EFFECTS OF ETHANOL ON INSTRUMENTAL LEARNING AND PLASTICITY WITHIN THE SPINAL CORD

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

Effects of Ethanol on Instrumental Learning And Plasticity Within The Spinal Cord (March 2013)

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Due to its high prevalence as a recreational substance in our society, especially in populations with spinal cord injury (Tate, 1993), it is imperative that we study the relationship between ethanol and its possible effects on instrumental learning within the spinal cord. Previous studies have demonstrated that the spinal cord is capable of making plastic changes in response to stimuli without the influence of the brain. Many substances have been shown to facilitate or inhibit this ability. I hypothesize that ethanol (EtOH) will have an inhibitory effect on both adaptive and maladaptive plasticity within the spinal cord. Rats were be used to test for this effect. The rats had their spinal cords transected at the second thoracic (T2) vertebrae to eliminate spinal cord communication with the brain. The next day, the rats were administered ethanol at concentrations designed to achieve a blood ethanol content (BEC) known to impair behavioral and neural function. The rats were behaviorally tested for learning and changes in spinal plasticity. In addition, I examined whether ethanol affects the development of a maladaptive form of plasticity, the central sensitization produced by peripheral inflammation.

The behavioral data acquired supports my hypothesis that ethanol intake has an inhibitory effect on adaptive plasticity in the spinal cord.

ACKNOWLEDGEMENTS

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NOMENCLATURE

Abbreviations:

EtOH	Ethanol
IP	Intraperitoneal
T2	Second thoracic
BEC	Blood Ethanol Content
SCI	Spinal Cord Injury

CHAPTER I

INTRODUCTION

Fifty percent of the population in the United States currently drinks ethanol three times a week or more (Understanding Ethanol). Ethanol consumption is a normative social action that has been integrated into our culture for generations. However, some populations drink more than others. The rates of ethanol consumption in SCI patients are significantly higher than those of the normal population (Tate, 1993). This data paired with the onset of 12,000 new cases of spinal cord injury every year highlight the importance of studying the effects of ethanol on the spinal cord (NSCISC, 2011). Ethanol consumption in this population is a clinically relevant behavior that could influence recovery.

The spinal cord has been shown to demonstrate instrumental learning independent of the brain (Grau, 1998). This can be studied in rats that have undergone a mid-thoracic spinal cord transection. Rats that receive shock whenever a hind limb is extended exhibit a progressive increase in flexion duration that reduces net shock exposure, a form of learning known as instrumental learning. Rats that receive shock independent of leg position fail to learn and later exhibit a learning deficit when tested with controllable shock.

Ethanol acts as a benzodiazepine and works on the central nervous system by binding to GABA receptors in the brain and inhibiting neural transmission. Specifically, ethanol is a direct $GABA_A$ receptor agonist (Carlson, 2004). GABA can mute neural transmission by reducing depolarization, a prerequisite for inducing NMDA-receptor mediated plasticity. By engaging this inhibitory system, ethanol could inhibit spinal plasticity. Supporting this, the $GABA_A$ receptor

agonist muscimol inhibits instrumental conditioning in spinalized rats (Ferguson et al., 2003). Given this, I hypothesized that ethanol would also inhibit spinal learning.

Another form of spinal plasticity is known as central sensitization. It is produced by peripheral inflammation and yields a lasting increase in nerve excitability that enhances mechanical sensitivity (allodynia) and inhibits learning. This can be studied in the laboratory by subcutaneously injecting a small amount of capsaicin (the active ingredient in chili peppers) in the hind paw. Peripheral treatment with capsaicin induces spinal sensitization and impairs instrumental learning (Grau, 2002). As central sensitization is NMDA-mediated similar to instrumental learning, I also hypothesized that ethanol would block the induction of this maladaptive plasticity.

CHAPTER II

METHODS

Subjects

Sprague-Dawley male rats weighing between 275-300 grams were used in this experiment. Animal care and usage in this experiment was approved by the University Laboratory Animal Care Committee at Texas A&M University and all proper animal use protocols were followed. Subjects were given *ad libitum* access to food and water and were housed in pairs.

