CONTRIBUTION OF THE PERIPHERAL NERVOUS SYSTEM TO INSTRUMENTAL LEARNING AND PERFORMANCE

A Dissertation

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

DOCTOR OF PHILOSOPHY

August 2011

Major Subject: Psychology

Contribution of the Peripheral Nervous System to Instrumental Learning and Performance

CONTRIBUTION OF THE PERIPHERAL NERVOUS SYSTEM TO INSTRUMENTAL LEARNING AND PERFORMANCE

A Dissertation

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

DOCTOR OF PHILOSOPHY

Approved by:

Chair of Committee,	James W. Grau
Committee Members,	Mary Meagher
	Rajesh Miranda
	Mark Harlow
Head of Department,	Ludy T. Benjamin Jr.

August 2011

Major Subject: Psychology

ABSTRACT

Contribution of the Peripheral Nervous System to Instrumental Learning and Performance. (August 2011)

Kevin Corcoran Hoy Jr., B.A., Kent State University; M.S., Texas A&M University

Chair of Advisory Committee: Dr. James W. Grau

Previous research 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, which reduces net shock exposure (Grau et al., 1998). Prior studies have examined the mechanisms that mediate this learning, but little is known about how or where the consequences of learning are stored (memory). The goal of this dissertation proposal is to examine the neural modification(s) that preserve learned behavioral effects over time.

It is clear that the central nervous system plays an essential role in instrumental learning. During the acquisition of instrumental learning, the connections between the peripheral nervous system (PNS) and the central nervous system must remain intact (Crown et al., 2002a). Acquisition is also disrupted by intrathecal application of pharmacological agents (lidocaine) that inhibit spinal reflexes (Crown et al., 2002a). The experiments outlined in this dissertation are motivated by an unexpected

observation: while application of lidocaine to the spinal cord prior to training blocks acquisition of the instrumental response, inactivating spinal neurons has no effect on the maintenance of the instrumental response. These data suggest that, after the instrumental response is acquired, a peripheral component is capable of maintaining the instrumental response.

Aim 1 examined how inhibiting the spinal cord influenced the maintenance of instrumental learning. Intrathecal lidocaine inhibited a spinal withdrawal reflex and instrumental learning, but did not affect the maintenance of the learned response. Expanding on these results, Aim 2 examined how disconnecting the PNS from the spinal cord would influence the maintenance of instrumental learning. If a PNS to spinal cord connection is needed for the maintenance of instrumental learning, then removing that connection by a sciatic transection should disrupt performance of the instrumental response. Together, the results of Aims 1 & 2 confirm that a peripheral alteration contributions to the maintenance of instrumental behavior.

In Aim 3, I developed a procedure that would allow for drug delivery directly to the tibialis anterior muscle. If the neuromuscular junction is capable of influencing a spinal reflex, then blocking the neuromuscular junction with an antagonist (curare) should disrupt the acquisition and maintenance of the instrumental response. Based on the results of Aim 3, Aim 4 investigated how other pharmacological manipulations at the neuromuscular junction can influence the acquisition and maintenance of the instrumental response. Using glutamate receptor antagonists (CNQX and MK-801), I showed that glutamatergic signaling plays an essential role.

DEDICATION

I dedicate this dissertation to my family. I would like to thank Thomas L. Hoy, Keri C. Bove (*née* Hoy), Kevin C. Hoy Sr. and Lisa L. Hoy. Without their endless encouragement and support I would never have completed this work.

ACKNOWLEDGEMENTS

I would like to thank my committee chair, Dr. Grau, for his seemingly limitless patience. I would also like to acknowledge my committee members, Dr. Meagher, Dr. Miranda, and Dr. Harlow, for their input throughout the course of this research.

Thanks also go out to my friends, colleagues, the department faculty and staff for making my time at Texas A&M University a great experience. Specifically I would like to thank Dr. Baumbauer, Dr. Young, Dr. Huie, Dr. Puga, and Milly Lee for their involvement and support throughout this research.

TABLE OF CONTENTS

Page

Page

Page

LIST OF FIGURES

FIGURE Page

LIST OF TABLES

INTRODUCTION

Colloquially, the term *muscle memory* is used to describe the positive effects of training on a complex motor task, such as weightlifting or playing the piano. Though muscles do undergo functional changes after motor training, muscle memory is really a misnomer. The term muscle memory fails to acknowledge that neural circuits are responsible for the initiation and coordination of complex motor tasks. Furthermore, most training paradigms that test muscle memory utilize complete organisms with an intact cerebrospinal tract. In an intact organism, it is difficult to parse out the contribution made by peripheral versus central components. As a behavioral neuroscientist, I want to know where behavioral memory resides? In this dissertation, I examine the contributions of the peripheral nervous system and neuromuscular junction on spinal instrumental learning.

Before I outline how the neuromuscular junction may contribute to instrumental performance, I will briefly review the behavioral capabilities of the isolated spinal cord, our learning task, and pertinent experimental evidence regarding the mechanisms involved.

Spinal Learning

Research over the last half of the 20th century has shown that the spinal cord is a dynamic system capable of regulating reflexes in the absence of a brain. Early studies demonstrated that the isolated spinal cord can support single stimulus learning (Groves

---

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

& Thompson 1970). This work showed that the spinal cord can produce an exaggerated response due to prior stimulation (sensitization) and a reduced response to repeated presentations of the same stimulus (habituation).

The spinal cord has also been shown to be sensitive to stimulus-stimulus relationships, a form of learning known as Pavlovian or Classical conditioning (Durkovic, 1975; Durkovic & Domianopoulos, 1986). In classical conditioning a conditioned stimulus (CS; such as a light) is associated with an unconditioned stimulus (US; such as shock). As a result of these CS-US pairings, there is a change in the response elicited by the CS. Using spinalized cats, the Durkovic laboratory showed that the isolated spinal cord is capable of supporting a variety of Pavlovian phenomenon. In these studies, Durkovic used a shock to the sapheous nerve (which innervates the proximal portion of the lower limb) as a CS and stimulation of the peroneal nerve (which innervates the tibialis anterior) as a US. After repeated CS-US presentations, the CS elicited a conditioned increase in tibialis anterior flexion response reaching asymptote (Durkovic, 1975). Additionally, the Durkovic laboratory showed that the US must be at an intensity that excites A-fibers, not C-fibers, in order to produce a strong conditioned response (CR) at test (Misulis & Durkovic, 1984), and that optimal learning occurs when the CS is presented within 0.5 seconds of the US (Misulis & Durkovic, 1984; Durkovic & Domianopoulos, 1986). These intervals were important whether the stimulus pairings were used in forward (CS-US) or backward (US-CS) conditioning (Durkovic & Domianopoulos, 1986).

Our laboratory also provided evidence that the rat spinal cord is sensitive to stimulus-stimulus relationships (Illich et al., 1994; Joynes & Grau, 1996). In these studies, a weak leg shock (CS) was paired with an intense shock to the tail (US). Subjects subsequently exhibited reduced nociceptive reactivity (antinociception) during the paired CS (Illich et al., 1994; Joynes & Grau, 1996). These studies also demonstrated that the spinal cord exhibits a range of Pavlovian phenomena, including: latent inhibition (preexposure to the CS undermines the CS-US pairing), overshadowing (a more salient CS undermines learning about a less salient cue), and blocking (a pretrained CS disrupts learning about an added cue) (Illich et al., 1994).

Further studies showed that the spinal cord is capable of instrumental learning as well. In instrumental learning, the key relationship is between a response (R) and an outcome (O). The response can vary from simple to complex and the outcome can be either the cessation or actuation of an event (e.g. shock). Previously studies exploring this form of learning were dismissed on methodological grounds (Buerger & Chopin 1976; Church, 1964). These studies lacked proper controls, baseline measurements, common testing parameters, and had incomplete experimental designs. In order to address these prior shortfalls, our laboratory published a seminal work on the ability of the spinal cord to encode R-O relationships (Grau et al., 1998). In this study, the spinal cord was able to acquire the R-O relationship between elicited leg position (response) and shock onset (outcome). Furthermore, we addressed the methodological issues that previously hindered the empirical testing of R-O contingencies (Table 1) in the isolated spinal cord and defined instrumental learning with succinct criteria.

Table 1. Criteria for Instrumental Learning. Adapted from Grau, Barstow, and Joynes (1998).

Minimum Criteria for Instrumental Learning

- Instituting a relationship between a response and an outcome produces a change in behavior (performance).
- The effect is neurally mediated.
- The modification outlasts (extends beyond) the environmental contingencies used to induce it.
- The behavioral modification depends on the temporal relationship between the response and the outcome.

To study spinally mediated instrumental learning, our laboratory uses a modified master-yoke paradigm. Subjects receive a complete spinal transection at the second thoracic vertebra (T2) and are lightly 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 (Figure 1). Over time, these subjects learn to maintain the shocked leg in a flexed position that reduces net shock exposure (response-contingent shock). Yoked animals receive shocks concurrently with master animals, independent of hind leg 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 (savings effect), while yoked animals fail to learn (learning deficit).

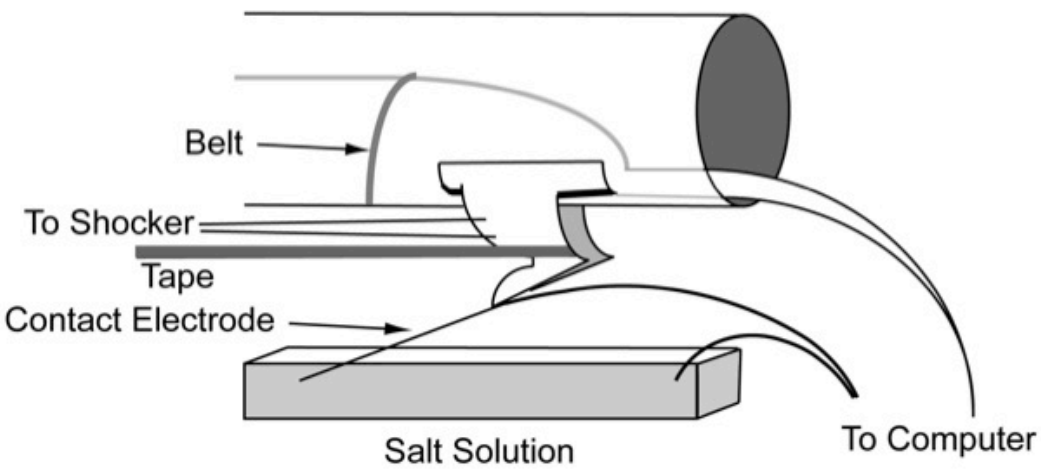

Figure 1. Apparatus used for instrumental learning. The hind limbs of the subject hang loosely restrained above the salt solution. When the contact electrode touches the salt solution shock is applied to the tibialis anterior. Over time subjects will maintain a flexion response, minimizing net shock exposure.

Evidence for Spinal Mediation of Instrumental Learning

The observation of the savings effect and the learning deficit (Grau et al., 1998) provided evidence that instrumental learning was mediated by spinal neurons. The savings effect showed that repeated exposure to the behavioral test improved overall performance at the learning task, which implied that spinal systems are sensitive to the response-outcome contingency. Interestingly, the learning deficit mimicked another phenomenon known as learned helplessness (Seligman & Maier, 1967). After uncontrollable stimulation (yoked to the master rat), subjects that were exposed to response-contingent shock failed to exhibit an increase in response duration (Grau et al., 1998). These experiments suggested that behavioral contingencies can have a lasting effect on spinal function.

Behavioral evidence of spinal mediation of instrumental learning was provided by the phenomenon of contralateral transfer. Contralateral transfer refers to a situation wherein training on one side of the body affects performance when subjects are tested on the opposite side (Crown & Grau, 2001; Crown et al., 2002a; Crown et al., 2002b; Joynes et al., 2003). If the behavioral experience of one leg (ipsilateral) affects the behavior of the opposite leg (contralateral), then a common system (the spinal cord) is implicated. Contralateral transfer is seen in the facilitation of instrumental learning. Subjects are first trained at normal electrode depth of 4 mm, and are then tested on the contralateral leg at an electrode depth of 8 mm, increasing task difficulty to a level where naïve subjects normally fail to acquire the response-outcome contingency (Crown et al., 2002a). Subjects that receive 30 minutes of training at 4 mm and are tested on the contralateral leg at an 8 mm electrode depth can acquire the task, whereas untrained controls fail to learn (Crown et al., 2002a). Contralateral transfer is also implicated in the induction of the learning deficit. Subjects given uncontrollable shock to one leg using the master yoke training paradigm (Crown & Grau, 2001), or variable intermittent stimulation (Crown et al., 2002b), exhibit a learning deficit when tested on the contralateral leg.

The learning deficit can also be induced using tailshock (Crown et al., 2002b). Using the same uncontrollable leg shock parameters, intermittent stimulation of the tail disrupted instrumental learning for 24-48 hours. Moreover, a later study showed that as little as 6-minutes of uncontrollable tail shock was sufficient to induce a strong learning deficit (Ferguson et al., 2006). These data showed that stimulating spinal neurons with

uncontrollable shock was sufficient to induce a behavioral deficit of the leg, even though the shock was applied to the tail (Crown et al., 2002b; Ferguson et al., 2006). The data from these studies (Crown et al., 2002b; Ferguson et al., 2006) imply that the spinal cord is the key component to the instrumental learning deficit and that the memory for the learning deficit lies solely within the spinal cord.

If instrumental learning requires spinal neurons, then inhibiting the spinal cord or severing the sciatic to spinal connection, should disrupt the acquisition of instrumental learning. The sciatic nerve is the largest peripheral nerve in the mammalian nervous system, and provides a direct link between terminus of the nervous system (where shock is applied) to the spinal cord. Our laboratory showed that spinal neurons must be active during the acquisition of the instrumental response (Crown et al., 2002a). Inhibiting the spinal cord prior to the acquisition of instrumental learning with an injection of intrathecal lidocaine (Na+ channel blocker) blocked the acquisition of instrumental learning (Crown et al., 2002a). Furthermore, these experiments showed that severing the sciatic nerve blocked the acquisition of instrumental learning (Crown et al., 2002a. These results confirmed that a spinal to sciatic connection is necessary for the acquisition of instrumental learning.

Further studies showed that instrumental learning is mediated by neurochemical changes within the spinal cord. Using the opioid antagonist naltrexone, our laboratory demonstrated that an intrathecal injection prior to testing blocked the expression of the learning deficit (Joynes & Grau, 2004). Other studies suggested that engaging C-fibers is sufficient to induce a learning deficit (Joynes et al., 2004; Grau et al., 2006). C-fibers

are implicated in slow pain and impact spinal neurons through the release of Substance-P, which engages neurokinin (NK) receptors. Building on these findings, Baumbauer showed that the NK-1 receptor agonist Substance P induces the learning deficit (Baumbauer et al., 2007b). Further, blocking NK-1 receptors with intrathecal administration of the antagonist L-703,606 prior to uncontrollable blocked the induction of the deficit in a dose dependent fashion (Baumbauer et al., 2007b). Other studies demonstrated that inhibiting new protein synthesis after uncontrollable stimulation blocked the induction of the learning deficit (Baumbauer et al., 2006). The inhibition of new protein synthesis is significant because it opened new avenues to test specific protein targets for their influence on instrumental learning. PKC and CaMKII are protein kinases that are directly linked to learning in the brain and pain processing after injury in the spinal cord (Figure 2; Ji et al., 2003). Studies showed that inhibiting either PKC or CaMKII prior to uncontrollable shock blocked the induction of the learning deficit (Ferguson et al., 2006; Baumbauer et al., 2007a). These findings are important because they implicate the glutamatergic system in instrumental learning and the induction of the learning deficit.

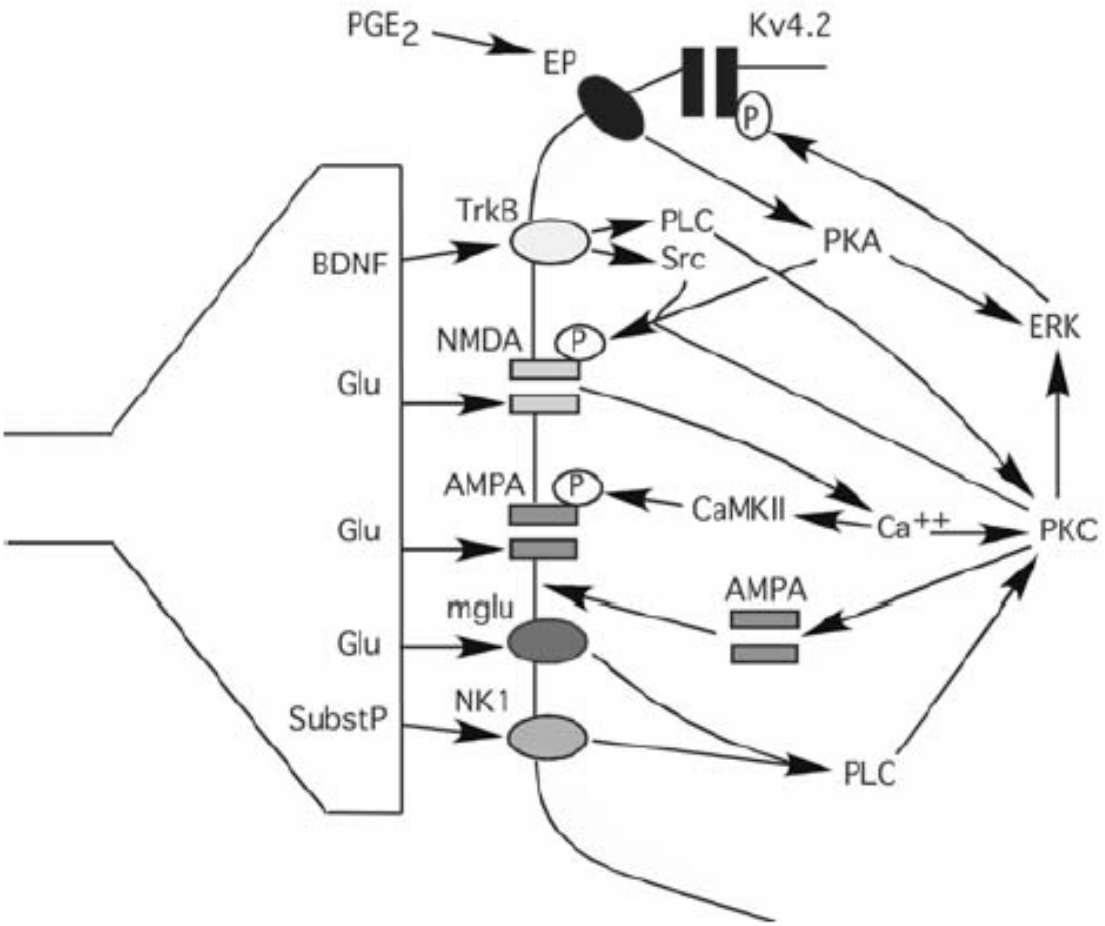

Figure 2. Proposed mechanisms of spinal activation. Adapted from Ji et al., 2003.

