug administration was analyzed for each subject. Mean shock intensities ranged from 0.60 ($\pm$ 0.04) to 0.70 ($\pm$ 0.05) mA. An ANOVA revealed no significant differences in baseline reactivity, $F(3,52) < 1.00$, $p > .05$. Initial flexion durations ranged from 0.13 ($\pm$ 0.01) to 0.11 ($\pm$ 0.01) and these differences were not significant, $F(3,52) < 1.00$, $p > .05$.

Subjects that received AMPA (0.125 nmol) or its vehicle (saline), were able to learn under normal conditions, but were not able learn at a higher criterion (Figure 13).

An ANOVA, of response duration, confirmed that raising the response criterion impacted learning $F(1,52)= 6.01$, $p < .05$, Trials X Testing Criterion $F(52,1508)= 1.731$, $p < .01$, but did not yield a significant effect of Drug treatment, $F(1,52) < 1.00$, $p > .05$. A direct comparison of the AMPA treated and vehicle treated controls yielded no significant differences, $F(1,26) < 1.00$, $p > .05$.

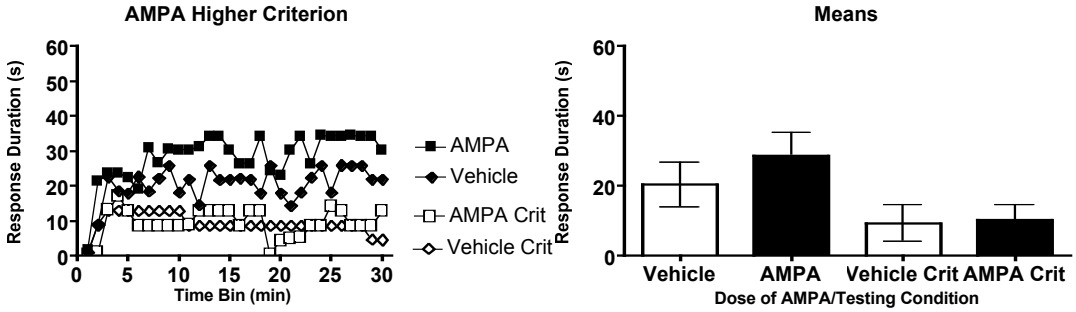

Figure 13. Response duration across all time bins (left) and group means (right). Error bars represent standard error of the mean (SEM).

As usual, groups that were tested at the normal criterion exhibited a lower overall rate of responding (Figure 14). However, analysis of response number revealed no significant differences of drug treatment, $F(1,52) < 1.00$, $p > .05$, or shock condition, $F(1,52) < 1.00$, $p > .05$. A direct comparison of the AMPA treated and vehicle treated controls yielded no significant results as well, $F(1,26) < 1.00$, $p > .05$.

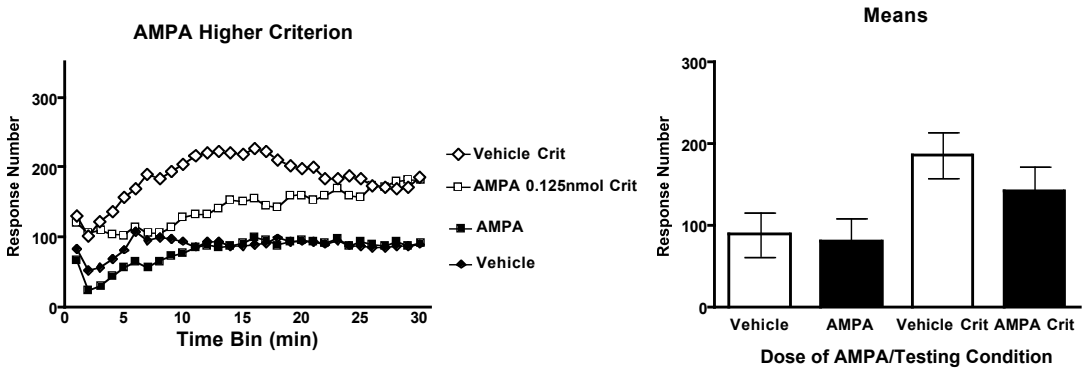

Figure 14. Response number across all time bins (left) and group means (right). Error bars represent standard error of the mean (SEM).

Discussion

The results of Experiment 6 yielded no evidence that a low dose of AMPA fosters instrumental learning or performance. Clearly, if such an effect does exist, it must be limited to a narrow range of conditions.

CONCLUSIONS

The results of these experiments indicate that the AMPAR plays a critical role in spinal plasticity. In the next sections, I review the results and discuss their relations to past work, and present some potential clinical implications.