Experiment 1

Surgery

Subjects were initially anesthetized using an isoflurane gas chamber. Upon the subject's unconsciousness, the subject was moved from the chamber to a stereotaxic apparatus equipped with a nose cone to continue isoflurane administration. The subject's chest was supported with a small gauze pillow that was placed under his chest and his body temperature was kept stable by a heating pad placed underneath the stereotax. The thoracic section of the rats back was shaved and the second thoracic (T2) vertebrae was located by touch. Approximately an inch-long incision was made over the T2 vertebrae. The tissue caudal to T1 and rostral to T2 was removed until the spinal cord was reached. Once exposed the spinal cord was cauterized at this section. The wound was closed and stapled up with Michel clips. After closure, the rats hind legs were shaved for future electrode placement, the rats were given an intraperitoneal (IP) injection of 4 ml of 0.9% saline for hydration, and their legs were taped up to avoid awkward stiffening overnight. After surgery, the rats rested overnight in a temperature-controlled (75 degrees)

recovery room where they had *ad libitum* access to food and water. The rats rested for an average of 18-24 hours before instrumental testing.

Instrumental Testing

The subjects were taken from the recovery rooms the next day and were expressed. The subject's legs were untaped and they were given an IP injection of 10 ml (5 ml to each side) of one of four solutions: 0.9% saline, 0.63g/10ml EtOH-Saline solution, 1.25g/10ml EtOH-Saline solution, or 2.5g/10ml EtOH-Saline solution. Ten minutes were given after administration of the vehicles to allow for proper absorption of ethanol into the central nervous system. After the absorption period, instrumental testing with controllable leg shock was conducted using an apparatus similar to that used in previous studies (Grau et al., 1998). Briefly, rats were restrained in ventilated plexiglas tubes with their hind legs hanging down freely over a rectangular plastic dish (11.5 cm [width (w)] × 19 cm [length (l)] × 5 cm [depth (d)]) containing a saline solution positioned 7.5 cm below the restraining tube (Figure 1). To monitor leg position, a stainless-steel rod [7 cm (l), 0.46 mm (w)] was attached to the pad of one foot (contact electrode) extending past the toes. The contact electrode was taped to the plantar surface of the rat's foot [Orthaletic, 1.3 cm (width); Johnson and Johnson] with the end positioned directly in front of the plantar protuberance. Heat-shrink tubing electrically insulated the rod from the paw. A fine wire (0.01 mm^2 [36 American wire gauge (AWG)], magnet wire single beldsol) was attached to the end of the rod at a point under the insulation. This wire extended from the rear of the foot and was connected to a digital input board that was monitored by a Macintosh G4 computer. To minimize lateral leg movements, a piece of porous tape [Orthaletic, 1.3 cm (width)] was wrapped around the leg above the tarsus and attached under the front panel of the restraining tube. Two electrodes were then inserted into one hind leg. The first electrode was a piece of stainless-steel wire [0.05 mm^2

(30 AWG)] and was inserted through the skin over the tibia, 1.5 cm from the tarsus. The second was made of fine wire [0.01 mm^2 (36 AWG), magnet wire single beldsol] and was inserted perpendicular to the leg, through the body of the tibialis anterior muscle, 1.7 cm above the first electrode. Legshock was applied by attaching one lead from a constant current AC shock generator (Model SG-903; BRS/LVE) to the electrode inserted into the tibialis anterior muscle. The second lead was attached to the wire implanted in the skin over the tibia. Shock intensity was adjusted for each subject to reach a proper flexion response. Once the animals were prepared, the 30 minute instrumental testing session began. Whenever the subjects' legs were extended, the end of the rod made contact with the saline solution and completed an electrical circuit. When the circuit was closed, shock was delivered to the tibialis anterior muscle, which elicited a flexion response. The flexion response removed the electrode from the solution breaking the circuit and terminating the shock. The flexion number, time spent in the solution, and flexion duration were recorded by the computer. The data was collected in 30 separate one-minute data bins constituting the 30 minute testing.

Experiment 2

Subjects were transected using the same methods described for Experiment 1 above.

Tactile Reactivity Testing

The day after surgery, rats were removed from the recovery room, expressed, and untaped. Subjects were then placed in ventilated Plexiglas tubes with their hind legs hanging down freely. Von Frey filaments were pressed into the subjects hind paws to test their baseline thresholds for reactivity. Filaments of increasing force were used until a muscle spasm could be detected by the experimenter. This baseline was recorded and the subject’s were then given an IP injection of 10

ml (5 ml to each side) of EtOH or its vehicle. For this experiment, the highest dose of 2.5 g/ml was used. After a 10 minute absorption period, the subjects were given 0.05 ug of a capsaicin solution intradermally into one of their hind paws, counter-balanced across legs. After one hour, tactile reactivity testing began and, as described above, filaments of increasing force were used to test for a change in reactive threshold (muscle flexion). An hour passed between each testing and there were a total of 4 tactile reactivity tests. After testing, the rats were expressed and returned to the recovery room with *ad libitum* access to food and water.

Defecit Testing

The next day the subjects were taken from the recovery room and instrumentally tested as described for Experiment 1. No alcohol or saline was administered during this testing.

