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Effects of Traumatic Brain Injury on Oxycodone Reinstatement and Physical Dependence

Neil B. Varshneya
Virginia Commonwealth University

EFFECTS OF TRAUMATIC BRAIN INJURY ON OXYCODONE REINSTATEMENT AND PHYSICAL DEPENDENCE

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by

Neil B. Varshneya, B.S.

College of William and Mary 2014

Director: Katherine L. Nicholson, DVM, Ph.D.

Assistant Professor

Department of Pharmacology and Toxicology

Virginia Commonwealth University

Richmond, Virginia

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ABSTRACT

Epidemiological data indicate that patients who experience a traumatic brain injury (TBI) have an elevated risk of developing a substance use disorder (SUD), but the underlying neurobiological connections remain unclear. To further understand the relationship between TBI and SUD, we investigated the effects of TBI on the abuse-related effects of oxycodone in preclinical models. Our evaluation utilized a lateral fluid percussion injury of moderate severity in adult male Sprague-Dawley rats. In the first aim, we tested the hypothesis that moderate TBI increases the risk for relapse to an opioid use disorder as measured by reinstatement of lever-pressing behavior following extinction in an intravenous oxycodone self-administration procedure. In the second aim, we tested the hypothesis that moderate TBI increases physiological dependence to oxycodone as measured by decreases in food-reinforced lever-pressing behavior and increases in other withdrawal behaviors in both precipitated withdrawal and spontaneous withdrawal. In tests for self-administration, brain-injured subjects, relative to non-injured subjects, showed no significant differences in the number of oxycodone-reinforced sessions required to meet stable maintenance criteria for lever-pressing behavior. Likewise, brain-injured subjects showed no significant differences in the number of non-reinforced sessions to meet extinction criteria for lever-pressing behavior relative to non-injured subjects. In tests for reinstatement, non-injured subjects reinstated responding under oxycodone-associated cue- and oxycodone prime-induced conditions, however, brain-injured subjects did not reinstate lever-pressing behavior under any conditions. In tests for physical dependence, brain-injured subjects showed no significant differences from non-injured subjects with regards to their mean withdrawal scores or food-reinforced lever-pressing behavior. Overall, these data suggest that brain-injured patients with no significant pre-morbid history of opioid abuse are at a lesser risk of relapse to opioid use disorders. Moreover, the characteristic withdrawal syndrome in opioid-dependent patients may not contribute to continued opioid abuse to a greater degree in brain-injured patients than compared to non-injured patients.

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Adviser:
Dr. Katherine L. Nicholson (Primary Adviser and Graduate Advisory Committee Chair)

Committee Members:
Dr. Matthew L. Banks
Dr. Louis J. De Felice
Dr. Keith L. Shelton

Laboratory Staff:
Ellen C. Soehngen
James C. Gillespie

Others:
Dr. Candace L. Floyd
Dr. Hamid I. Akbarali
Matthew E. Tracy

Family:
Deepak Varshneya and Lynn Varshneya
Austin Varshneya

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CHAPTER 1: INTRODUCTION

Substance abuse is a major public health concern that imposes a broad range of costs on society. The Diagnostic and Statistical Manual of Mental Disorders (DSM) characterizes substance abuse as “a maladaptive pattern of substance use manifested by recurrent and significant adverse consequences related to the repeated use of substances” [1]. In the earliest human records, psychoactive substances were used by priests for religious ceremonies, healers for medicinal purposes, and by the general population in a variety of ways [2]. Today, regular drug use may evolve into a problem of drug abuse that taxes our healthcare system and results in lost workplace productivity and accidental hazards. In 2004, the World Health Organization estimated that at least 15.3 million people worldwide have drug use disorders and in 2007, the United States National Drug Intelligence Center estimated that the total economic costs of substance abuse exceeded 193 billion dollars annually [3,4]. In 2013, the National Survey on Drug Use and Health estimated that 21.6 million Americans aged 12 or older abused illicit drugs in the past year based on the DSM criteria for substance abuse [5]. A recent report by the National Institute of Health Office of Budget indicated that the research awards supported by the National Institute on Drug Abuse for the fiscal year of 2014 totaled nearly 770 million dollars [6]. Substance abuse and dependence are well-recognized public health problems and remain of great interest to researchers and research institutions alike.