Building on this work, studies have shown that the acquisition of instrumental learning can be influenced by glutamatergic signals in the spinal cord (Joynes et al., 2004; Ferguson et al., 2006; and Hoy et al., in prep). These studies showed that blocking spinal ionotropic glutamate receptors (NMDAR & AMPAR) prior to instrumental testing disrupts the acquisition of the instrumental response (Joynes et al., 2004; Hoy et al., in prep). Furthermore, blocking the NMDAR or the AMPAR prior to uncontrollable stimulation blocked the induction of the learning deficit (Ferguson et al., 2006; Hoy et

al., in prep). Though all of these previous findings (contralateral transfer, central induction of the deficit, intrathecal pharmacological interventions) suggest that the spinal cord is the seat of the behavioral memory, an empirical investigation of the peripheral component of instrumental learning has not been completed.

Peripheral Plasticity: Implication for the Maintenance of Instrumental Learning

Shifting gears, at the beginning of this document I introduced the concept of muscle memory and its under-represented neural component. The interaction between the isolated spinal cord and the peripheral nervous system (PNS) has been largely unexplored in our instrumental learning paradigm. Because our laboratory has shown that the spinal cord is responsible for the expression of the learning deficit, is it safe to assume that all components of instrumental learning are spinal? We know that the neuromuscular junction is sensitive to Hebbian (LTP, LTD) like phenomena (Dan & Poo, 1992), which are related to learning and memory in the brain and spinal cord. Further, the PNS has an inherent capacity for change (plasticity), and this could contribute to instrumental performance. We also know that at the site of the neuromuscular junction there are also glutamatergic receptors (Mays et al., 2009). Furthermore, neurons at the neuromuscular junction have been shown to co-release acetylcholine (the common neurotransmitter of the neuromuscular junction) and glutamate (Nishimaru et al., 2005), and drugs that block the acquisition of instrumental learning by inhibiting the glutamatergic signal (CNQX & MK-801) also produce ataxia in muscles (Shih, 1990; Gmiro & Serdyuk, 2006). These data suggest that there is

glutamatergic modulation of the neuromuscular junction, but it is not know whether these contribute to behavioral alteration associated with learning.

The experiments outlined in this dissertation are motivated by an unexpected observation that suggested we might have underestimated the potential contribution of the PNS (Experiment 1B). While application of lidocaine to the spinal cord prior to training blocks acquisition of the instrumental response, inactivating spinal neurons appears to have no effect on the maintenance of the instrumental response. These data suggest that when the spinal cord is "shut down", a peripheral component is capable of maintaining the instrumental response.

Specific Aims

The set of experiments described in this dissertation were designed to examine the contribution of the PNS and the neuromuscular junction to the acquisition and maintenance of instrumental behavior. The central hypothesis of this dissertation is that a modification at the neuromuscular junction contributes to the maintenance (memory) of instrumental behavior.

From a learning perspective, if the PNS is not involved in the maintenance of the instrumental response, then inhibiting the spinal cord pharmacologically should cause an acquired response to wane. Aim 1 examined how inhibiting the spinal cord with intrathecal lidocaine influences the maintenance of instrumental behavior. Expanding on these results, Aim 2 examined how disconnecting the PNS from the spinal cord affects instrumental performance. If a PNS to spinal cord connection is needed for the

maintenance of instrumental learning, then removing that connection (by cutting the sciatic nerve) should disrupt the maintenance of the instrumental response. The results of Aims 1 and 2 revealed that spinal neurons are required for new learning, but not the maintenance of instrumental behavior.

In Aim 3, I developed a procedure to apply drugs directly to the tibialis anterior muscle, which allowed me to explore the neurochemical systems involved at the neuromuscular junction. If the neuromuscular junction is capable of influencing a spinal reflex, then blocking the neuromuscular junction with an antagonist should disrupt the acquisition and maintenance of the instrumental response. Based on the results of Aim 3, Aim 4 further investigated how pharmacological manipulations of the neuromuscular junction influence the acquisition and maintenance of the instrumental response.

GENERAL METHOD

Subjects

All subjects, male Sprague-Dawley rats (100-120 days old 300-450g), were obtained from Harlan Laboratories (Houston, TX). Subjects were dual 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.

Spinal Transection

All subjects received 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 then performed to expose the cord rostral to T2. Heat cautery was used to transect the exposed cord and the cavity formed was filled with gelfoam (Harvard Apparatus, Holliston, MA). The incision was closed using Michel Clips (Fine Science Tools, Foster City, CA). Subjects were injected with 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 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 examination of the spinal cord in a random sample of subjects.

Sciatic Dissection

In experiments 2B, 2C, and 4G, subjects received a transverse incision across their leg exposing the biceps femoris and vastus lateralus muscles. The sciatic nerve was exposed within the popliteal fossa. A chromic catgut suture was placed underneath the exposed sciatic nerve to allow for transection during instrumental training.

Tibialis Anterior Catheter

In Aims 3 and 4, a 27g needle tip was inserted into the tibialis anterior muscle prior to training. The 27g needle tip was connected to a 29 cm long PE-50 tube, which was attached to a 1ml syringe for drug delivery.

Apparatus

Instrumental testing was conducted while rats are loosely restrained in tubes (23.5 cm X 8 cm). Two slots (5.6 cm X 1.8 cm) 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). 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 was 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 the solution. Extension of the ankle joint caused the contact electrode to touch the saline solution, completing the computer-monitored circuit.

Shock intensity was adjusted to elicit a flexion force of 0.4 N prior to testing. 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. Three test shocks were then applied to determine the correct level of shock needed to elicit a 0.4 N flexion force. The strain gauge has been calibrated by determining the relationship between a change in voltage and force in Newtons.

Tail- flick Test

During experiment 1A, thermal nociceptive thresholds were assessed using a radiant heat tail flick device. This device consisted of a 375-W movie light and a condenser lens positioned 8 cm below the light that focused it onto the subject's tail. A photocell located under the subject's tail was used to detect movements. Tail movements exceeding 0.5 cm terminated the light source, latency was recorded to the nearest 0.01 s.

Instrumental Learning Behavioral Procedure

Prior to instrumental testing, the hind limbs were shaved and marked for electrode placement. To minimize lateral leg movements, a piece of porous tape (Orthaletic, 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 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 was raised above the solution, response number was 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: $\text{Response Duration}_i = (60 \text{ s} - \text{time in solution}_i) / (\text{Response Number}_i + 1)$, where i is the current time bin.

Statistics

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

RESULTS

Aim 1: Prior Training Implicates the Periphery in Spinal Learning

Aim 1 used the voltage gated sodium channel blocker lidocaine to assess the impact of inactivating spinal neurons. Prior studies have shown that intrathecal lidocaine administration inhibits spinally mediated reflexes (Crown et al., 2002a). Experiment 1A verified that treatment with lidocaine inhibits spinal reflexes. Experiments 1B and1C examined how lidocaine administration influences the maintenance and facilitation of instrumental learning. These experiments provided preliminary evidence that the peripheral nervous system contributes to the maintenance of the learned behavior.

Experiment 1A: Time Course of Spinal Lidocaine on the Tail Flick Test

Work from our laboratory has shown that lidocaine is effective at inhibiting spinally mediated reflexes (Crown et al., 2002a). What we do not know from previous work is the latency with which lidocaine affects spinal reflexes. The rest of the experiments in this aim depend on lidocaine having maximal efficacy within minutes of application. Experiment 1A verified how quickly lidocaine inhibits the spinal tail-flick reflex.

Procedure

Subjects in experiment 1A were placed in restraining tubes for 5 min to acclimate to the testing room, which was maintained at 26.7° C. Two baseline measures of reactivity were taken approximately 1 min apart for each subject. After baseline testing

(Table 2), subjects received lidocaine (20μl, 40%) or saline intrathecally. Two tail-flicks were performed during drug administration. Subjects then received 3 more measures of thermal reactivity spaced 1 min apart. If the subject did not respond to radiant heat in eight seconds, the test was terminated.

Results

Baseline tail-flicks ranged from 4.37 (± 0.26) to 5.06 (± 0.36). These baseline differences did not approach significance, $F < 1.00$, $p > 0.05$. Subjects that then received lidocaine showed a marked inhibition of the spinal tail-flick response within minutes of administration. An analysis of covariance (ANCOVA), using the mean baseline tail-flicks as the covariate, yielded a significant effect of lidocaine administration and a significant Tail-flick X Lidocaine interaction, all $Fs > 3.20$, $p < .05$, as indicated in Figure 3. *Post hoc* comparisons of the scores at each time points using a student's *t*-test with a Bonferroni correction for 5 comparisons ($p = .05$, corrected .01) showed that the lidocaine had a significant effect after one minute of drug application.

Table 2. Procedure for Experiment 1A.

Transection	24-hours	Baseline 2 Tail flicks	2 Tail Flicks Lidocaine or Saline	3 Test Tail flicks

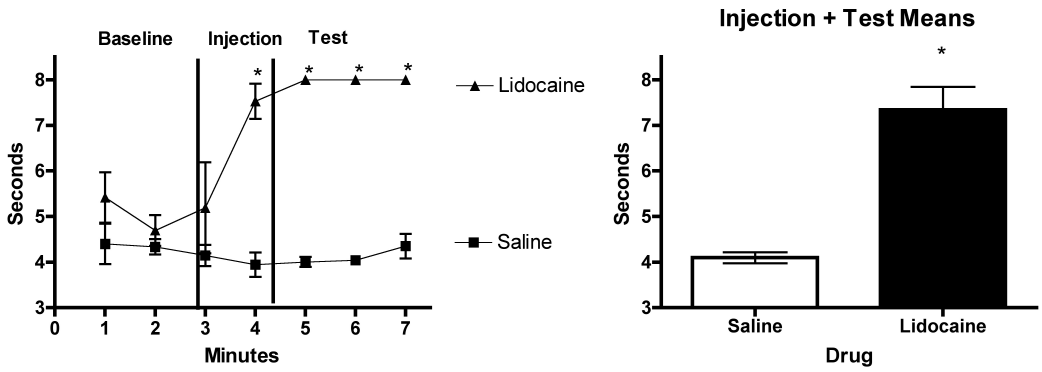

Figure 3. Tail flick latencies after lidocaine exposure. Time course of intrathecal lidocaine on the, spinally mediated, tail flick reflex. Subjects that received lidocaine showed a marked attenuation of the tail-flick response within 1-minute of drug administration. Error bars represent SEM (n=4). * indicates significant difference $p<0.05$.

Discussion

Subjects that received lidocaine showed a marked reduction in nociceptive reactivity within one minute of drug administration. This experiment establishes the efficacy and latency of lidocaine as an inhibitor of spinally mediated reflexes.

Experiment 1B: Intrathecal Lidocaine Administration Does Not Block the Maintenance of Instrumental Learning

Previous research has shown that lidocaine blocks the acquisition of instrumental learning (Crown et al., 2002a), implying that spinal neurons play an essential role in instrumental learning. This experiment examined whether lidocaine affects the performance of the instrumental response after learning has reached a stable asymptote. Experiment 1A showed that intrathecal lidocaine rapidly inhibits a spinally dependent

behavior. If the maintenance of instrumental responding depends on the spinal cord, then intrathecal lidocaine administration should disrupt performance within minutes of application.

Procedure

A 2X2 factorial design was used for this experiment (n=6). After subjects were prepared for instrumental testing (Table 3), half received 30 minutes of training with response contingent shock. The remaining subjects received no training, and served as a positive control to verify that lidocaine treatment disrupts instrumental learning. Twenty-five minutes into the session, half of the subjects in each condition (trained, or positive control) received an intrathecal lidocaine injection (20µl, 40%) or saline. During the last 30 minutes of the testing session all subjects were treated with response contingent shock.

Results

Baseline measurements confirmed that the groups did not differ prior to treatment. The average shock intensities needed to elicit a 0.4N flexion force ranged from 0.52 (± 0.06) to 0.59 (± 0.04) mA. Initial flexion response means ranged from 0.09 (± 0.01) to 0.12 (± 0.02). These differences did not approach significance, $Fs < 1.0$, $p > .05$.

The two pretrained groups exhibited a progressive increase in response duration and did not differ prior to drug treatment, $F(1,10) < 1.00$, $p > .05$. During testing, saline

treated subjects were able to acquire and maintain the instrumental response. Untrained subjects pretreated with lidocaine prior to testing (lidocaine positive controls) did not exhibit an increase in response duration during the 30-minute testing session. Lidocaine had no effect on subjects that had 25 minutes of training prior to the testing session (Figure 3). A two-way repeated measures ANOVA of the 30 minute testing session revealed a significant main effect of drug treatment, training condition, time, a Drug X Condition interaction, Time X Condition interaction, and a Time X Drug X Condition interaction, all $Fs > 1.45$, $p < .05$. *Post hoc* comparisons showed that the lidocaine untrained group (lidocaine (+), Figure 4) was significantly different from all other groups ($p < .05$).

Table 3. Procedure for Experiment 1B.

Transection	24-hours	No-training	Lidocaine	30-min Testing
			Saline	
		30-min Training	Lidocaine	
			Saline	

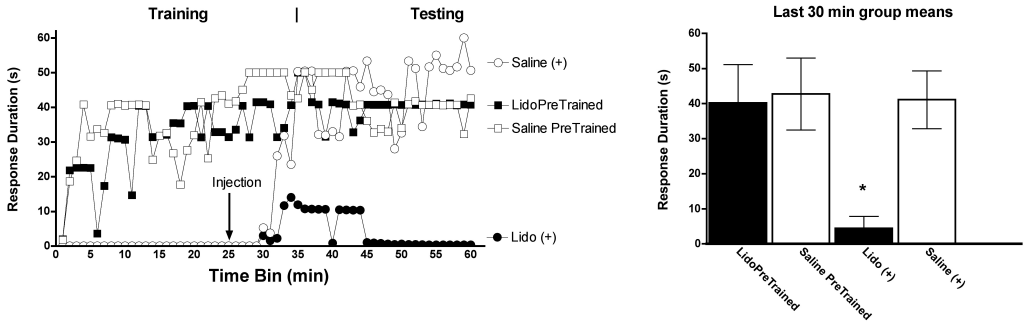

Figure 4. Intrathecal lidocaine and response duration. Subjects that received training prior to lidocaine administration did not show a reduction in response duration. These results indicate that prior training may engage non-spinal mechanisms. (*) indicates statistically significant $p < .05$.

Subjects that exhibited an increase in flexion duration made fewer instrumental responses (Figure 5). During training there was a significant Time X Drug interaction $F(29,290) = 2.00$, $p < .01$. No other comparisons were significant during training. During testing subjects that were not pretrained and received lidocaine showed an increase in response number during the testing session compared to all other groups. A two-way repeated measures ANOVA of the 30 minute testing session revealed a significant main effect of drug treatment, training condition, time, a Drug X Training condition interaction, a Time X Training condition interaction, a Time X Drug interaction, and a Time X Drug X Training condition interaction, $Fs > 8.00$, $p < .01$. *Post hoc* comparisons revealed that the lidocaine untrained (lidocaine (+), Figure 5) was significantly different from all other groups ($p < .05$).

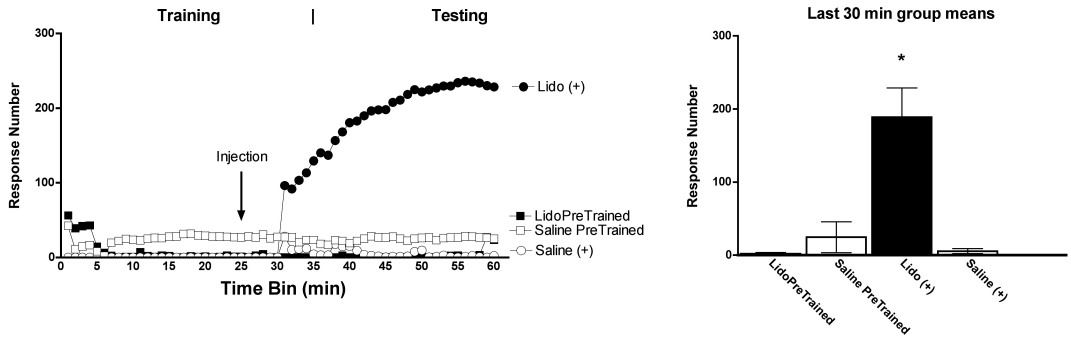

Figure 5. Intrathecal lidocaine and response rate. Response number throughout the training and testing session. Error bars represent standard error of the mean (SEM). (*) indicates statistically significant, $p < .05$.

Discussion

Prior research has shown that lidocaine blocks the acquisition of instrumental learning (Crown et al., 2002a), and a similar effect was observed here. Lidocaine did not however, affect the performance of the instrumental response in pretrained subjects. These results indicate that after training, non-spinal neurons may contribute to the maintenance of learning. These results were the main driving force in generating the hypothesis that the peripheral nervous system plays an active role in the maintenance of instrumental learning. In the experiments that follow, I work to elucidate how the peripheral nervous system contributes to the maintenance of the instrumental response.