CHAPTER 3

RESULTS

Experiment 1

As in prior studies (Grau et al., 1998), saline-treated rats given response-contingent shock exhibited a progressive increase in response duration across the thirty minutes of testing. Ethanol treatment disrupted learning (Figures 2 and 3). While the main effect of ethanol treatment was not statistically significant, $F_{(3,28)}=2.32$, $p=.098$, trend analysis revealed a significant linear component, $F_{(1)}=5.57$, $p=.025$. This indicates that learning was disrupted by ethanol treatment in a dose-dependent manner.

Experiment 2

Capsaicin treatment enhanced mechanical reactivity in the saline-treated controls (Figure 4). Ethanol produced a hyporeactivity that countered the effect of capsaicin treatment. An ANOVA confirmed that during tactile reactivity testing, the ethanol group was significantly hyporeactive compared to the saline group, $F_{(1,14)}=196.185$, $p=.0001$ Relative to Experiment 1, saline treated rats given capsaicin performed poorly when tested in the instrumental paradigm twenty four hours later (Figure 5). Pretreatment with ethanol had no effect on this learning impairment, $F_{(1,14)}=.007$, $p=.936$.

CHAPTER 4

CONCLUSIONS

Experiment 1

I found that ethanol treatment disrupted spinally-mediated instrumental learning in a dose-dependent manner. Relative to the $GABA_A$ agonist muscimol, ethanol had a lesser effect. A possible reason for this is that ethanol is an allosteric modulator not a specific GABA receptor agonist.

Experiment 2

The extreme hyporeactivity demonstrated by the ethanol group in tactile testing suggests that ethanol has a short-term combative effect on central sensitization by inhibiting nociceptive processing. However, capsaicin pharmacologically outlives ethanol and so ethanol does not have any long-term protective effects. This is indicated by the instrumental learning data twenty-four hours after capsaicin treatment as both groups exhibited the learning deficit. Future studies will examine whether a higher dose of ethanol attenuates the long-term effect of peripheral inflammation.

General Conclusions

These results suggest that ethanol treatment can have a short-term effect that counters inflammation-induced pain. However, this acute effect is accompanied by an inhibition of spinal learning (Experiment 1). I found no evidence that ethanol treatment affects the long-term effect of peripheral inflammation. Thus, alcohol use in SCI recovery patients would appear to be detrimental as it would impair important learning mechanisms within the spinal cord. Furthermore, any anti-nociceptive benefits are too short term to have any lasting benefits.

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Figures:

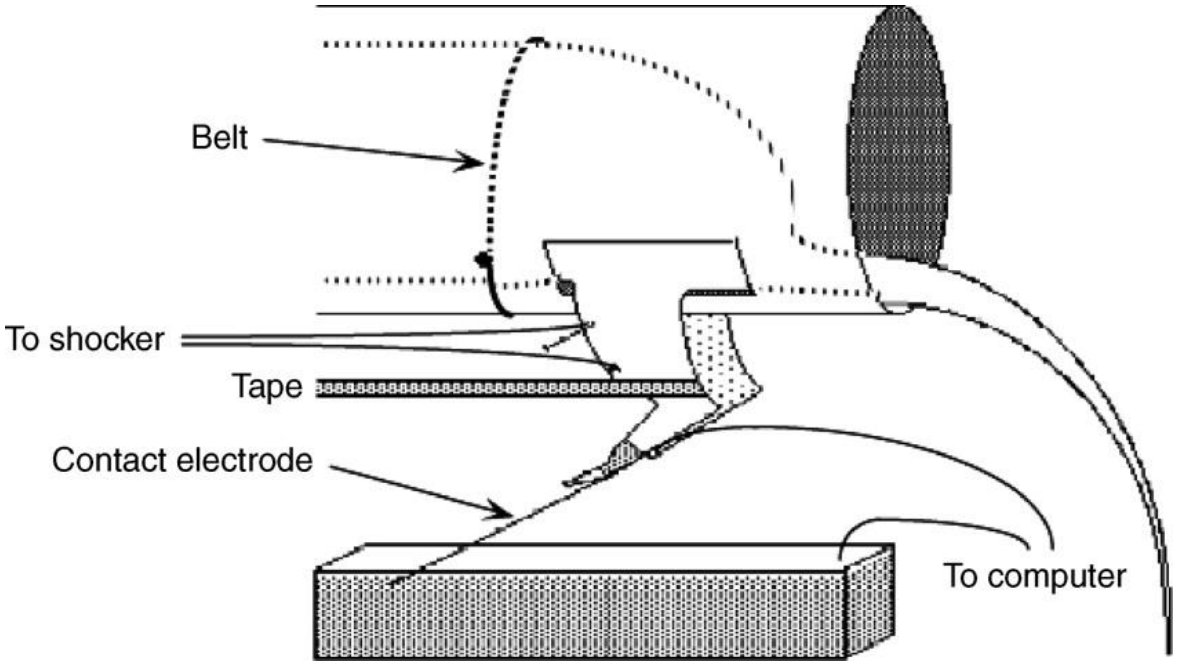

Figure 1. Instrumental learning apparatus.