Over the past century, substance abuse research has produced various therapeutics that aid the cessation of substance abuse, but those treatments are not always efficacious and are not without side-effects. Relapse to substance abuse remains a possibility even after successful treatment or sustained abstinence. Withdrawal symptoms can persist at low intensities for days, weeks, or months depending on the particular drug and doses at which the user became dependent. In this case, resumption of substance abuse behaviors serves to alleviate the persistent withdrawal dysphoria. In addition, limited periods of controlled, non-problematic drug use can lead to rapid escalation in consumption after abstinence. In 2004, the National Survey on

Drug Use and Health reported that only 3.8 million Americans of the 22.5 million Americans that were classified with substance dependence received treatment in that year [5]. Research in the abuse of substances has yielded numerous advances in our understanding of relapse to substance abuse, however, it remains a considerable public health issue that requires additional research to adequately address.

Of interest to drug abuse researchers is the relationship between traumatic brain injury (TBI) and substance use disorders (SUD). In 2006, the National Center for Injury Prevention and Control estimated that 1.7 million people in the United States sustain a TBI each year, of which 275,000 are hospitalized and 1.365 million are released from an emergency department [7]. Similar estimates by the National Institutes of Health in 1998 indicated that 70,000 to 90,000 individuals that experience a TBI suffer from substantial long-term loss of physical, cognitive, emotional, and behavioral function [8]. At present, the relationship between TBI and SUD has been investigated almost entirely from the perspective of drug intoxication as the cause of trauma [9]. It has been well-established that drug intoxication itself increases the risk of TBI and that the chief causes of that trauma include motor vehicle accidents, falls, or involvement in acts of violence [10,11].

Clinical evidence has emerged, however, of a correlation between the incidence of TBI and SUD in patients that have no history of significant substance use prior to injury. In a 2000 study, it was reported that the relative risk of substance abuse in patients with TBI (22%) was 1.3 fold greater than the risk of substance abuse in patients without TBI (16.7%) [12]. In a 2004 study of 188 TBI patients of whom 70 percent did not self-report substance abuse pre-injury, the prevalence of substance abuse rose from 14 percent at 1-year post-injury to 17 percent 3-years post-injury [13]. A 2004 study reported that TBI patients with no evidence of mental illness or substance abuse-related service utilization in the year prior to injury had a 4.5 odds ratio of substance abuse within the first year post-injury and still had a 1.4 times greater risk when evaluated at 25-36 months post injury [14].

Overall, epidemiological data support a connection between experiencing a TBI and developing a SUD. It is unclear, however, if the correlation between TBI and SUD reflects a coping mechanism or if neuroanatomical and neurochemical changes induced by brain injury result in increased vulnerability to development of substance use disorders. At the inception of this project, the manifestation of SUD as a result of TBI had yet to be investigated in preclinical models [9]. Currently, there is only one published study on the effects of TBI in a preclinical model of self-administration, more specifically a rodent model of ethanol self-administration where changes in ethanol intake in ethanol experienced subjects were assessed before and after TBI [15]. However, no research has been published regarding the TBI-induced changes in intake of any other abused substance in drug naïve or drug experienced subjects.

Since bodily trauma is often comorbid with TBI, clinical management of pain with opioid analgesics is common in TBI patients. It follows, then, that TBI patients are likely to be exposed to opioid analgesics during the course of their medical care. Over the last decade, as prescription opioid sales sharply increased, rates of prescription opioid abuse have also continued to rise, and as a result treatment admissions and deaths due to overdose are at epidemic levels [16,17]. Mortality rates due to opioid-analgesic poisonings nearly quadrupled in the years between 1999 and 2011, and in 2011 alone there were 41,340 deaths due to drug poisoning, 41% (16,917 deaths) of them involved opioid analgesics [18]. In cases of prescription opioid abuse that do not result in death, continued abuse of medication is common. In cases of prescription opioid abuse where abstinence is an outcome, relapse remains a possibility [19]. Moreover, repeated use of pain medication is likely to lead to the development of physical dependence [20] and withdrawal-induced dysphoria after abrupt cessation of opioid intake may contribute to continued drug taking [21].

Given the greater incidence of substance abuse in brain-injured patients and high probability of exposure to opioids prescribed in the course of treatment following brain injury, we investigated the effects of TBI on the response to oxycodone in preclinical models of abuse-

related behaviors in rats. In the first aim, we tested the hypothesis that moderate TBI increases the risk for relapse to an opioid use disorder as measured by reinstatement of lever-pressing behavior following extinction in an intravenous oxycodone self-administration procedure. In the second aim, we tested the hypothesis that moderate TBI increases physiological dependence to oxycodone as measured by decreases in food-reinforced lever-pressing behavior and increases in other withdrawal behaviors in both precipitated withdrawal and spontaneous withdrawal.