Experiment 1C: Intrathecal Lidocaine and the Facilitation of Instrumental Learning

Prior work in our laboratory has shown that instrumental training enables subsequent instrumental learning. In untrained rats, instrumental learning is normally observed when subjects are tested at an electrode depth of 4 mm. If the response criterion is raised to 8 mm, untrained rats fail to learn (Crown et al., 2002a). However, when subjects receive training at 4 mm prior to testing at 8 mm, they can learn at the raised learning criterion (facilitation). Experiment 1C examined whether lidocaine administration after training disrupts the facilitation of instrumental learning. A key difference between experiments 1B and 1C is that in 1C subjects will have to reacquire the learning task when the electrode depth is changed from 4 mm to 8 mm. My hypothesis was that reacquisition (new learning) and memory depend on different processes, and that only the former requires input from the spinal cord.

Procedure

In this experiment, all subjects received 30 minutes of instrumental training (Table 4) at a 4 mm electrode depth. Twenty-five minutes into the training session subjects received either lidocaine (20μl, 40%) or saline. Immediately following the 30-minute training session the water level was raised to 8 mm and subjects were tested for 30 minutes.

Results

Baseline measurements confirmed that the groups did not differ prior to training. The mean shock intensities needed to elicit a 0.4N flexion force ranged from 0.53 (± 0.05) to 0.63 (± 0.04) mA. Initial flexion response average ranged from 0.17 (± 0.03) to 0.18 (± 0.03). These differences were not significant, $Fs > 2.60$, $p > .05$.

During training both groups exhibited an increase in response duration (Figure 6) and did not differ prior to drug administration, $F(1,10) < 1.00$, $p > .05$. During the subsequent 30-minute testing session, at the 8 mm higher criterion, saline treated controls were able to acquire the instrumental response and learn at a higher criterion during the test session, replicating previous findings (Crown et al., 2002a). Subjects treated with lidocaine showed a marked learning deficit. A repeated measures ANOVA revealed a significant main effect of drug treatment, $F(1,10) = 20.50$, $p < .01$, and a significant Drug X Time interaction, $F(29,290) = 2.24$, $p < .01$.

Table 4. Procedure for Experiment 1C.

Transection	24-hours	30-min Training	Lidocaine	30-min testing High Crit.
			Saline	

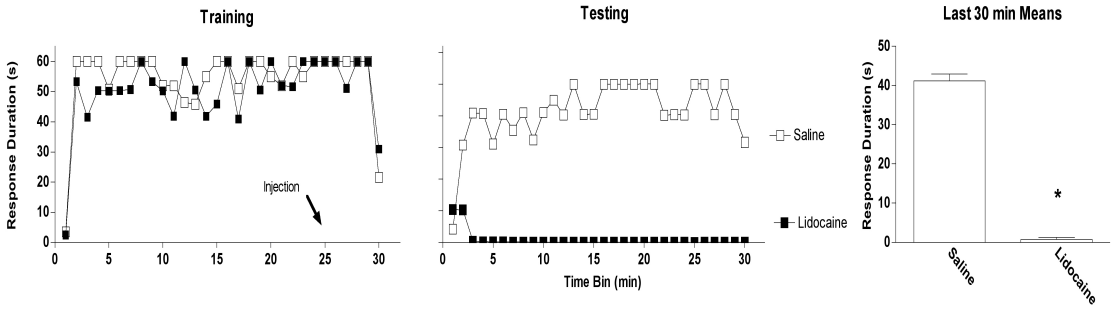

Figure 6. Intrathecal lidocaine facilitation and response duration. Subjects that received lidocaine after training could not learn when the response criterion was raised prior to testing. (*) indicates $p < .01$.

An examination of response number during the training session revealed a significant main effect of time (Figure 7), $F(29,290) = 6.80$, $p < .01$. No other term was significant, Fs > 1.43, $p > .05$. Subjects that received lidocaine showed an increase in response number during the testing session, yielding a main effect of drug treatment $F(1,10) = 48.48$, $p < .01$, time $F(29,290) = 2.01$, $p < .01$, and a Time X Drug interaction $F(29,290) = 2.01$, $p < .01$.

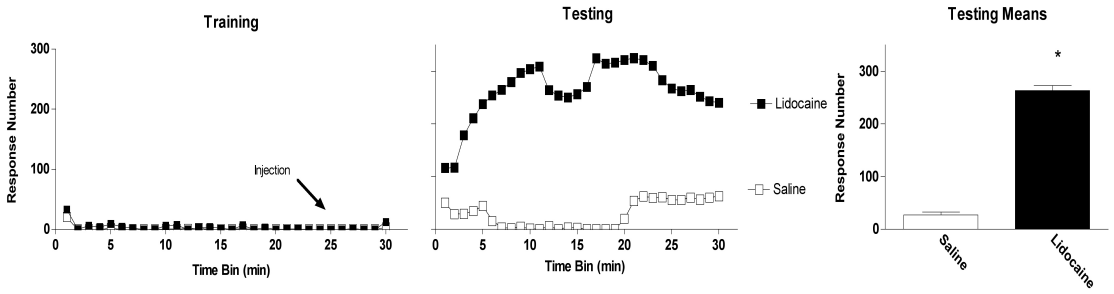

Figure 7. Intrathecal lidocaine facilitation and response rate. Subjects that received lidocaine after training showed an increase in response number compared to saline treated subjects. (*) indicates $p < .01$.

Discussion

Subjects that received lidocaine after instrumental training failed to learn when tested with a higher response criterion. These data indicate that the facilitation of instrumental learning requires active spinal neurons. Furthermore, these data (when taken with the results from 1B) suggest that spinal neurons are needed to learn about new instrumental relations, but not to maintain a response after it is acquired.

Aim 2: The Peripheral Nervous System can Control Behavioral Outcomes After Training

Aim 1 confirmed that intrathecal lidocaine inhibits a spinal reflex within minutes of application and blocks the acquisition of instrumental learning, as reported by Crown et al. (2002b). Lidocaine did not disrupt the maintenance of instrumental learning. It did, however, inhibit learning when subjects were tested at a higher response criterion. These observations suggest that spinal mechanisms must be active to acquire a response,

but are not needed to maintain the response. Aim 2 further examines how disrupting the link between the peripheral and central nervous system influences the maintenance of instrumental learning. In the rat nervous system, the sciatic nerve innervates all sensory and muscular associated areas from L4 to S3 of the spinal column (Figure 8). If interfering with sciatic nerve function causes a performance deficit after training, then we can conclude that the maintenance of instrumental learning has a peripheral component.

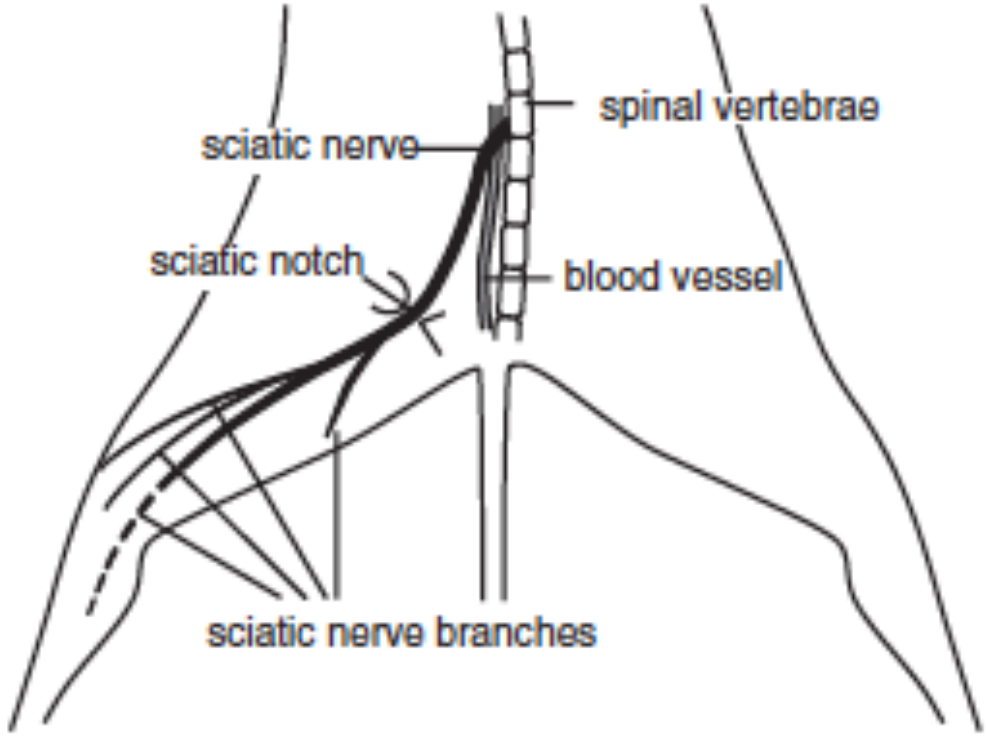

Figure 8. Anatomy of the rat the spinal cord and sciatic nerve. Adapted from Weinstein & Wu, 2001.

Experiment 2A: Direct Lidocaine Application to the Sciatic Nerve Does Not Disrupt the Maintenance of Instrumental Learning

Experiment 1B revealed that after training, i.t. lidocaine is ineffective at disrupting instrumental performance. These results suggest that neural activity within the spinal cord is not necessary to maintain the performance of the instrumental response. The corollary to this is that the key modification may live in the periphery, perhaps at the neuromuscular junction. If this is true, then disrupting communication along the sciatic nerve should disrupt the acquisition, but not the maintenance of instrumental behavior. Experiment 2A addresses this issue by inhibiting sciatic nerve activity using lidocaine.

Procedure

A 2X2 factorial design (Table 5) was used for this experiment (n=6). Prior to behavioral testing, subjects had their sciatic nerve exposed. After subjects were prepared for instrumental testing, half received 30 minutes of training with response contingent shock. Twenty-five minutes into the training session, half of the subjects in each condition [trained or untrained] received a lidocaine bath of the exposed sciatic nerve (50μl, 40%) or saline. During the last 30 minutes of the testing session, all subjects were treated with response contingent shock.

Results

Baseline measurements confirmed that the groups did not differ prior to treatment. The mean shock intensities needed to elicit a 0.4N flexion force ranged from 0.57 (± 0.04) to 0.65 (± 0.02) mA. Initial flexion response means ranged from 0.14 (± 0.03) to 0.18 (± 0.02). These differences were not significantly different, $Fs < 1.00$, $p > .05$.

The two pretrained groups exhibited a progressive increase in response duration and did not differ prior to drug treatment, $F(1,10) < 1.00$, $p > .05$. During the subsequent 30-minute testing session, subjects that were not pretrained and received lidocaine showed a deficit in response duration compared to all other groups (Figure 9). A two-way repeated measures ANOVA of the 30-minute testing session revealed a significant main effects of drug treatment, and time, and a significant interaction between Drug X Training, Time X Drug, Time X Training, and a Time X Drug X Training, all $Fs > 1.78$, $p < .05$. *Post hoc* comparisons showed that the lidocaine-untrained group (lidocaine (+), Figure 9) was significantly different from all other groups ($p < .05$).

Table 5. Procedure for Experiment 2A.

Transection	24-hours	None	Lidocaine	30-min Testing
			Saline	
		30-min Training	Lidocaine	
			Saline	

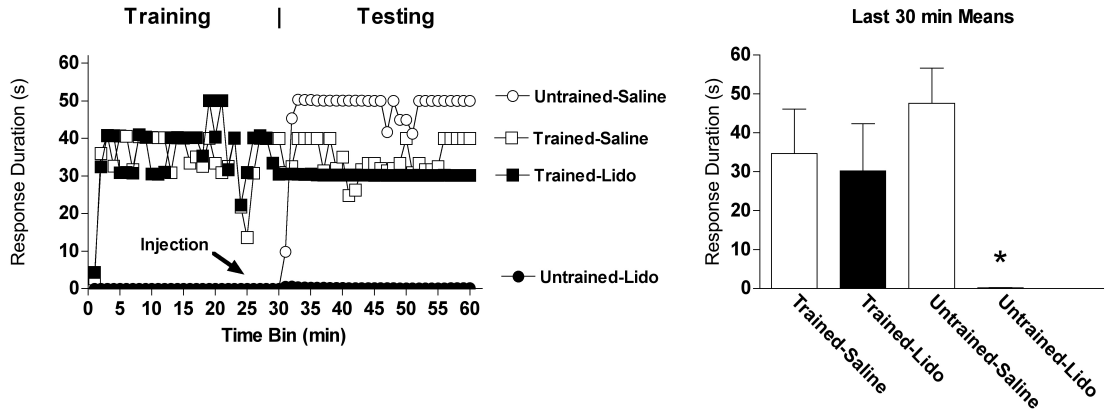

Figure 9. Sciatic lidocaine exposure and response duration. Subjects that received training prior to lidocaine administration did not show a reduction in response duration. (*) Indicates statistically significant $p < .05$.

Subjects that exhibited an increase in flexion duration made fewer instrumental responses (Figure 10). During training there was a significant main effect of time, $F(29,290) = 2.99$, $p < .01$. No other comparisons were significant during training, all Fs > 1.05, $p > .05$. During testing, subjects that were not pretrained and received lidocaine showed an increase in response number during the testing session compared to all other groups. A two-way repeated measures ANOVA of the 30-minute testing session revealed a significant main effect of time, and Training X Drug, Time X Training, Time X Drug, and Time X Drug X Training condition interactions, all $Fs > 2.53$, $p < .05$. *Post hoc* comparisons revealed that the lidocaine-untrained group (Figure 10) was significantly different from the Saline-untrained group ($p < .05$). No other groups were statistically different.

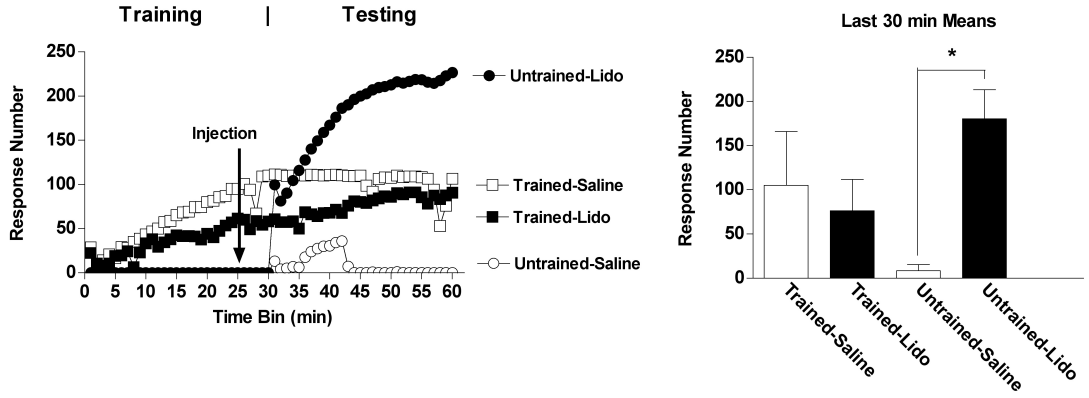

Figure 10. Sciatic lidocaine exposure and response rate. Subjects that received training prior to lidocaine administration responded at a lower rate during testing. (*) indicates statistically significant $p < .05$.

Discussion

Saline treated subjects were able to acquire and maintain a normal level of instrumental learning during training and testing. Subjects that did not receive training prior to lidocaine administration were unable to acquire the instrumental response. Subjects that received 25 minutes of training prior to lidocaine administration showed no learning deficit during the testing session. These results replicate the results from experiment 1B, but in the peripheral nervous system. The findings suggest that continued performance of the instrumental response does not require continued input from the spinal cord after the response is acquired. This implies that the effective behavioral memory does not lie within the spinal cord.

Experiment 2B: Sciatic Transection after Training Does Not Block the Maintenance of Instrumental Learning

Results from Experiment 2A indicate that sciatic lidocaine exposure does not disrupt the maintenance of the instrumental response. Similarly, experiments within Aim 1 suggest i.t. lidocaine has no effect on instrumental memory. However, one could argue that lidocaine did not disrupt maintenance because the drug only partially blocked neural activity. From this perspective, lidocaine might only impact acquisition because it requires greater neural activity. To address this possibility, the next experiment will examine whether cutting the sciatic nerve after the instrumental response is learned affects the maintenance of the instrumental response. Prior work established that cutting the sciatic nerve blocks acquisition of the instrumental response (Crown et al., 2002a).

Procedure

A 2X2 factorial design was used (Table 6) for this experiment (n=8). Prior to behavioral testing, subjects had their sciatic nerve exposed. After subjects were prepared for instrumental testing, half received 30 minutes of training with response contingent shock. Twenty-five minutes into the training session, half of the subjects in each condition (trained, or positive control) received a complete sciatic transection or nothing. During the last 30 minutes of the testing session, all subjects received response contingent shock.

Results

Baseline measurements confirmed that the groups did not differ prior to treatment. The average shock intensities needed to elicit a 0.4N flexion force ranged from 0.58 (± 0.04) to 0.64 (± 0.03) mA. Initial flexion response means ranged from 0.13 (± 0.01) to 0.17 (± 0.02). These differences were not significant, $Fs < 1.00$, $p > .05$.

The two pretrained groups exhibited a progressive increase in response duration and did not differ prior to drug treatment, $F(1,14) < 1.00$, $p > .05$. During the subsequent 30-minute testing session, subjects that were not pretrained and received lidocaine showed a deficit in response duration compared to all other groups (Figure 11). A two-way repeated measures ANOVA of the 30-minute testing session revealed a significant main effect of condition (transected/none), training, time, a Training X Condition interaction, and a Time X Condition interaction, all $Fs > 1.72$, $p < .05$. No other relationships were significant. *Post hoc* comparisons showed that the Untrained Transected group (Untrained-Cut, Figure 11) was significantly different from all other groups ($p < .05$).