CNQX disrupts the maintenance of instrumental learning and protects against the effects of uncontrollable shock

In Experiment 1 we tested whether AMPAR activation was necessary for instrumental learning in the rat spinal cord. Subjects that received CNQX prior to instrumental testing did not show an increase in flexion duration compared to vehicle treated controls. These findings suggest that AMPAR activation is a necessary component of spinal instrumental learning. Similar effects have been observed in the induction of LTP and the recall of spatial learning tasks (Bast et al., 2005).

Experiment 2 examined how blocking the AMPAR after the subject had already acquired the increase in flexion response affected the maintenance of learning. Rats that received CNQX after the acquisition of learning did not maintain the previously learned response. A similar effect has been observed in the presence of an NMDAR antagonist. These findings, in conjunction with past work (Joynes et al., 2004; Ferguson et al., 2006), suggest that the AMPAR and NMDAR are critical to both the acquisition and maintenance of spinal learning.

Experiment 3 investigated the induction of the learning deficit. Previous work in our laboratory has shown that a robust instrumental deficit can be produced using 6-minutes of uncontrollable shock to the tail 24-hours prior to testing (Crown et al., 2002).

The induction of this deficit is blocked by pretreatment with an NMDAR antagonist (Ferguson et al., 2006). Previous work had also shown that a GABA antagonist (Ferguson et al., 2003) and protein synthesis inhibition (Patton et al., 2004) blocks the induction of the learning deficit. Similarly, subjects that received CNQX prior to uncontrollable shock exposure showed no deficit in instrumental learning when tested 24-hours later. By blocking the activation of the AMPAR, CNQX was able to have a protective affect against the induction of the learning deficit.

AMPA administration has detrimental effects on spinal instrumental learning

Experiment 4 explored how activation of the AMPAR prior to instrumental testing affects learning. Subjects that received AMPA showed a dose-dependant decrease in flexion durations. This diminished learning could reflect a saturation of plasticity (Moser et al., 1998) due to pharmaceutical AMPAR activation.

Previous research has shown that uncontrollable shock (Crown et al., 2002), and mGluR activation (Ferguson et al., 2006), can produce a learning deficit that lasts at least 24-hours. Experiment 5 examined whether an AMPA dose that produces an acute deficit has a lasting effect. Subjects that received AMPA 24-hours prior to instrumental testing showed no behavioral deficit compared to the positive control group that received acute AMPA treatment. These results indicate that AMPAR activation, by itself, does not produce a long-term effect.

Experiment 6 investigated whether a low dose of AMPA promotes learning. Using both a moderate and a high response criterion, pre-treatment with AMPA had no effect. Though previous work in the brain (Ikemoto et al., 2004) had shown AMPA

administration increases learning, the effect observed in this experiment was small at best.

The AMPAR has parallel contributions to spinal learning and LTP

The induction of LTP is dependant upon the rapid activation of the AMPAR followed by NMDAR activation (Watt et al., 2004). Just as blocking the activation of the AMPAR with CNQX blocks the induction of LTP (Bast et al., 2005), CNQX disrupted instrumental learning. These results parallel work with the NMDAR antagonist AP-5, which blocked both spinal learning (Joynes et al., 2004) and the induction of LTP (Bast et al., 2005). Furthermore, electrical stimulation of the glutamatergic system caused a saturation of plasticity that inhibited learning (Moser et al., 1998), similarly to how activating the AMPAR in Experiment 4 caused a decrease in learning. These converging pieces of evidence indicate a similar influence of the AMPAR on both LTP and spinal learning.

Implications of the AMPAR for neuropathic pain after SCI

Previous research has characterized two phenomena that involve the glutamatergic system and neuropathic pain: central sensitization (Ji et al., 2005) and excitotoxicity (Yezierski, 2005). Central sensitization is observed after peripheral injury/inflammation, which produce an increase in neural excitability within the spinal cord that has been linked to heightened pain. Central sensitization and spinal LTP are thought to depend on common neurochemical systems, including the AMPAR and NMDAR (Ji et al., 2005). Though central sensitization is implicated in increased receptivity to pain, it is not intrinsically linked to the loss of tissue. Excitotoxicity is

associated with chronic activation of the AMPARs that causes a large influx of positive ions, followed by increased pain behaviors and tissue loss (Gorman et al., 2001; Yezierkski, 2005). Furthermore, pretreatment with CNQX blocked the induction of excitotoxicity in cell culture (Brorson et al., 1994).