CHAPTER 2: PART 1

2.1 OVERVIEW OF CLASSICAL CONDITIONING

In 1927, Ivan Pavlov published a report titled *Conditioned Reflexes, An Investigation of the Physiological Activity of the Cerebral Cortex*. Pavlov systematically investigated the adjustments organisms make in response to the presentation of various environmental and proprioceptive stimuli [22]. In a typical Pavlovian experiment, a *neutral stimulus* (NS), which initially elicits no physiological response, is repeatedly paired with the presentation of an *unconditional stimulus* (US), a stimulus that alone is capable of eliciting a physiological response termed the *unconditioned response* (UR). Organisms learn to associate the NS with the US, and after several couplings, the NS alone can trigger a response that is similar to the response triggered by the US, known as the *conditioned response* (CR). At this point, the NS is no longer neutral since it has gained the ability to elicit a physiological response and is now referred to as the *conditional stimulus* (CS). In Pavlov's experiments, dogs were conditioned to salivate (CR) upon presentation of a tone (CS) after repeated pairing with food powder (US). In the case of drug-related behaviors, a NS is predictably followed by a US, the effects of the drug. As a result of repeated pairings, the CS elicits a CR which is similar to that of the UR, or the drug effects. Conditioned cues that are present during drug administration serve as conditioned reinforcers of drug-seeking behaviors if the effects of the drug are positively reinforcing [23].

In 1935, Clark Hull expanded on the understanding of Pavlov's experiments by introducing the *drive reduction theory*. Motivation, Hull proposed, has both drive and incentive components. He states, "the incentive is that substance or commodity in the environment which satisfies a need, i.e., which reduces a drive" [24]. In 1948, Abraham Wikler applied Hull's theory to the phenomenon of substance abuse relapse. He proposed that through a process of associative learning, neutral stimuli in the environment can, over the course of many pairings with drug taking, come to elicit conditioned withdrawal responses in drug dependent subjects [25]. For example, if the sight of a needle, syringe, or white powder (NS) is repeatedly paired with a diminishing drug

level (UR) during withdrawal (US), those cues may then serve as conditional stimuli (CS), which elicit a conditioned withdrawal response (CR). If the conditioned cues are presented after a period of abstinence from substance abuse, the subject's desire to relieve the conditioned dysphoria may result in a relapse to drug consumption [26,27]

2.2 OVERVIEW OF OPERANT CONDITIONING

Over the last several decades, animal models have been used extensively to investigate the neurobiological and behavioral mechanisms underlying vulnerability to relapse. A "reinstatement model" allows researchers to analyze drug-seeking and relapse-like behavior. Many experimental models in substance abuse research, including the reinstatement model as it is used in self-administration, are fundamentally dependent on the principles of operant conditioning. In 1930, Burrhus Skinner first described the use of operant chambers to study animal behavior in a paper titled *On the Conditions of Elicitation of Certain Eating Reflexes* [28–30]. In 1938, Skinner published *The Behavior of Organisms* which set forth the principles for the experimental analyses that we perform today. In a typical Skinnerian experiment, organisms adjust their behavior according to the behavior-consequence contingencies specified by the investigators. If a consequence increases the probability of the behavior antecedent to it, it is termed a *reinforcer*. If a consequence decreases the probability of a behavior antecedent to it, it is termed a *punisher*. If a reinforcing or punishing stimulus is presented it is termed *positive*, however, if it is removed from the system it is termed *negative*. The relationship between the behaviors and their consequences is referred to as a *two-term contingency*. If for any two-term contingency, the probability of emission of the behavior is increased or decreased only in the presence of a given stimulus, it is known as a *three-term contingency* and the modulating stimulus is known as *discriminative stimulus* [31]. For example, a light in an illuminated state may signal that the emission of a lever press behavior will consequently be followed by the presentation of a food pellet, however, when the light is not illuminated the contingency does not apply. In this regard, the illuminated light serves as the discriminative stimulus and the presentation of the food

pellet is a form of *positive reinforcement* since the stimulus is added to the system and increases the probability of the behavior antecedent to it. In these experiments, the effect of a specified consequence on a particular behavior can be evaluated by measuring the total number of emitted behaviors or the rate of the emission of those behaviors.