Table 6. Procedure for Experiment 2B.

Transection	24-hours	None	Cut	30-min Testing
			Un-cut	
		30-min Training	Cut	
			Un-cut	

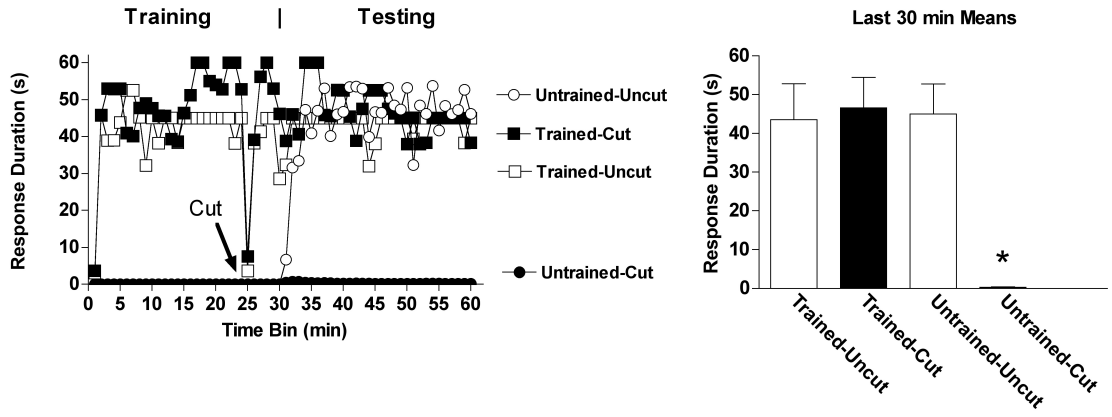

Figure 11. Sciatic transection and response duration. Subjects that received training prior to sciatic transection did not show a reduction in response duration. (*) indicates statistically significant $p < .05$.

Subjects that exhibited an increase in flexion duration made fewer instrumental responses, (Figure 12). During training there was a significant Time X Condition interaction $F(29, 406) = 1.85$, $p < .01$. No other comparisons were significant during training. During testing, subjects that were not pretrained and received a sciatic transection showed an increase in response number compared to all other groups. A two-way repeated measures ANOVA of the 30-minute testing session revealed a significant main effect of condition, training, time, and a Condition X Training, Time X Training, Time X Condition, and a Time X Condition X Training interactions, all $Fs > 2.71$, $p < .05$. *Post hoc* comparisons revealed that the Untrained Transected group (Untrained-Cut, Figure 11) was significantly different from all other groups ($p < .05$).

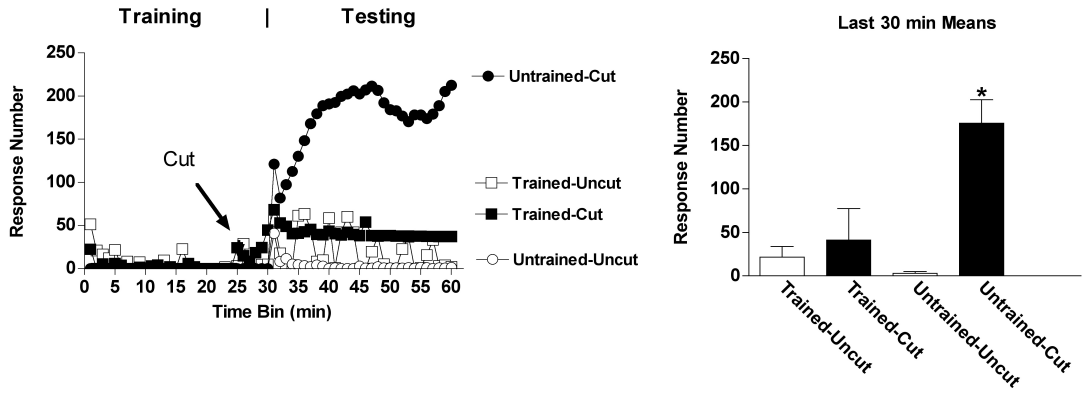

Figure 12. Sciatic transection and response rate. Response number throughout the entire training/testing session. Error bars represent standard error of the mean (SEM). (*) indicates statistically significant, $p < .05$.

Discussion

Subjects that did not receive training prior to complete sciatic transection were unable to acquire the instrumental response. These results replicate previous work in our laboratory (Crown et al., 2002). Subjects that had 25 minutes of training prior to complete sciatic transection showed no performance deficit during the testing session. These results, taken with those from Experiment 2A, show that the spinal cord is necessary for the acquisition of instrumental learning, but is not needed to maintain instrumental performance after the behavior has been acquired.

Experiment 2C: Sciatic Transection Disrupts the Facilitation of Instrumental Learning

The previous experiment (2B) examined how prior training can allow the isolated sciatic nerve to maintain a learned response. This experiment will examine how a

complete sciatic transection influences the facilitation of instrumental learning. The difference between Experiments 2B and 2C is that the latter requires subjects to acquire a stronger instrumental response. My hypothesis is that sciatic transection after training will disrupt learning when subjects are tested with a higher criterion (facilitation).

Procedure

Prior to behavioral testing subjects had their sciatic nerve exposed. All subjects (n=8) received 30 minutes of instrumental training (Table 7) at a 4 mm electrode depth. Twenty-five minutes into the training session, subjects received either a complete sciatic transection or nothing. Immediately after the water level was raised to 8 mm, performance was tested for an additional 30 minutes.

Results

Baseline measurements confirmed that the groups did not differ prior to treatment. The average shock intensities needed to elicit a 0.4N flexion force ranged from 0.55 (± 0.03) to 0.64 (± 0.03) mA. Initial flexion response means ranged from 0.16 (± 0.02) to 0.17 (± 0.02). These differences were not significant, $Fs < 1.00$, $p > .05$.

During training (Figure 13) there was a main effect of time, $F(29,406) = 10.14$, $p < .01$. No other effects were significant, all Fs > 1.85, $p > .05$. Subjects that received a complete sciatic transection showed a marked learning deficit. A repeated measures ANOVA revealed a significant main effect of a sciatic transection and a significant

Transection X Time interaction, all *Fs* > 3.16, $p < .01$. No other terms were significant, all *F*s > 1.00, $p > .05$.

Table 7. Procedure for Experiment 2C.

Transection	24-hours	30-min Training	Un-Cut	30-min Testing High Criterion
			Cut	

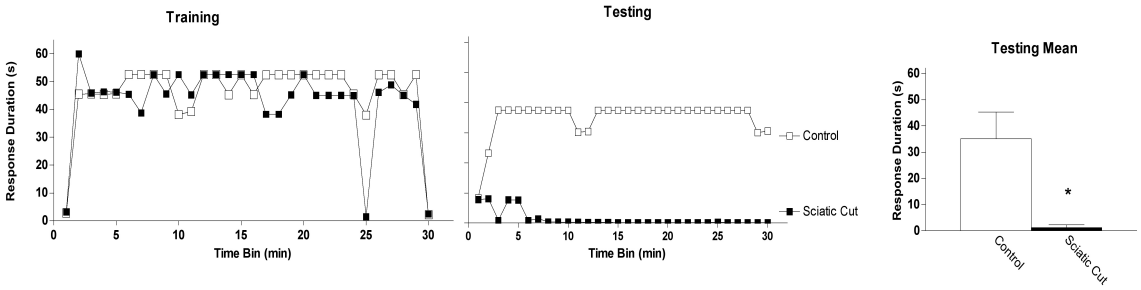

Figure 13. Sciatic transection facilitation and response duration. Subjects that received a sciatic transection after training were unable to learn when tested with a higher response criterion. (*) indicates $p < .01$.

During the training session there was a change in response number over time, $F(29,406) = 5.23$, $p < .01$. No other term was significant, all *F*'s > 1.85, $p > .05$. During testing, subjects that received a complete sciatic transection showed an increase in response number (Figure 14). An ANOVA yielded a main effect of condition (transected/none), $F(1,14) = 4.93$, $p < .05$. No other relationship was significant, all *F*s > 1.20, $p > .05$.

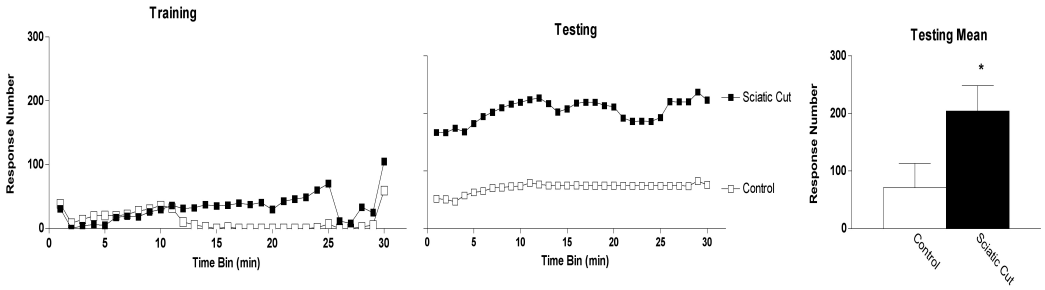

Figure 14. Sciatic transection facilitation and response rate. Subjects that received a sciatic transection after training showed an increase in response number compared to non-transected controls. (*) indicates $p < .05$.

Discussion

Subjects that had an intact sciatic nerve were able to acquire the instrumental response at a higher criterion. Subjects that had a sciatic transection were unable to learn at a higher criterion. These results indicate that the spinal cord is needed to learn a new response, to maintain the leg at a higher criterion.

Aim 3: Alteration of the Neuromuscular Junction

In Aim 1, I examined the influence of spinal inhibition on the maintenance of learning. Preliminary data suggested that the peripheral nervous system contributes to the maintenance of the instrumental response (Experiment 1B). Aim 2 took this a step further, by examining the impact of disrupting communication between the spinal cord and the neuromuscular junction. Here too, data imply (Experiments 2A and 2B) that peripheral mechanisms contribute to the maintenance of the learned response. Aim 3 extends these observations to examine how the terminus of the peripheral nervous

system, the neuromuscular junction, contributes to instrumental learning/performance. In Experiments 3A and 3B the nicotinic acetylcholine receptor antagonist curare was used to block the neuromuscular junctions within the tibialis anterior muscle.

Experiment 3A: Curare Administration Disrupts the Acquisition of Instrumental Learning

To examine the role of the neuromuscular junction, curare was administered into the tibialis anterior muscle. Curare blocks the nicotinic acetylcholine receptors at the neuromuscular junction that induce muscle flexion. My aim is to use this drug manipulation to verify that the maintenance of the flexion response does not reflect an alteration within the muscle. To establish an effective dose, the present experiment will determine the concentration of curare needed to block acquisition.

Procedure

This experiment used 3 groups (control and 2 drug doses) (n=6). Prior to behavioral testing, subjects had a catheter implanted into their tibialis anterior muscle. This catheter allowed drug administration directly into the muscle that controls the flexion response. Subjects received a dose of curare (120ug, 240ug), or saline, twenty-five minutes (Table 8) prior to thirty minutes of instrumental testing.

Results

Baseline measurements confirmed that the groups did not differ prior to treatment. The mean shock intensities needed to elicit a 0.4N flexion force ranged from 0.55 (± 0.03) to 0.60 (± 0.05) mA. Initial flexion response means ranged from 0.14 (± 0.03) to 0.18 (± 0.02). These measures were not significantly different, $Fs > 1.00$, $p > .05$.

The effect of curare on the acquisition of instrumental learning is depicted in Figure 15. Subjects that received a saline injection into the tibialis anterior muscle prior to testing were able to acquire the flexion response over the thirty minute testing session. Rats pretreated with curare (120ug, 240ug) failed to learn. A one-way repeated measures ANOVA revealed a significant main effect of drug treatment, $F(2,15) = 5.56$, $p < .05$, time $F(29,435) = 2.71$, $p < .01$, and a Time X Drug interaction $F(58, 435) = 2.91$, $p < .01$. *Post hoc* analysis revealed that both curare treated groups (120ug, 240ug) were significantly different from the saline treated controls, $p < .01$, and the curare treated groups did not differ, $p > .05$.

Table 8. Procedure for Experiment 3A.

Transection	24-hours	Saline	30-min Testing
		Curare 120ug	
		Curare 240ug	

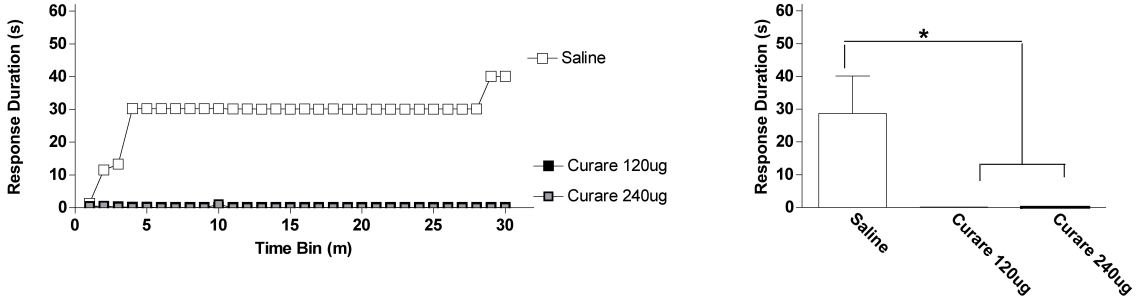

Figure 15. Intramuscular curare and acquisition. Subjects that received curare prior to instrumental testing showed a significant decrease in response duration. (*) indicates statistically significant $p < .01$.

Curare had a non-monotonic effect on performance of the shock-elicited response. The lowest dose of curare enhanced responding, whereas the highest dose disrupted performance. A one-way repeated measures ANOVA of the 30 minute testing session revealed a significant main effect of drug, $F(2,15) = 4.59$, $p < .05$, and a Time X Drug condition interaction, $F(58,435) = 1.61$, $p < .01$. *Post hoc* comparisons revealed that curare treated groups were significantly different from each other ($p < .05$), but neither drug group was significantly different relative to the saline treated subjects.

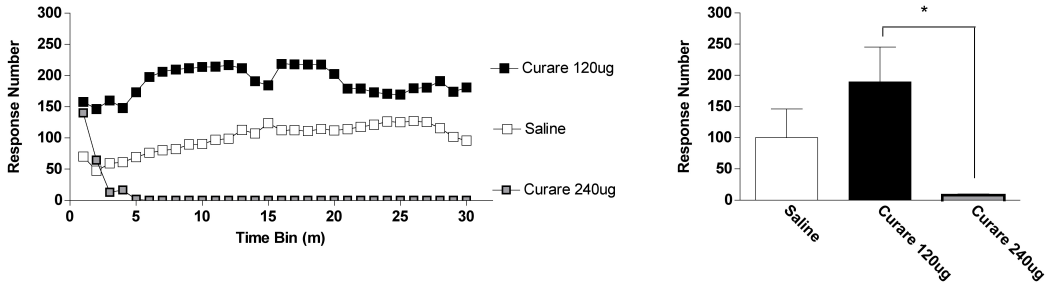

Figure 16. Intramuscular curare and acquisition rate. Subjects that received the lowest dose, 120ug, of curare showed an increase in response rate compared to subjects treated with the higher dose, 240ug, of curare . (*) indicates statistically significant $p < .05$.

Discussion

The results indicate that implanting a catheter in the muscle does not disrupt the acquisition of instrumental learning and allows for local drug delivery. Furthermore, as expected, acetylcholine release was necessary for the acquisition of instrumental learning. Saline treated subjects were able to acquire and maintain a normal level of instrumental learning during testing. Curare administration prior to instrumental learning disrupted the acquisition of the flexion response. Subjects that received the highest dose of curare (240ug) eventually habituated to the shock stimulus, whereas the lower dose (120ug) produced an increased rate of responding. These results confirm that the acquisition of the instrumental response requires a functional neuromuscular junction.

Experiment 3B: Curare Administration Disrupts the Maintenance of Instrumental Learning

Results from Aim 1 & 2 show that manipulations that block the acquisition of instrumental learning do not necessarily disrupt the maintenance of instrumental learning. We have assumed that the maintenance of instrumental learning is dependant on the continued release of acetylcholine from the peripheral nerves. This experiment examines how curare administration influences instrumental learning after 30 minutes of training. If a functional neuromuscular junction is needed for the maintenance of instrumental learning, then curare should disrupt the maintenance of the instrumental response.

Procedure

A 2X2 factorial design (Table 9) was used for this experiment (n=8). Prior to behavioral testing, subjects had a catheter implanted into their tibialis anterior muscle. After subjects were prepared for instrumental testing, half received 30 minutes of training with response contingent shock. Twenty-five minutes into the training session, half of the subjects in each condition (trained, or positive control) received a drug injection (curare 240ug) or saline. During the last 30 minutes of the testing session, all subjects were treated with response contingent shock.

Results

Baseline measurements confirmed that the groups did not differ prior to treatment. The mean shock intensities needed to elicit a 0.4N flexion force ranged from 0.56 (± 0.04) to 0.64 (± 0.03) mA. Initial flexion response means ranged from 0.12 (± 0.01) to 0.18 (± 0.02). These measures were not significantly different, $Fs > 1.56$, $p > .05$.

The two pretrained groups exhibited a progressive increase in response duration and did not differ prior to drug treatment, $F(1,14) = < 1.00$, $p > .05$. During the subsequent 30-minute testing session, subjects that received curare showed a rapid decline in response duration relative to the saline treated controls. As in Experiment 3A, curare also disrupted learning in the untrained positive controls (Figure 17). A two-way repeated measures ANOVA of the 30 minute testing session revealed a significant main effect of drug treatment, $F(1,28) = 19.18$, $p < .01$, a Time X Drug interaction $F(29,812)$

= 2.22, $p < .01$, and a Time X Training interaction $F(29,812) = 3.06$, $p < .01$. No other relationships were significant, all Fs > 1.28, $p > .05$. *Post hoc* comparisons showed that the curare treated groups (Figure 16) were significantly different the saline treated controls ($p < .05$).