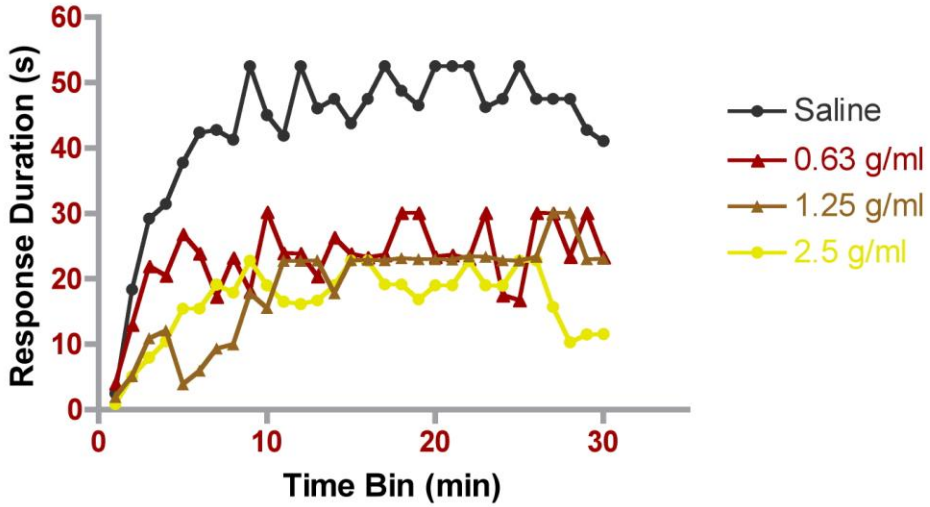

Figure 2. Response duration over time in spinally transected rats tested with response-contingent leg shock. Saline treated rats exhibited an increase in response duration indicative of learning. This learning was impaired by ethanol treatment.

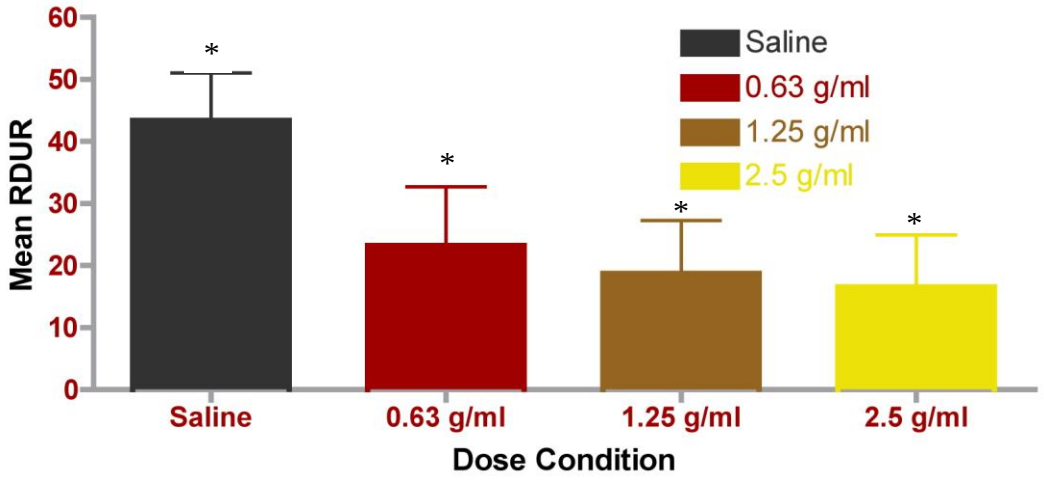

Figure 3. Mean performance, collapsed across the thiry minutes of testing, in Experiment 1. Ethanol treatment dose-dependently disrupted instrumental learning.