AMPAR/opioid interactions: Implications for recovery of function after SCI

Neuronal excitation within AMPA-dependant nociceptive pathways can be modified by both endogenous and exogenous opioids (Fundytus 2001; Abraham et al., 2001). These findings suggest that opioid-glutamatergic interactions can impact spinal plasticity. Prior research has shown that morphine exposure can produce an up-regulation in AMPARs that produces hyperexcitability in rats (Glass et al., 2005; Suzuki et al., 2006). Rats exposed to the AMPAR receptor agonist quisqualic acid show increase expression of mRNA of the endogenous opioids propredynorphin (PPD) and propreenkephalin (PPE) (Abraham et al., 2001). Furthermore, the increased levels of PPD and PPE were only found in subjects that showed excessive grooming and pain-like behaviors (Abraham et al., 2001). Increased hind paw withdrawal latencies (to both mechanical and thermal stimulation) produced by an AMPAR antagonist can be reversed using the opioid receptor antagonists (Kong & Yu, 2006). Moreover, morphine tolerance can be reversed by the administration of an AMPAR antagonist (Kest et al., 1997; King et al., 2005). Researchers have applied these concepts showing that co-administration of an AMPAR antagonist and morphine can: prevent excitotoxicity sparing tissue (Brorson et al., 1994), reduce the amount of morphine needed to produce analgesia (Kest et al., 1997), and alleviate acute morphine tolerance in the dorsal horn

(Fundytus 2001). These studies show a link between the glutamatergic system and opioid system at the level of pain processing. Elucidating how these systems interact could suggest new therapeutic strategies for the treatment of pain and secondary injury after SCI. Below ,in figure 15, is a theoretical model describing this interaction.

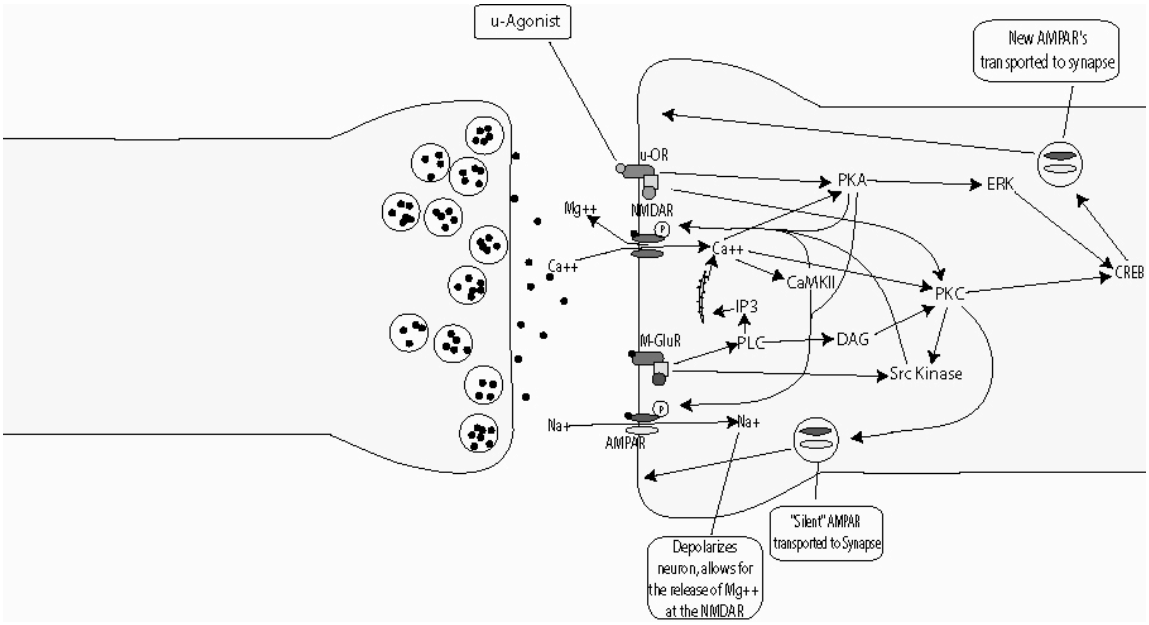

Figure 15. This model demonstrates how the opioid system interacts with the glutamatergic system to induce a state of excitotoxicity by up-regulation of AMPARs. ●= Glutamate

Summary

This study assessed the role of the AMPAR in instrumental learning. The first three experiments using CNQX (AMPAR antagonist) suggests the AMPAR plays a critical role in the acquisition of instrumental learning, the acquisition of the learning deficit, and the maintenance of learning. I then showed that, acute activation of the AMPAR prior to testing also disrupted learning. These results show that the AMPAR is an essential component of the learning process in the spinal cord. Further studies are being conducted to examine how instrumental training impacts the AMPAR at a biochemical level and how these modifications impact clinically relevant phenomena.

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VITA

Kevin Corcoran Hoy Jr.
Department of Psychology
TAMU MS 4235
College Station, TX 77843
(979) 862-4852
hoyaiag@tamu.edu

Education:

B.A. Kent State University, 2005
Major: Psychology/Biology

Professional Organizations:

The Society for Neuroscience