Table 9. Procedure for Experiment 3B.

Transection	24-hours	No-Training	Saline	30-min Testing
			Curare	
		30-min Training	Saline	
			Curare	

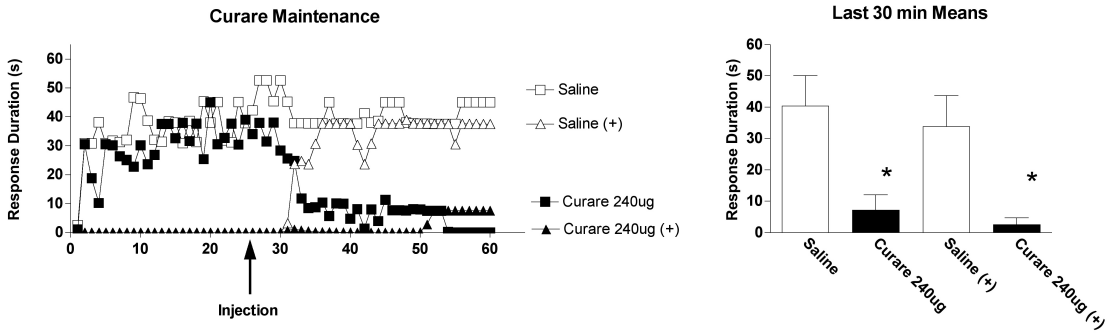

Figure 17. Intramuscular curare and maintenance. Subjects that received curare show a reduction in response duration. (*) indicates statistically significant $p < .05$.

Response rate was also analyzed. As usual, learning yielded a significant effect of time, $F(29, 406) = 1.79$, $p < .01$. By chance, 4 of the 8 subjects that were subsequently given curare exhibited a high rate of responding 5-15 minutes into the training period, yielding a significant Time X Drug interaction $F(29,406) = 2.91$, $p < .01$. Importantly, the overall group difference was not significant (Figure 18) and both groups

exhibited nearly identical performance during the second half of the training period, all Fs > 1.14, p > .05. The subsequent testing session showed no significant relationships, all Fs > 1.0, p > .05.

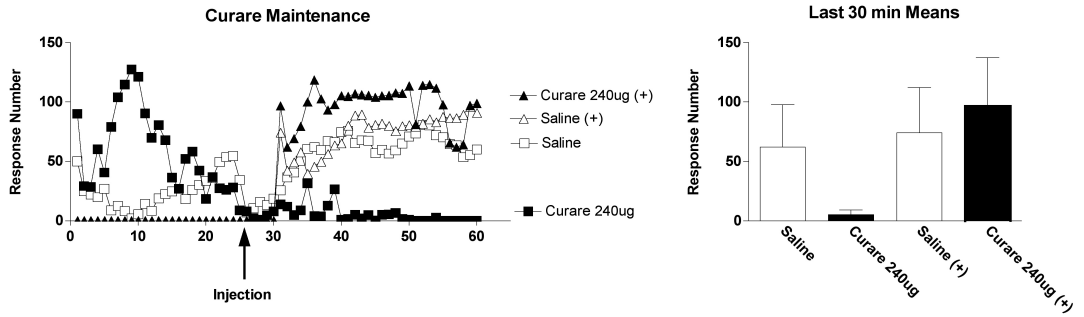

Figure 18. Intramuscular curare and maintenance response rate. Response number throughout the entire training/testing session. Error bars represent standard error of the mean (SEM).

Discussion

The results of this experiment indicate that a continued release of acetylcholine is required to maintain the instrumental response. Furthermore, the experiments in this aim demonstrate that drugs can be delivered in real-time to the tibialis anterior muscle during instrumental training. Developing this method of drug delivery will allow for further pharmacological manipulation at the site of the neuromuscular junction.

Aim 4: The Location of the Behavioral Memory, the Neuromuscular Junction

The final aim of this dissertation (Aim 4) will examine the mechanisms that contribute to the peripheral mediated behavioral memory observed in Aims 1-3. Aim 1 showed that after training, intrathecal lidocaine did not disrupt maintenance of the instrumental response. In Aim 2, I examined this phenomenon further. Transecting the sciatic nerve after instrumental training (Aim 2) also did not disrupt the maintenance of instrumental behavior. These experiments suggest that the periphery is capable of driving the tibialis anterior muscle after training. In Aim 3, I examined how the release of acetylcholine from the terminus of the peripheral nerves contributes to instrumental behavior. Aim 3 showed that acetylcholine release is required to maintain the response. Together, these results indicate that, after training the peripheral nervous system (Aims 1-2) can regulate the release of acetylcholine (Aim 3) to maintain instrumental behavior. Aim 4 examined the mechanisms that allow the peripheral nervous system (figure below) to regulate the release of acetylcholine at the neuromuscular junction.

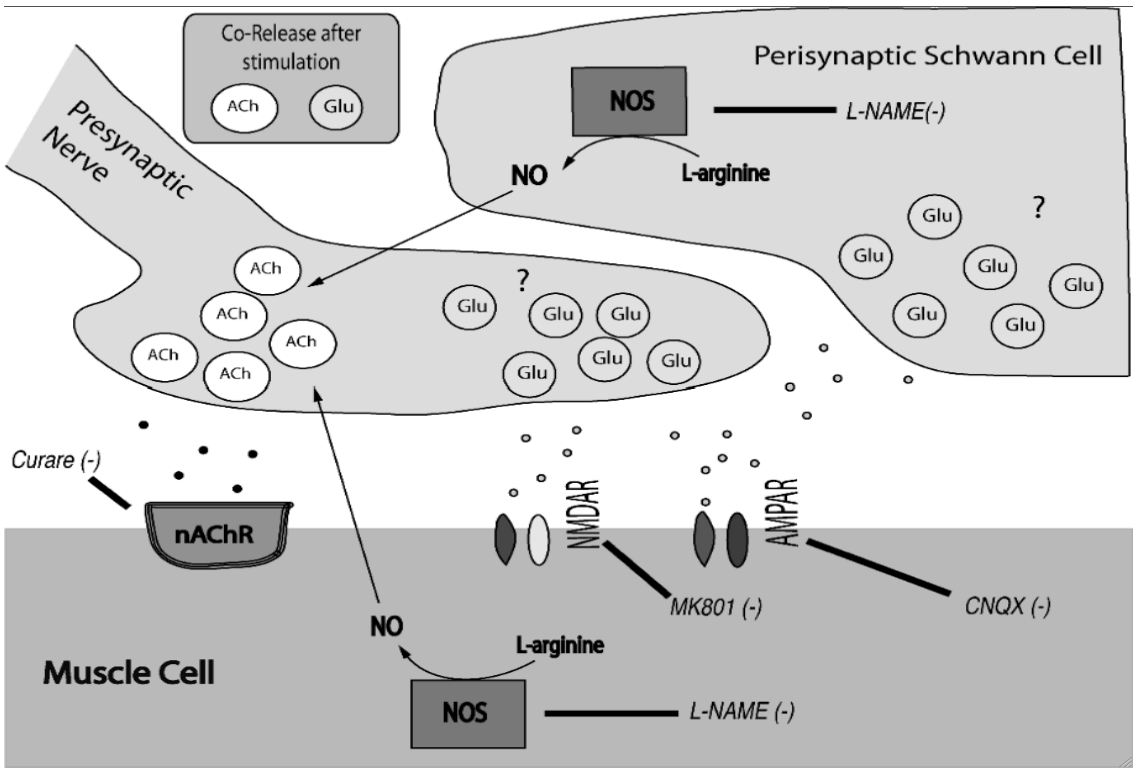

Figure 19. Proposed mechanisms for peripheral memory. Aim 4 will examine the co-release of glutamate & acetylcholine, and the retrograde NO signal. Arrows indicate activation. Pharmacological manipulations are labeled in *italics*: *Curare, L-NAME, MK801, & CNQX.* Question marks indicate unknown source of glutamate.

Research from other laboratories has shown that: 1) NMDA receptors are co-localized at the neuromuscular junction with acetylcholine receptors (Mays et al., 2009), 2) motor neurons co-release glutamate and acetylcholine at the neuromuscular junction (Nishimaru et al., 2005), 3) glutamate release regulates the retrograde NO signal (Malomouzh et al., 2005; Malomouzh et al., 2003; Vyskocil et al., 2009), and 4) the retrograde NO signal in turn regulates the facilitated release of acetylcholine at the neuromuscular junction (Malomouzh et al., 2007; Vyskocil et al., 2009). The experiments in this aim examined how retrograde NO (Experiment 4A) signaling and

glutamate release (Experiment 4B-G), at the neuromuscular junction, influence the maintenance and acquisition of instrumental learning.

Experiment 4A: Retrograde Nitrous Oxide Does Not Influence the Acquisition of Instrumental Learning

In the presence of the NO-synthase inhibitor L-NAME rats undergoing treadmill training showed a marked decrease in hindlimb muscular blood flow and a reduction in acetylcholine release (Musch et al., 2001; Hirai et al., 1994), which suggest that NO release contributes to the improvement in treadmill performance. Likewise, retrograde NO signaling may modulate instrumental performance. My hypothesis is that the release of nitric oxide increases the production of acetylcholine at the neuromuscular junction, and thereby augments flexion duration. This experiment will explore this issue by testing whether a NO-synthase inhibitor, L-NAME, affects instrumental learning/performance.

Procedure

This experiment (Table 10) used 3 groups (Saline & 2 drug doses) (n=6). Prior to behavioral testing, subjects had a catheter implanted into their tibialis anterior muscle. This catheter allowed drug administration directly into the muscle that controls the flexion response. Subjects received a dose of L-NAME (3 or 10mg) or saline twenty-five minutes prior to thirty minutes of instrumental testing (Musch et al., 2001).

Results

Baseline measurements confirmed that the groups did not differ prior to treatment. The mean shock intensities needed to elicit a 0.4N flexion force ranged from 0.52 (± 0.04) to 0.62 (± 0.03) mA. Initial flexion response means ranged from 0.10 (± 0.01) to 0.13 (± 0.02). These measures were not significantly different , $Fs > 1.06$, $p > .05$.

The effect of L-NAME on the acquisition of instrumental learning is depicted in Figure 20. Subjects that received a saline injection into the tibialis anterior muscle prior to testing were able to acquire the flexion response over the thirty minute testing session. Rats pretreated with L-NAME (3 or 10mg) were also able to learn. A one-way repeated measures ANOVA revealed a significant main effect of time $F(29,435) = 2.84$, $p < .01$. No other relationships were significant.

Table 10. Procedure for Experiment 4A.

Transection	24-hours	Saline	30-min Testing
		L-NAME 3mg	
		L-NAME 10mg	

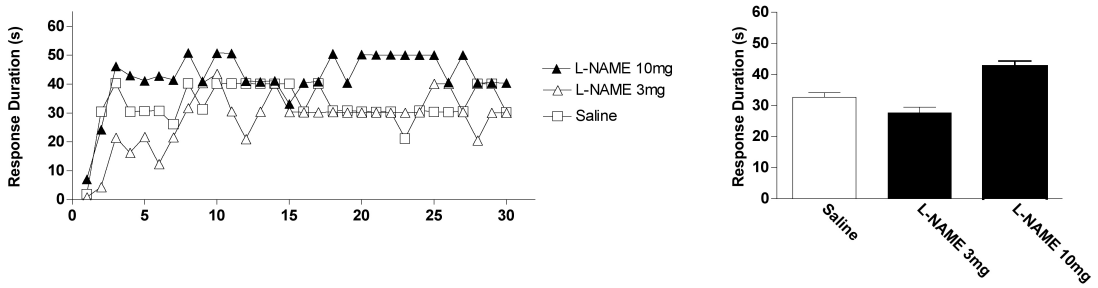

Figure 20. Intramuscular L-NAME and acquisition. Subjects that received L-NAME did not show a reduction in response duration.

Saline treated subjects responded at a similar rate as the L-NAME treated groups (Figure 21). A one-way repeated measures ANOVA of the 30 minute testing session revealed a significant main effect of time, $F(29,435) = 1.93$, $p < .01$. No other relationships were significant.

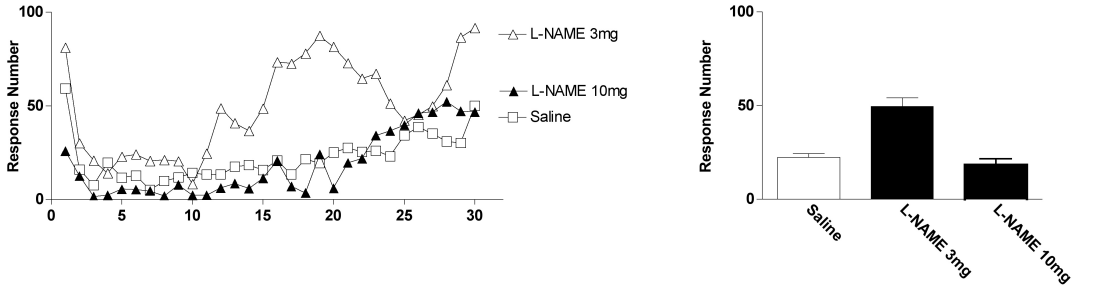

Figure 21. Intramuscular L-NAME and acquisition response rate. Subjects that received L-NAME prior to testing responded at a similar rate as saline treated subjects.

Discussion

As usual, saline treated subjects were able to acquire the instrumental response during testing. L-NAME administration had no measureable effect on instrumental performance. These results lend little support for the modulatory role of nitric oxide in instrumental responding.

Experiment 4B: Peripheral CNQX Administration Blocks the Acquisition of Instrumental Learning

Ionotropic glutamate receptors at the neuromuscular junction may contribute to the acquisition of instrumental learning. CNQX has previously been used in our laboratory to block the acquisition of instrumental learning and maintenance through intrathecal application. This experiment will examine the influence of CNQX applied to the muscle. Prior work using intramuscular injections has shown that CNQX can induce ataxia (Melis et al., 1992). This experiment will examine whether intramuscular CNQX affects instrumental learning/performance.

Procedure

This experiment used (Table 11) 3 groups (saline & 2 drug doses) (n=6). Prior to behavioral testing, subjects had a catheter implanted into their tibialis anterior muscle. Subjects received a dose of CNQX (10 or 20ug) or saline twenty-five minutes prior to thirty minutes of instrumental testing. These doses were chosen on the basis of past work demonstrating that CNQX causes ataxia after i.m. injection (Melis et al., 1992).

Results

Baseline measurements confirmed that the groups did not differ prior to treatment. The mean shock intensities needed to elicit a 0.4N flexion force ranged from 0.61 (± 0.03) to 0.63 (± 0.05) mA. Initial flexion response means ranged from 0.12 (± 0.01) to 0.13 (± 0.02). These measures were not significantly different, $Fs < 1.00$, $p > .05$.

The effect of CNQX on the acquisition of instrumental learning is shown in Figure 22. Subjects that received a saline injection into the tibialis anterior muscle prior to testing were able to acquire the flexion response over the thirty minute testing session. Rats pretreated with CNQX displayed a dose-dependent disruption in learning/performance. A one-way repeated measures ANOVA revealed a significant main effect of drug, $F(2,15) = 11.19$, $p < .01$, and time, $F(29,435) = 4.66$, $p < .01$. *Post hoc* analysis showed that the 20ug CNQX group was significantly different from all other groups. No other relationships were significant.

Table 11. Procedure for Experiment 4B.

Surgery	24-hours	Saline	Test 30min
		10ug CNQX	
		20ug CNQX	

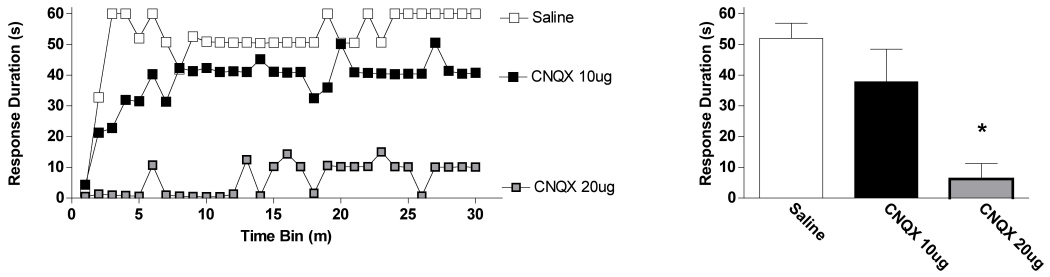

Figure 22. Intramuscular CNQX and acquisition. Subjects that received CNQX showed a reduction in response duration. (*) indicates statistically significant $p < .05$.

Saline treated subjects responded at a similar rate as the 10ug CNQX treated group (Figure 23). A one-way repeated measure ANOVA revealed a significant main effect of drug, $F(2,15) = 9.85$, $p < .01$. *Post hoc* analysis revealed that the 20ug CNQX treated group was significantly different from all other groups ($p < .05$).

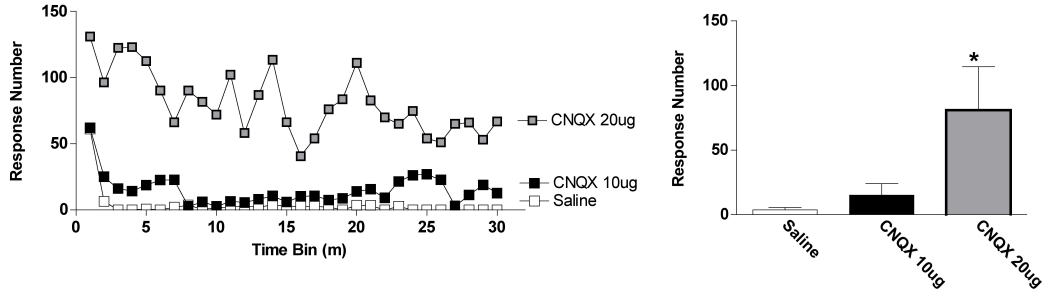

Figure 23. Intramuscular CNQX and acquisition response rate. Response rate during the 30-min testing session. (*) indicates statistically significant $p < .05$.