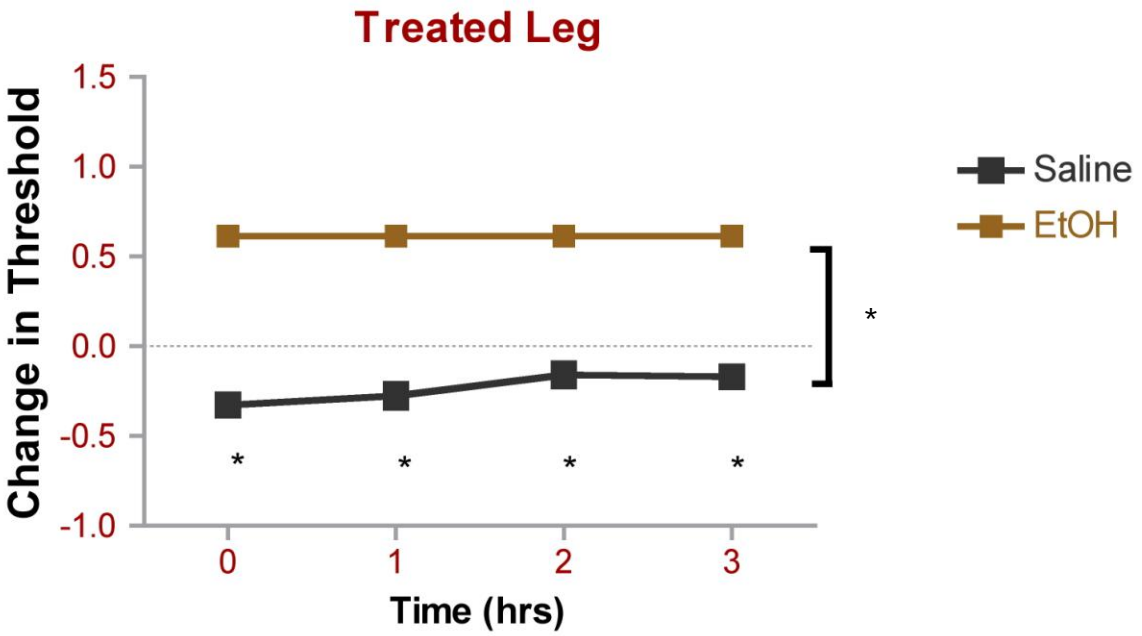

Figure 4. Tactile reactivity after capsaicin treatment. Peripheral treatment with capsaicin enhanced mechanical reactivity in the saline treated animals. Pretreatment with ethanol induced a hyporeactivity that countered the effect of capsaicin treatment.

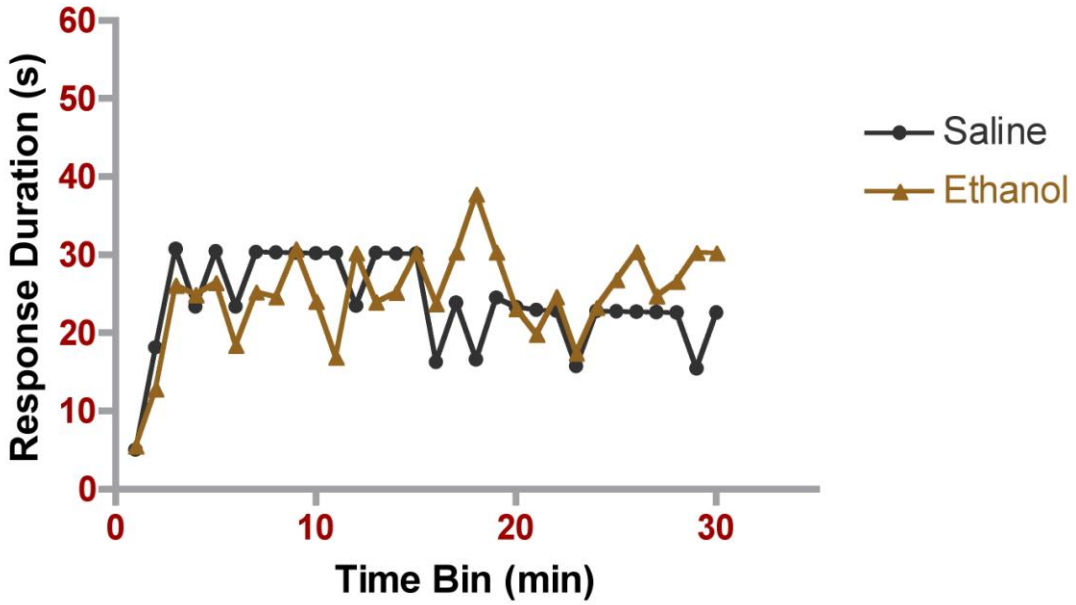

Figure 5. Long term effect of capsaicin treatment on instrumental learning. As previously reported, rats given saline prior to capsaicin treatment exhibited poor learning when tested twenty-four hours later. Pretreatment with ethanol had no effect.