Discussion

Saline treated subjects were able to acquire the instrumental response. Subjects that received the highest dose (20ug) of CNQX showed a marked learning deficit relative to the lower dose (10ug) of CNQX and Saline treated animals. These results are similar to the data collected on CNQX administration interthecally (unpublished observation). These results also indicate that functional AMPA-receptors are present at the neuromuscular junction, and that AMPA-receptors at the neuromuscular junction are necessary for instrumental learning.

Experiment 4C: Peripheral CNQX Blocks the Maintenance of Instrumental Learning

In the previous experiment (4B) I examined whether CNQX affects the acquisition of instrumental learning. Experiment 4C examined how CNQX influences the maintenance of instrumental behavior.

Procedure

A 2X2 factorial design (Table 12) was used for this experiment (n=8). Prior to behavioral testing subjects had a catheter implanted into their tibialis anterior muscle. After subjects were prepared for instrumental testing, half received 30 minutes of training with response contingent shock. Twenty-five minutes into the training session, half of the subjects in each condition (trained, or positive control) received a drug injection (CNQX 20ug) or saline. During the last 30 minutes of the testing session, all subjects were treated with response contingent shock.

Results

Baseline measurements confirmed that the groups did not differ prior to treatment. The mean shock intensities needed to elicit a 0.4N flexion force ranged from 0.56 (± 0.04) to 0.62 (± 0.02) mA. Initial flexion response means ranged from 0.11 (± 0.01) to 0.19 (± 0.01). These measures were not significantly different, $Fs > 1.69$, $p > .05$.

The two pretrained groups exhibited a progressive increase in response duration and revealed a main effect of time, $F(29, 406) = 8.09$, $p < .01$, and a Time X Drug interaction, $F(29, 406) = 2.88$, $p < .01$. During the subsequent 30-minute testing session, subjects that received CNQX showed a deficit in response duration compared to all other groups (Figure 22). A two-way repeated measures ANOVA of the 30-minute testing session revealed a significant main effect of drug treatment, time, and a Time X Drug interaction, $Fs > 3.48$, $p < .01$. No other relationships were significant. *Post hoc* comparisons showed that both CNQX treated groups (Figure 24) were significantly different from the saline treated controls ($p < .05$).

Table 12. Procedure for Experiment 4C.

Transection	24-hours	No-Training	Saline	30-min Testing
			CNQX	
		30-min Training	Saline	
			CNQX	

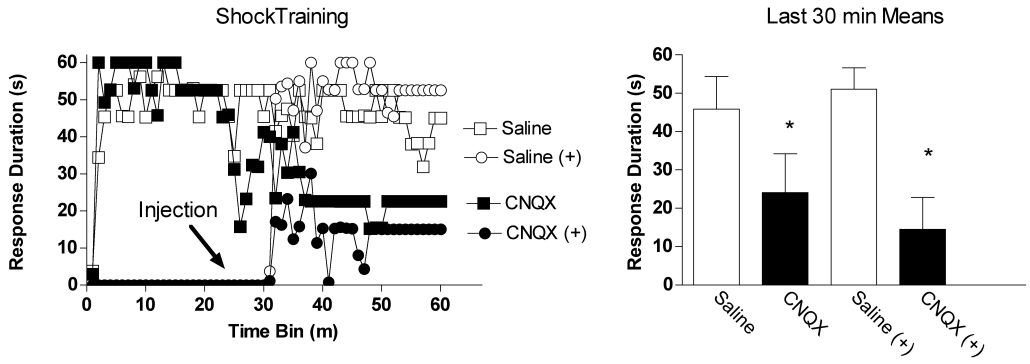

Figure 24. Intramuscular CNQX and maintenance. Subjects that received CNQX show a reduction in response duration. (*) indicates statistically significant $p < .05$.

Subjects that exhibited an increase in flexion duration made fewer instrumental responses (Figure 25). During training there was a significant main effect of time, $F(29,406) = 2.82$, $p < .01$, and a Time X Drug interaction, $F(29,406) = 2.59$, $p < .01$. No other comparisons were significant during training. A two-way repeated measures ANOVA of the 30-minute testing session revealed a significant main effect of drug, time, and a Drug X Training condition interaction, $Fs > 2.23$, $p < .01$. *Post hoc* comparisons revealed that the Untrained CNQX group (CNQX (+), Figure 25) was significantly different from both saline treated groups ($p < .05$).

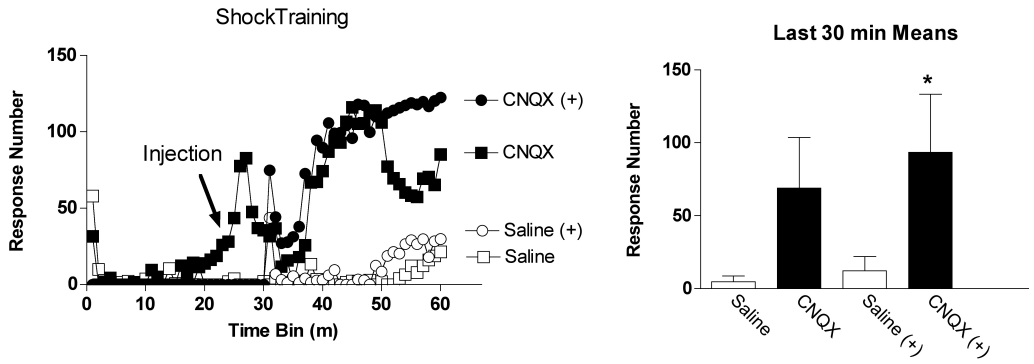

Figure 25. Intramuscular CNQX and maintenance response rate. Response number throughout the entire training/testing session. Error bars represent standard error of the mean (SEM). (*) indicates statistically significant $p < .05$.

Discussion

In this experiment, subjects that received a saline injection into their tibialis anterior muscle were able to acquire and maintain a normal level of instrumental responding. Subjects that received CNQX displayed a marked learning deficit regardless of training condition. These results indicate that active AMPA-receptors are required for both the acquisition and maintenance of instrumental responding.

Experiment 4D: Intramuscular MK-801 Exposure Disrupts the Acquisition of Instrumental Learning

Previous work has shown that i.m. administration of MK-801 (0.3mg/kg-1.0mg/kg) induces ataxia within 10 minutes of administration (Shih, 1990; Gmiro & Serdyuk, 2006). To determine if NMDA receptor activation at the neuromuscular

junction influences the acquisition of instrumental learning, subjects were given MK-801 directly into the tibialis anterior muscle prior to instrumental testing.

Procedure

This experiment used (Table 13) 3 groups (Saline & 2 drug doses) (n=6). Prior to behavioral testing, subjects had a catheter implanted into their tibialis anterior muscle. Subjects received a dose of MK-801 (50 or 200ug) or saline twenty-five minutes prior to thirty minutes of instrumental testing. These doses were based on prior work demonstrating that MK-801 can induce ataxia (Shih, 1990; Gmiro & Serdyuk, 2006).

Results

Baseline measurements confirmed that the groups did not differ prior to treatment. The mean shock intensities needed to elicit a 0.4N flexion force ranged from 0.58 (± 0.05) to 0.65 (± 0.04) mA. Initial flexion response means ranged from 0.11 (± 0.01) to 0.12 (± 0.01). These measures were not significantly different, $Fs < 1.00$, $p > .05$.

The effect of MK-801 on the acquisition of instrumental learning is shown in Figure 26. Subjects that received a saline injection into the tibialis anterior muscle prior to testing were able to acquire the flexion response over the thirty minute testing session. Rats pretreated with MK-801 displayed a dose-dependent disruption in learning. A one-way repeated measures ANOVA revealed a significant main effect of drug, $F(2,15) = 8.77$, $p < .01$, and time, $F(29,435) = 2.38$, $p < .01$. *Post hoc* analysis showed that the

200ug MK-801 group was significantly different from all other groups ($p < .05$). No other relationships were significant ($p > .05$).

Table 13. Procedure for Experiment 4D.

Surgery	24-hours	Saline	Test 30min
		50ug MK-801	
		200ug MK-801	

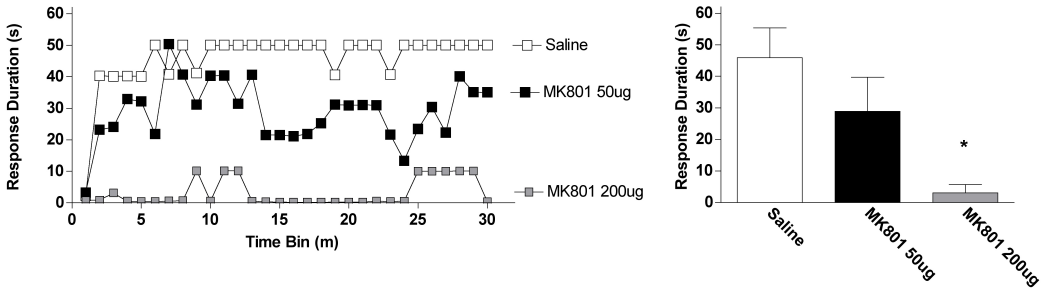

Figure 26. Intramuscular MK-801 and acquisition. Subjects that received MK-801 showed a reduction in response duration. (*) indicates statistically significant $p < .05$.

Saline treated subjects responded at a similar rate as the both MK-801 treated groups (Figure 27). A one-way repeated measure ANOVA found no significant difference.

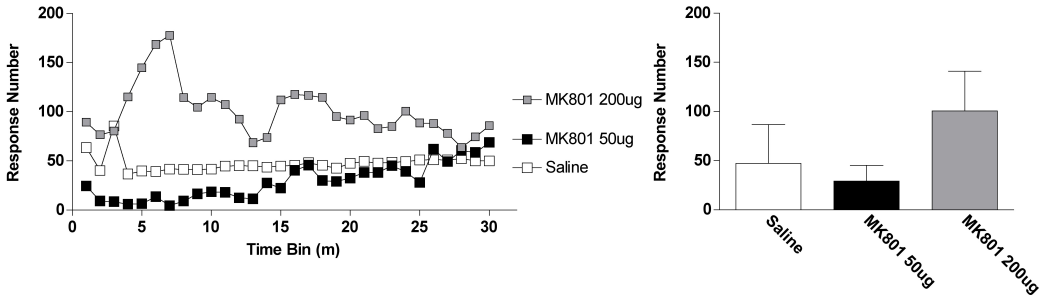

Figure 27. Intramuscular MK-801 and acquisition response rate. Response rate during the 30-min testing session. (*) indicates statistically significant $p < .05$.

Discussion

Saline treated subjects were able to acquire the instrumental learning response. Subjects that received the highest dose (200ug) of MK-801 showed a marked learning deficit when compared to the lower dose (50ug) of MK-801 and Saline treated animals. These results are similar to the data collected on MK-801 administration interthecally (Ferguson et al., 2006). These findings suggest that, not only are functional AMPA-receptors present at the neuromuscular junction, but NMDA-receptors are as well. Furthermore, NMDA-receptors at the neuromuscular junction are necessary for the acquisition of instrumental learning.

Experiment 4E: Peripheral MK-801 Blocks the Maintenance of Instrumental Learning

In the previous experiment (4D) I examined how i.m. MK-801 affects the acquisition of instrumental learning. Experiment 4E examined whether MK-801 influences the maintenance of instrumental behavior.

Procedure

A 2X2 factorial design (Table 14) was used for this experiment (n=14). Prior to behavioral testing subjects had a catheter implanted into their tibialis anterior muscle. After subjects were prepared for instrumental testing, half received 30 minutes of training with response contingent shock. Twenty-five minutes into the training session, half of the subjects in each condition (trained, or positive control) received a drug injection (MK-801, 200ug) or saline. All subjects then received 30-minutes of response-contingent shock.

Results

Baseline measurements confirmed that the groups did not differ prior to treatment. The mean shock intensities needed to elicit a 0.4N flexion force ranged from 0.51 (± 0.02) to 0.57 (± 0.03) mA. Initial flexion response means ranged from 0.13 (± 0.01) to 0.16 (± 0.01). These differences were not significantly different, $Fs < 1.00$, $p > .05$.

The two pretrained groups exhibited a progressive increase in response duration and revealed a main effect of time, $F(29,754) = 10.19$, $p < .01$. During the subsequent 30-minute test session, subjects that received MK-801 showed a deficit in response duration compared to all other groups (Figure 28). A two-way repeated measures ANOVA of the 30-minute testing session revealed a significant main effect of drug treatment, training condition treatment, time, a Time X Training Condition interaction, a Time X Drug interaction, and a Time X Training X Drug interaction, all $Fs > 1.95$, $p <$

.01. The three-way interaction indicates that the change in response duration observed over time depends on both training and drug treatment. The Drug X Training interaction was not significant, $F = 2.04$, $p > .05$. *Post hoc* comparisons revealed that the MK-801 positive control differed from all other groups, and the MK-801 experimental group was significantly different than the saline experimental group ($p < .05$). No other comparisons were significant.

Table 14. Procedure for Experiment 4E.

Transection	24-hours	No-Training	Saline	30-min Testing
			MK-801	
		30-min Training	Saline	
			MK-801	

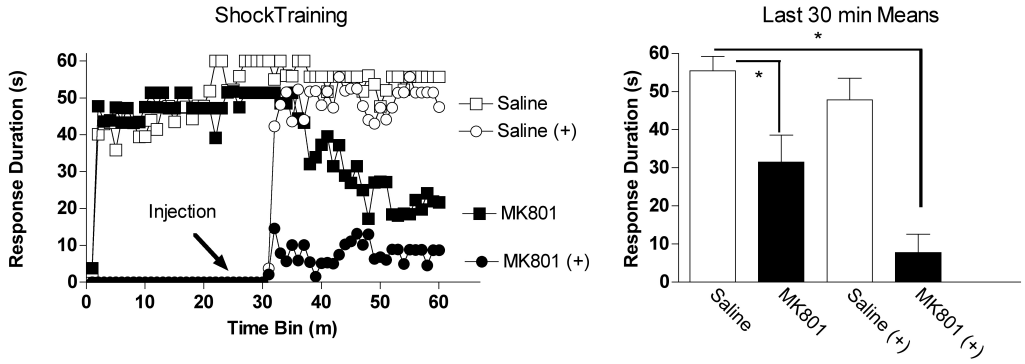

Figure 28. Intramuscular MK-801 and maintenance. Subjects that received MK-801 show a reduction in response duration. (*) indicates statistically significant $p < .05$.

Subjects that exhibited an increase in flexion duration made fewer instrumental responses (Figure 29). During training there was a significant main effect of time, $F(29,754) = 3.68$, $p < .01$. No other comparisons were significant during training, all Fs > 1.32, $p > .05$. A two-way repeated measures ANOVA of the 30-minute testing session revealed a significant main effect of drug, training, a Training X Drug interaction, a Time X Training interaction, and a Time X Training X Drug interaction, all $Fs > 2.00$, $p < .05$. *Post hoc* comparisons revealed that the MK-801 positive control differed from all other groups ($p < .05$). No other comparisons were significant.

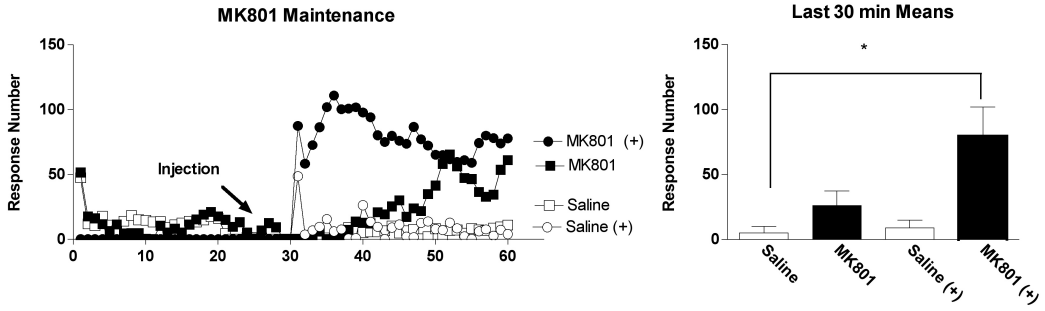

Figure 29. Intramuscular MK-801 and maintenance response rate. Response number throughout the entire training/testing session. Error bars represent standard error of the mean (SEM). (*) indicates statistically significant, $p < .05$.

Discussion

This experiment, combined with 4C, 4D, and 4E, showed that the acquisition and maintenance of instrumental learning is dependent on ionotropic glutamate receptors present at the neuromuscular junction. These results indicate that the neuromuscular

junction is influenced by glutamate signals and raise the possibility that a form of NMDA-receptor (NMDAR) mediated plasticity contributes to the maintenance of the instrumental response at the neuromuscular junction.

Experiment 4F: Peripheral MK-801 Administration Ipsilateral to a Sciatic Transection Disrupts the Maintenance of Instrumental Learning

Using intrathecal lidocaine (Aim 1) and a sciatic transection (Aim 2), I showed that a peripheral modification contributes to the maintenance of instrumental behavior. The results of Experiment 4E suggest that a form of NMDA-receptor mediated plasticity, at the neuromuscular junction, may be involved in regulating this behavior. However, Experiment 4E was done using subjects that had an intact sciatic to spinal cord connection. For this reason, pharmacological manipulation within the tibialis anterior muscle may evoke a neural response within the spinal cord, which in turn could disrupt the maintenance of the instrumental response. To address this possibility, and conclusively show that the MK801 is affecting the peripheral nervous system in the absence of spinal input, this experiment combined a sciatic nerve transection with tibialis anterior drug administration of MK-801. If MK-801 exerts its effect locally, drug treatment should disrupt the maintenance of the response after a sciatic nerve transection.

Procedure

Prior to behavioral testing, subjects had a catheter implanted into their tibialis anterior muscle and their sciatic nerve was also exposed. All subjects received 30 minutes (Table 15) of instrumental training. Twenty-five minutes into the training session, all subjects received a sciatic transection followed by an injection of either MK801 (200μg) or saline. During testing, subjects continued to receive response contingent shock.

Results

Baseline measurements confirmed that the groups did not differ prior to treatment. The mean shock intensities needed to elicit a 0.4N flexion force ranged from 0.65 (± 0.03) to 0.66 (± 0.04) mA. Initial flexion response means ranged from 0.11 (± 0.01) to 0.14 (± 0.02). These measures were not significantly different, $Fs > 1.53$, $p > .05$.

During training both groups exhibited an increase in response duration (Figure 30) and revealed a main effect of time, $F(29,290) = 5.87$, $p < .01$, and a Time X Drug interaction, $F(29,290) = 1.74$, $p < .05$. During the subsequent 30-minute testing session, subjects treated with MK-801 showed a marked learning deficit. A repeated measures ANOVA revealed a significant main effect of drug treatment, time, and a Drug X Time interaction, all $Fs > 1.40$, $p < .01$.

Table 15. Procedure for Experiment 4F.

Spinal Transection	24 hour	30-min Train	Sciatic Transection	Saline MK-801	30-min Testing

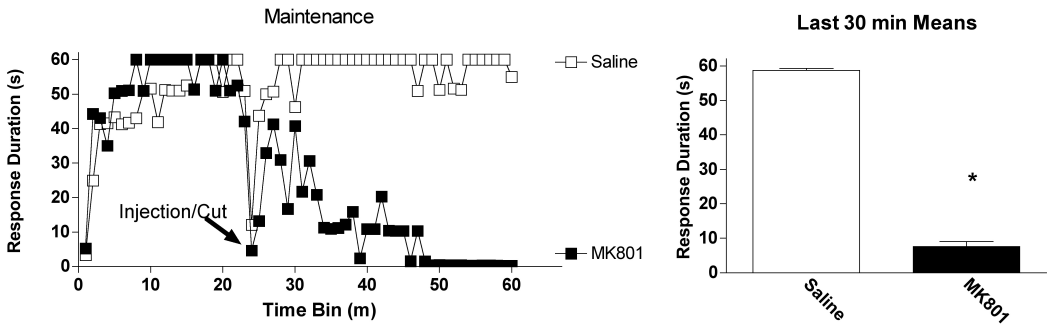

Figure 30. Intramuscular MK-801/Sciatic transection and maintenance. Subjects that received MK-801 after training were unable to display a facilitation in learning. (*) indicates $p < .01$.

During the training session, response number varied over time, $F(29,290) = 3.99$, $p < .01$. No other term was significant, all Fs < 1.00, $p > .05$. Subjects that received MK-801 showed an increase in response number during the testing session (Figure 31). An ANOVA revealed a main effect of drug treatment, time, and a Time X Drug interaction, all $Fs > 2.47$, $p < .01$.

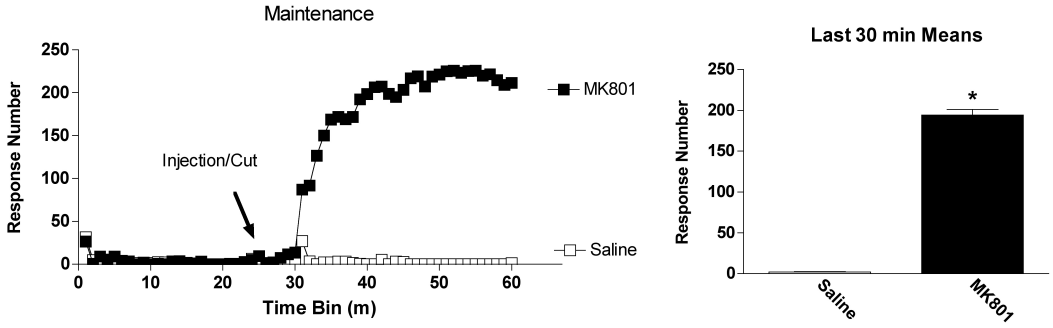

Figure 31. Intramuscular MK-801/Sciatic transection and maintenance response rate. Subjects that received MK-801 after training showed an increase in response number compared to saline treated subjects. (*) indicates $p < .01$.

Discussion

Pretrained saline treated subjects were able to maintain performance of the instrumental response after the sciatic nerve was cut, replicating the results of Experiment 2B. Subjects that received MK-801 after a sciatic transection were unable to maintain the previously learned task. These results indicate that MK-801 has a direct, local, effect on the peripheral mechanism that maintains the instrumental response; the impact of i.m. MK-801 does not, in any way, depend on continued communication with neurons within the spinal cord.

GENERAL DISCUSSION SUMMARY

A common view of neural processing involves a form of top-down processing, wherein the brain is the "master" of the system and the spinal cord is simply a subsystem within the network of the central nervous system. The top-down view allows for the analysis of the system as a whole, but may neglect the role of lower-level systems. A bottom-up approach to examining the nervous system allows us ask questions that would not be possible within the whole system. It allows us to look at specific parts of the system when they are operating alone, and examine what those components are capable of as separate entities. In this dissertation, I have used a bottom-up approach to understanding how spinal instrumental learning works, first by examining the role of the spinal cord and finally by examining how the terminus of the nervous system (the neuromuscular junction) contributes to instrumental performance.

The goal of this dissertation was to establish evidence for peripheral mediation of spinal instrumental learning. Doing so was no simple task; previous work from my laboratory had implied that memory for instrumental learning laid within the spinal cord (Grau et al., 2006). All previous interpretations of instrumental learning data ignored the PNS component (for review Grau et al., 2006) or only examined the contribution of the peripheral component to the acquisition of instrumental learning (Crown et al., 2002a). This dissertation provided the first empirical study on the role of the PNS in spinal instrumental learning during acquisition, facilitation (reacquisition) and maintenance.

The Spinal Cord is Required for the Acquisition of Instrumental Learning

I was able to confirm the results from previous studies (Crown et al., 2002a), that the spinal cord is required for the acquisition of instrumental learning. Furthermore, I was able to demonstrate this using multiple methods. In Experiment 1A, subjects that received intrathecal lidocaine without prior instrumental training did not exhibit an increase in response duration, my index of instrumental learning. These results replicated the findings of Crown et al. (2002a). Building on those results, I then applied the same neural inhibitor (lidocaine) directly to the sciatic nerve. In a similar fashion, lidocaine applied directly to the sciatic nerve without prior instrumental training caused a disruption in the acquisition of instrumental learning (Experiment 2A). Also replicating prior results (Crown et al., 2002a), Experiment 2B showed that cutting the sciatic nerve prior to training prevented instrumental learning. These 3 experiments (1B, 2A, & 2B) support the claim that the spinal cord is required for the acquisition of R-O relationships.

Expanding on prior work, I also examined how the spinal cord influences the facilitation of instrumental learning. Facilitation involves the reacquisition of learning at a higher response criterion. In Experiment 1C subjects that received intrathecal lidocaine prior to testing at a higher criterion were unable learn. Likewise, Experiment 2C showed that a complete sciatic transection disrupted the reacquisition of instrumental learning at a higher criterion. All of the experiments outlined here (1B, 1C, 2A, 2B, & 2C) support the notion that the acquisition of instrumental learning requires the spinal

cord. Procedures that disrupted PNS-spinal communication (2A, 2B, & 2C), or inhibited the spinal processing (1B, 2C), disrupted instrumental learning.

Evidence for Non-Spinal Mediation on the Maintenance of Instrumental Learning

Setting out to demonstrate peripheral mediation of the maintenance of instrumental learning was no minor task. Previous work from my lab (Grau et al., 1998; Crown et al., 2002a; Crown et al., 2002b) had indicated that the spinal cord was the seat of the memory for instrumental learning. These studies had focused on the acquisition of learning (Crown et al., 2002a) and the contralateral transfer of the learning deficit (Crown et al., 2002b). Using those methods, there seemed little doubt that neural tissue within the spinal cord played an essential role. Contrary to this dogma, my results suggest that a key component (part of the memory) lies outside the spinal cord.

In Experiment 1A, I confirmed that a single dose of intrathecal lidocaine inhibits the tail-flick reflex in spinally transected rats. If the maintenance of instrumental learning is mediated by spinal mechanisms alone, then a single dose of lidocaine to the spinal cord should block the maintenance of instrumental behavior. The results of Experiment 1B showed otherwise. In Experiment 1B, subjects that received 25 minutes of training prior to intrathecal lidocaine treatment showed no measureable drop off in learning. This suggested that a non-spinal component might contribute to the maintenance of instrumental learning.

To further investigate the potential role of a non-spinal mechanism, Experiment 2A tested whether inhibition of the sciatic nerve would disrupt the maintenance of

instrumental learning. If the memory for the instrumental response is located in the spinal cord, then inhibiting sciatic transmission with lidocaine should have disrupted the maintenance of instrumental learning. Paralleling the results of Experiment 1B, lidocaine had no effect on the maintenance of instrumental performance in Experiment 2A. However, one could argue that lidocaine did not have sufficient penetration into the exposed sciatic nerve, allowing some neural activity to propagate. To address this concern, I next examined the effect of cutting the sciatic nerve.

In Experiment 2B a complete sciatic transection was used to severe the connection between the spinal cord and sciatic nerve. If the maintenance of instrumental learning depends on spinal neurons, then cutting the sciatic nerve after training should disrupt the maintenance of instrumental responding. It did not. This finding was replicated within Experiment 4F. These data provided the first evidence that non-spinal mechanisms contribute to instrumental performance.

Using multiple methods (intrathecal lidocaine, sciatic lidocaine bath, and sciatic transection) I have demonstrated that there is a non-spinal component responsible for the maintenance of instrumental learning. Though these experiments produced novel results, they provided little information regarding the mechanisms that mediate this peripheral effect. As detailed below, the remaining experiments implicated an alteration at the neuromuscular junction.

Pharmacological Evidence that Non-Spinal Mechanisms Influence the Outcomes of Instrumental Learning

Experiments 1B, 2B, 2C, and 4F provided evidence for a non-spinal influence of instrumental learning, but failed to address what mechanisms are responsible for these effects. To examine the mechanism(s) involved, I developed a method to deliver drugs directly into the tibialis anterior muscle (the muscle that produces the flexion response during instrumental learning).

The first target that I chose was the nicotinic acetylcholine receptor. These receptors are present at all mammalian skeletal neuromuscular junctions. It has been well established that these receptors are responsible for skeletal muscle flexion. In Experiment 3A, I examined how blocking the nicotinic acetylcholine receptor with curare influences the acquisition of instrumental learning. If the nicotinic acetylcholine receptor is essential to performing the flexion response, then blocking the receptor with curare should disrupt instrumental learning. Non-surprisingly, curare had this effect. To verify that continued acetylcholine release was needed to maintain the instrumental response, Experiment 3B examined whether curare given after training affects performance. As expected, curare caused the instrumental response to wane.

I used Aim 3 to develop the method of drug delivery into the tibialis anterior muscle. In doing so, I was able to verify that nicotinic acetylcholine receptors must be functional in order to produce increase response durations during instrumental testing. All subjects did not, however, respond as I would have predicted. Curare in nature and in medicine is used to paralyze muscles. Though some of my subjects habituated to the

flexion eliciting shocks after curare administration, not all subjects responded in that fashion. Indeed, some subjects responded at rates (350 responses/min) seldom seen in our instrumental testing paradigm. It is unclear why curare treatment had this effect in a subset of the subjects.

Having established a method to deliver drugs directly to the tibialis anterior during testing, Aim 4 explored the neurochemical mechanisms involved. Previous studies have shown that, in the presence of the NO-synthase inhibitor L-NAME, rats undergoing treadmill training produced a marked decrease in hindlimb muscular blood flow and a reduction in acetylcholine release (Musch et al., 2001; Hirai et al., 1994), which suggests that NO release could contribute to an increase in flexion duration. Administration of L-NAME during the acquisition of instrumental learning produced no measureable effect on instrumental performance. It is not clear why L-NAME affects treadmill training, but not instrumental performance. A key difference is that the studies examining treadmill training used subjects with an intact nervous system.

Other pharmacological manipulations within Aim 4 yielded positive results. Drugs that affect the glutamatergic signaling (ionotropic glutamate receptor antagonists) produce ataxia within minutes of administration (Melis et al., 1992; Shih, 1990; Gmiro & Serdyuk, 2006). If activating muscular ionotropic glutamate receptors is necessary for the acquisition of instrumental learning, then inhibiting ionotropic glutamate receptors should disrupt the acquisition of instrumental learning. In Experiment 4B intramuscular CNQX, (an AMPAR antagonist) blocked the acquisition of instrumental learning in a dose dependent fashion. Likewise, in Experiment 4D intramuscular MK-801, (an

NMDAR antagonist) blocked the acquisition of instrumental learning in a dose dependent fashion. These results indicate that ionotropic glutamate receptor activation at the neuromuscular junction is required for the acquisition of instrumental learning.

In Experiments 4B and 4D, I established that functional intramuscular ionotropic glutamate receptors are necessary for the acquisition of instrumental learning. In Experiments 4C and 4E, I examined their role during the maintenance of instrumental learning. Experiment 4C showed that CNQX disrupts the maintenance of instrumental learning. Similarly, Experiment 4E, showed that MK-801 disrupts maintenance. Because these experiment used subjects with an intact sciatic nerve, a peripheral drug manipulation could engage a spinally-mediated process that impacts instrumental performance. To determine whether the isolated neuromuscular junction can maintain instrumental performance in the presence of MK-801, I combined the methods from Experiment 2B (sciatic transection) with intramuscular administration of MK-801 (Experiment 4E). Experiment 4F demonstrated that after a sciatic transection, intramuscular MK-801 disrupts the maintenance of instrumental behavior.

These experiments represent the first empirical study designed to examine the influence of the peripheral nervous system on spinal instrumental learning. I have shown that, 1) the acquisition of instrumental learning requires spinal neurons (Experiments 1B, 1C, 2A, 2B, & 2C), 2) the maintenance of instrumental learning has a peripheral component (Experiments 1B, 2A, 2B, 4C, 4E, & 4F), and 3) muscular ionotropic glutamate receptors may have a modulatory role in the maintenance of instrumental behavior (Experiments 4C, 4E, & 4F). These results mark a change in the

way instrumental learning will be interpreted from now on. Formerly, this form of behavioral plasticity was assumed to be spinally mediated. Just as prior studies forced researchers to recognize that the spinal cord is not simply a conduit for the brain, my data suggest a further step and implicate a modification at the neuromuscular junction. Now, for the first time, I have shown that of instrumental memory has a peripheral component.

Comparison of Spinal Manipulations Versus Peripheral Manipulations in Maintenance Experiments

Previous studies from our laboratory have shown that the maintenance of instrumental learning is dependent on spinal NMDARs. These studies demonstrated that NMDAR antagonists administered intrathecally disrupt the maintenance of instrumental learning (Joynes et al., 2004; Ferguson et al., 2006). Administration of an NMDAR antagonist to the muscle caused a similar pattern of results (4E &F). Looking at the data qualitatively, Experiment 4F shows the most robust disruption of maintenance compared to other experiments in this dissertation and previous studies (Joynes et al., 2004; Ferguson et al., 2006). This result may be, in part, due to the combination of a complete sciatic transection and intramuscular MK-801. In the previous studies (Joynes et al., 2004; Ferguson et al., 2006), the maintenance of instrumental responding seems to degrade in a linear fashion, whereas in Experiment 4F the fall off in response duration was abrupt.

Encoding Memory at the Neuromuscular Junction: Glutamatergic Mediation

In this dissertation I have shown that the PNS plays an active role in the maintenance of instrumental behavior. I have demonstrated that there are non-spinal processes responsible for the maintenance of instrumental learning. In Aims 1 and 2, I showed that when the spinal cord was inhibited (Experiment 1A), or disconnected from peripheral inputs (Experiment 2B), the maintenance of instrumental performance was unaffected. These experiments provided new insight on the influence of non-spinal mechanisms during instrumental responding, but these results did not provide a mechanism responsible for the peripherally mediated behavioral memory observed.

In Aim 4, I examined the role of glutamatergic mediation at the neuromuscular junction. I hypothesize that the prolonged flexion durations observed during instrumental learning are not exclusively controlled by acetylcholine release. In addition, experimental evidence suggests that the maintenance of the flexion response is mediated by ionotropic glutamate receptors at the neuromuscular junction. Because acetylcholine is broken down very quickly by acetylcholinesterase, it is unlikely that prolonged flexion durations are only caused by increased acetylcholine release. Recent studies have provided evidence that glutamate and acetylcholine are co-released at the neuromuscular junction (Nishimaru et al., 2005; Mays et al., 2009). Further behavioral evidence shows that drugs that block ionotropic glutamate receptors also produce ataxia (Shih, 1990; Gmiro & Serdyuk, 2006; Melis et al., 1992). Experiments in Aim 4 (B, C, D, E, and F) demonstrated that blocking the glutamatergic signal at the neuromuscular junction disrupts the acquisition and maintenance of instrumental learning. Experiment

4F showed that even in the absence of all spinal input, blocking the glutamatergic signal disrupts the maintenance of instrumental learning. These results lend credence to the hypothesis that the glutamatergic signal mediates prolonged flexion durations in the spinal rat. Based on the results from Aim 4, I hypothesize that prolonged flexion responses are produced due to a training induced increase in glutamatergic transmission at the neuromuscular junction. Further studies are needed to verify that this hypothesis holds true, and to determine the source (glial or neuronal) of glutamate at the neuromuscular junction.

Clinical Implications for Peripheral Plasticity and Spinal Cord Injury

Examining the phenomenon of spinal cord injury (SCI) is not an acutely spinal problem. After injury to the spinal cord, the entire nervous system goes through changes. Whether it is the vagus nerve, the cerebral cortex, the peripheral nervous system, or the spinal cord, injury induces a state that is very different from non-injured system. Viewing SCI as a series of interactions (spinal cord X brain, spinal cord X inflammation, spinal cord X peripheral nervous system, spinal cord X immune activation, etc…) allows for a more complete approach to the recovery of function after injury. The interactions between the injured spinal cord and other systems will yield the most beneficial results after spinal cord jury. For instance, if a therapeutic training method was developed that showed great regeneration in the spinal cord, but made the patients clinically depressed, the recovery outcomes would be poor due to the interaction of the brain (depression) X spinal cord (regeneration treatment). Because the

interaction of the injured spinal cord with another system could be detrimental to the recovery of function after SCI, it is important to focus on these interactions when investigating SCI. In this dissertation, I examined how the injured spinal cord interacts with the peripheral nervous system and how the PNS contributes to a spinal reflex. It is important to ask, can the PNS affect the spinal reflexes after injury, and how does the PNS influence behavior that was thought to be exclusively spinal?

Several empirical studies in humans and rats after SCI have focused on the peripheral consequences after injury. In humans, acute SCI is associated with a hyperexcitability of motorneurons below the level of injury (Leis et al., 1996; Boland et al., 2009). These studies show that the PNS undergoes plastic changes that induce hyperexcitability after spinal trauma. Another consequence of human SCI is the loss of motorneurons in the chronic phase of SCI (Chang, 1998). These results in humans have been replicated in rat models of SCI (Collazos-Castro et al., 2005; Burns et al., 2007; Ollivier-Lanvin et al., 2009). Together, these studies highlight that SCI has many effects outside of the spinal cord. Moreover, these changes induce a hyper-excitable plastic state in the PNS acutely after SCI, but then lead to motorneuron loss during the chronic stages. Can we find away to preserve the motorneurons that normally degenerate after SCI? Should we be developing therapies that try to reduce the excitability of the PNS during the acute stage of SCI? Does sparing motorneurons in the chronic phase of SCI improve outcomes? Further work is needed to address these questions.

Peripheral Plasticity and Neuropathy

Using animal models of peripheral neuropathy, researchers have characterized the electrophysiological properties of the damaged sciatic nerve and the dorsal horn after peripheral injury. Peripheral sensitization occurs at the level of the sciatic nerve, contributing to initial induction of pain and over-excitability. Over time the pathological hyperexcitability in the periphery contributes to cellular changes in the central nervous system (spinal dorsal horn) that can produce centrally mediated hyperexcitability known as central sensitization (Ji et al., 2003).

Using animal models of sciatic neuropathy, researchers have reported hyperexcitability in the injured sciatic nerve (Abdulla,et al., 2003, Matzner & Devor, 1994). Electrophysiological studies of the injured sciatic nerve demonstrate a robust increase in excitability post injury (Abdulla et al., 2003, Hogan et al., 2000). These changes are induced immediately after sciatic nerve injury and the hyperexcitability is observed for weeks after injury. Peripheral sensitization is associated with reduced withdrawal threshold to heat (hyperalgesia) and a spontaneous self-mutilation called autotomy (Abdulla et al., 2003, Hogan et al., 2000). Subjects showing autotomy (Figure 32) after sciatic nerve transection also exhibited the most excitability from electrical stimulation.

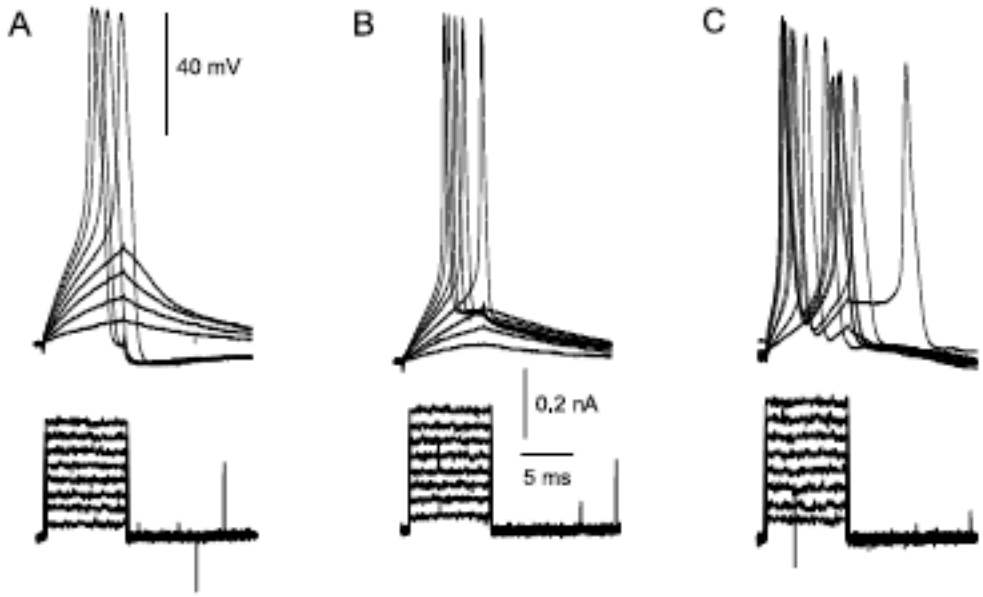

Figure 32. Peripheral sensitization to injury. This figure depicts electrophysiological recording from DRG neurons after sciatic nerve injury: (A) sham; (B) sciatic nerve injury; and (C) sciatic nerve injury with autotomy. The upper portion shows the recordings of DRG neurons, and the lower portion shows the electrical stimulation presented. Notice how in A & B not all stimulations elicit action potentials. In C all stimulations (even below threshold for A & B) elicit action potentials. Adapted from Abdulla et al. (2003).

Future Directions and Conclusion

Many aspects of this dissertation need further investigation to determine the role of peripheral memory in instrumental behavior. Aim 4 began with an experiment that examined how NO synthase inhibition influences the acquisition of instrumental learning. Though the results of the experiment were negative, many other studies showed that NO synthase inhibition has an effect on motorneuron behavior. Could a different cellular target, or possibly a different NO synthase inhibitor, produce a disruption in the acquisition of instrumental learning?

The final 5 experiments of Aim 4 examined the ionotropic glutamatergic signal present at the neuromuscular junction. Does training increase overall receptor

population at the NMJ? Does training change the ionotropic glutamate receptor make up (NMDAR : AMPAR ratio)? Are there metabotropic glutamate receptors present at the NMJ, and do they influence learning in a similar fashion as the ionotropic receptors? To expand on the influence of the glutamatergic signal at the neuromuscular junction, how do glutamate agonists influence the acquisition and maintenance of instrumental learning? Can glutamatergic agonists induce the facilitation of instrumental learning after a sciatic transection? Could glutamate alone induce the facilitation of instrumental learning without prior training? To address the issue of peripheral hyperexcitability, do glutamate agonists induce allodynia in the spinal rat? Finally, does peripheral injury/inflammation induce the learning deficit? Prior work has shown that peripheral injury/inflammation increases the excitability of the PNS, does this have a detrimental or beneficial influence on the acquisition and maintenance of instrumental learning? Could a chronic inflammatory sciatic nerve injury produce a lasting learning deficit?

In conclusion, the experiments in this dissertation confirmed prior findings that the spinal cord is necessary for the acquisition of instrumental learning. The novel finding is that the peripheral nervous system has the capability to maintain instrumental performance without the spinal cord. The ability of the PNS to maintain instrumental performance represents a new form of short-term behavioral memory that was previously not reported.

REFERENCES

Abdulla, F. A., Moran, T. D., Balasubramanyan, S., & Smith, P. A. (2003). Effects and consequences of nerve injury on the electrical properties of sensory neurons. *Canadian Journal of Physiology and Pharmacology*, *81*(7), 663-682.

Baumbauer, K. M., Young, E. E., Hoy, K. C., Jr, Abood, A., & Joynes, Robin L. (2007a). Administration of a Ca-super(2+)/calmodulin-dependent protein kinase II (CaMKII) inhibitor prevents the learning deficit observed in spinal rats after noncontingent shock administration. *Behavioral Neuroscience*, *121*(3), 570-578.

Baumbauer, K. M., Young, E. E., Hoy, K. C., Jr, France, J. L., & Joynes, Robin L. (2006). Intrathecal infusions of anisomycin impact the learning deficit but not the learning effect observed in spinal rats that have received instrumental training. *Behavioural Brain Research*, *173*(2), 299-309.

Baumbauer, K. M., Young, E. E., Hoy, K. C., Jr, & Joynes, Robin L. (2007b). Neurokinin receptors modulate the impact of uncontrollable stimulation on adaptive spinal plasticity. *Behavioral Neuroscience*, *121*(5), 1082-1094.

Boland, R. A., Bostock, H., & Kiernan, M. C. (2009). Plasticity of lower limb motor axons after cervical cord injury. *Clinical Neurophysiology*, *120*(1), 204-209.

Buerger, A. A., & Chopin, S. F. (1976). Instrumental avoidance conditioning in spinal vertebrates. *Advances in Psychobiology*, *3*, 437-461.

Burns, A. S., Jawaid, S., Zhong, H., Yoshihara, H., Bhagat, S., Murray, M., Roy, R. R., et al. (2007). Paralysis elicited by spinal cord injury evokes selective disassembly

of neuromuscular synapses with and without terminal sprouting in ankle flexors of the adult rat. *The Journal of Comparative Neurology*, *500*(1), 116-133.

Chang, C. W. (1998). Evident transsynaptic degeneration of motor neurons after spinal cord injury: A study of neuromuscular jitter by axonal microstimulation. *American Journal of Physical Medicine & Rehabilitation / Association of Academic Physiatrists*, *77*(2), 118-121.

Church, R. M. (1964). Systemic effect of random error in the yoked control design. *Psychological Bulletin*, *62*, 122-131.

Collazos-Castro, J. E., Soto, V. M., Gutiérrez-Dávila, M., & Nieto-Sampedro, M. (2005). Motoneuron loss associated with chronic locomotion impairments after spinal cord contusion in the rat. *Journal of Neurotrauma*, *22*(5), 544-558.

Crown, E. D., Ferguson, A. R., Joynes, R. L., & Grau, J. W. (2002a). Instrumental learning within the spinal cord: IV. Induction and retention of the behavioral deficit observed after noncontingent shock. *Behavioral Neuroscience*, *116*(6), 1032-1051.

Crown, E. D., Ferguson, A. R., Joynes, R. L., & Grau, J. W. (2002b). Instrumental learning within the spinal cord: II. Evidence for central mediation. *Physiology & Behavior*, *77*(2-3), 259-267.

Crown, E. D., & Grau, J. W. (2001). Preserving and restoring behavioral potential within the spinal cord sing an instrumental training paradigm. *Journal of Neurophysiology*, *86*(2), 845 -855.

Dan, Y., & Poo, M. (1992). Hebbian depression of isolated neuromuscular synapses in vitro. *Science*, *256*(5063), 1570 -1573.

Durkovic, R., & Damianopoulos, E. (1986). Forward and backward classical conditioning of the flexion reflex in the spinal cat. *The Journal of Neuroscience*, *6*(10), 2921 -2925.

Durkovic, R. G. (1975). Classical conditioning, sensitization and habituation in the spinal cat. *Physiology & Behavior*, *14*(3), 297-304.

Ferguson, A.R., Crown, E.D., & Grau, J.W. (2006). Nociceptive plasticity inhibits adaptive learning in the spinal cord. *Neuroscience*, *141*(1), 421-431.

Gmiro, V. E., & Serdyuk, S. E. (2007). Epinephrine potentiates antipsychotic, but not cataleptogenic effect of haloperidol in rats. *Bulletin of Experimental Biology and Medicine*, *143*(5), 617-619.

Grau, J W, Barstow, D. G., & Joynes, R L. (1998). Instrumental learning within the spinal cord: I. Behavioral properties. *Behavioral Neuroscience*, *112*(6), 1366-1386.

Grau, J. W., Crown, E. D., Ferguson, A. R., Washburn, S. N., Hook, M. A., & Miranda, R. C. (2006). Instrumental learning within the spinal cord: Underlying mechanisms and implications for recovery after injury. *Behavioral and Cognitive Neuroscience Reviews*, *5*(4), 191 -239.

Groves, P. M., & Thompson, R. F. (1970). Habituation: a dual-process theory. *Psychological Review*, *77*(5), 419-450.

Hirai, T., Visneski, M. D., Kearns, K. J., Zelis, R., & Musch, T. I. (1994). Effects of NO synthase inhibition on the muscular blood flow response to treadmill exercise in rats. *Journal of Applied Physiology*, *77*(3), 1288 -1293.

Hogan, Q. H., McCallum, J. B., Sarantopoulos, C., Aason, M., Mynlieff, M., Kwok, W. M., & Bosnjak, Z. J. (2000). Painful neuropathy decreases membrane calcium current in mammalian primary afferent neurons. *Pain*, *86*(1-2), 43-53.

Illich, P. A., Salinas, J. A., & Grau, James W. (1994). Latent inhibition, overshadowing, and blocking of a conditioned antinociceptive response in spinalized rats. *Behavioral and Neural Biology*, *62*(2), 140-150.

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*(12), 696-705.

Joynes, R. L., Ferguson, A. R., Crown, E. D., Patton, B. C., & Grau, J. W. (2003). Instrumental learning within the spinal cord: V. Evidence the behavioral deficit observed after noncontingent nociceptive stimulation reflects an intraspinal modification. *Behavioural Brain Research*, *141*(2), 159-170.

Joynes, R L, & Grau, J W. (1996). Mechanisms of Pavlovian conditioning: role of protection from habituation in spinal conditioning. *Behavioral Neuroscience*, *110*(6), 1375-1387.

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*(1), 35-51.

Joynes, R. L., Janjua, K., & Grau, J. W. (2004). Instrumental learning within the spinal cord: VI: The NMDA receptor antagonist, AP5, disrupts the acquisition and maintenance of an acquired flexion response. *Behavioural Brain Research*, *154*(2), 431-438.

Leis, A. A., Kronenberg, M. F., Stetkarova, I., Paske, W. C., & Stokic, D. S. (1996). Spinal motoneuron excitability after acute spinal cord injury in humans. *Neurology*, *47*(1), 231 -237.

Malomouzh, A. I., Mukhtarov, M. R., Nikolsky, E. E., & Vyskočil, F. (2007). Muscarinic M1 acetylcholine receptors regulate the non‐quantal release of acetylcholine in the rat neuromuscular junction via NO‐dependent mechanism. *Journal of Neurochemistry*, *102*(6), 2110-2117.

Malomouzh, A. I., Mukhtarov, M. R., Nikolsky, E. E, Vyskočil, F., Lieberman, E. M., & Urazaev, A. K. (2003). Glutamate regulation of non‐quantal release of acetylcholine in the rat neuromuscular junction. *Journal of Neurochemistry*, *85*(1), 206-213.

Malomouzh, A. I., Nikolsky, E. E., Lieberman, E. M., Sherman, J. A., Lubischer, J. L., Grossfeld, R. M., & Urazaev, A. K. (2005). Effect of N‐acetylaspartylglutamate (NAAG) on non‐quantal and spontaneous quantal release of acetylcholine at the neuromuscular synapse of rat. *Journal of Neurochemistry*, *94*(1), 257-267.

Matzner, O., & Devor, M. (1994). Hyperexcitability at sites of nerve injury depends on voltage-sensitive Na+ channels. *Journal of Neurophysiology*, *72*(1), 349-359.

Mays, T. A., Sanford, J. L., Hanada, T., Chishti, A. H., & Rafael‐Fortney, J. A. (2009). Glutamate receptors localize postsynaptically at neuromuscular junctions in mice. *Muscle & Nerve*, *39*(3), 343-349.

Melis, M. R., Stancampiano, R., & Argiolas, A. (1992). Effect of excitatory amino acid receptor antagonists on apomorphine-, oxytocin- and ACTH-induced penile erection and yawning in male rats. *European Journal of Pharmacology*, *220*(1), 43-48.

Misulis, K. E., & Durkovic, R. G. (1984). Conditioned stimulus intensity: Role of cutaneous fiber size in classical conditioning of the flexion reflex in the spinal cat. *Experimental Neurology*, *86*(1), 81-92.

Musch, T., McAllister, R., Symons, J., Stebbins, C., Hirai, T., Hageman, K., & Poole, D. (2001). Effects of nitric oxide synthase inhibition on vascular conductance during high speed treadmill exercise in rats. *Experimental Physiology*, *86*(6), 749 -757.

Nishimaru, H., Restrepo, C. E., Ryge, J., Yanagawa, Y., & Kiehn, O. (2005). Mammalian motor neurons corelease glutamate and acetylcholine at central synapses. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(14), 5245 -5249.

Ollivier-Lanvin, K., Lemay, M. A., Tessler, A., & Burns, A. S. (2009). Neuromuscular transmission failure and muscle fatigue in ankle muscles of the adult rat after spinal cord injury. *Journal of Applied Physiology*, *107*(4), 1190-1194.

Shih, T.-M. (1990). Anticonvulsant effects of diazepam and MK-801 in soman poisoning. *Epilepsy Research*, *7*(2), 105-116.

Vyskocil, F., Malomouzh, A. I., & Nikolsky, E. E. (2009). Non-quantal acetylcholine release at the neuromuscular junction. *Physiological Research / Academia Scientiarum Bohemoslovaca*, *58*(6), 763-784.

Weinstein, D. E., & Wu, R. (2001). Isolation and purification of primary Schwann cells. *Current Protocols in Neuroscience*. 3.17.1-3.17.9.

VITA

Name: Kevin Corcoran Hoy Jr.

Address: Texas A&M, Psychology Department
MS 4235
College Station, TX 77843

Email Address: hoyaiag@gmail.com

Education: B.A., Biology/Psychology, Kent State University, 2005
M.S., Psychology, Texas A&M University, 2008
Ph.D. Psychology, Texas A&M University, 2011