It is also possible to manipulate the way a reinforcer is delivered as a function of responding to investigate the ways in which organisms adjust to consequences of their behaviors. In operant conditioning, reinforcement schedules commonly consist of ratio schedules and interval schedules. In *ratio schedules*, a specified number of behaviors must be emitted for the delivery of reinforcement. For example, in a *fixed ratio schedule* (FR) the number of required responses is fixed whereas in a *variable ratio schedule* (VR), the number of required responses varies around a mean of the ratio of the schedule. In *interval schedules*, a specified amount of time must elapse after the last reinforced behavior before subsequent behaviors are reinforced. For example, in a *fixed interval schedule* (FI) the amount of time that must elapse is fixed whereas in a *variable interval schedule* (VR) the amount of time that must elapse varies around a mean of the interval of the schedule [32]. Schedules of reinforcement are frequently manipulated in the case of self-administration procedures and reinstatement procedures as discussed in the subsequent sections.

2.3 ORIGINS OF THE SELF-ADMINISTRATION PROCEDURE

In 1940, Sidney Spragg published a monograph titled *Morphine Addiction in Chimpanzees*. Spragg applied Hull's drive reduction theory to chimpanzees. In an experimental room separate from their living quarters, chimpanzees were administered morphine injections twice daily for an average four weeks or until they were opioid-dependent. Given a choice between food and drug, chimpanzees preferred the injection to food when experiencing withdrawal symptoms. Spragg's results suggest that relief of morphine-induced withdrawal symptoms may serve as sufficient reinforcement for the development of drug-seeking behavior and that the positive drug effects serve as conditioned reinforcers as later suggested by Wikler [25,33].

In 1957, Horace Beach published a report titled *Morphine Addiction in Rats* in which he used a Y-choice discrimination box to determine whether rats would seek stimuli associated with the drugs effects [34]. After habituation to the apparatus, subjects were given the choice of two distinctly different contexts with unique cues (goal boxes). After baseline preference determination, rats were administered morphine or saline injections once daily for twelve days and then immediately placed in either their preferred or non-preferred goal box. Given a choice between stimuli previously associated with the drug effects and stimuli not previously associated with the drug effects, rats showed a significant preference for the stimuli previously associated with drug effects as compared to their baseline preference. As suggested by Spragg in 1940 and Wikler in 1948, Beach's results support the hypothesis that both the euphoric effects of morphine and the action of morphine in relieving withdrawal distress are sufficiently reinforcing to promote the development of morphine-seeking behavior.

In 1962, James Weeks published a paper in Science titled *Experimental Morphine Addiction: Method for Automatic Intravenous Injections in Unrestrained Rats*, which laid the foundation for the self-administration procedures that are widely used in substance abuse research today. Weeks surgically implanted polyethylene cannulae into the jugular veins of albino female rats using a technique developed by Vojin Popovic [35]. With the ability to directly introduce morphine sulfate to the rodent circulatory system, Weeks applied Skinner's (1938) fundamental principles of operant conditioning to study the effect of drugs on animal behavior. In operant boxes, relatively unrestrained rodents could then utilize a self-injection technique to intravenously administer morphine sulfate by lever press activation of an automatic syringe driver. It was shown that the rate of self-administration of morphine varied inversely by the dose. It was also shown that morphine acted as a reinforcer that produced almost immediate relief from withdrawal in dependent subjects as shown previously by Spragg (1940) in chimpanzees and Beach (1957) in rats [36].

2.4 ORIGINS OF THE REINSTATEMENT PARADIGM

Substance abuse relapse is studied in animal models of reinstatement which often utilize self-administration procedures. In a typical self-administration experiment with a reinstatement design, subjects learn to press a lever in an operant chamber for an intravenous infusion of drug during the *acquisition* phase. Once the subjects reliably press a lever for drug infusion, and stable drug-taking behavior is reproducible, the subjects are considered to be in the *maintenance* phase. It is then possible to extinguish the learned contingency by replacing the drug infusion with a saline infusion or no infusion, of which the latter two do not serve as reinforcing stimuli when presented. Often this results in an *extinction burst* which is characterized by a sudden and temporary increase in the subject's response frequency. Once subjects learn that the reinforcement is no longer a consequence of lever-pressing behavior, they are considered to be in the *extinction* phase. A non-contingent, pre-session drug injection, referred to as a *drug priming*, may renew the previously extinguished expression of lever-pressing behavior, even when the emission of that behavior does not result in drug infusion. A drug priming model is designed to simulate an exposure to the drug that was abused or a related drug after treatment or abstinence in humans. Similarly, an exteroceptive cue, or *cue priming* and a noxious stimulus or *stress priming* will also result in the renewed expression of previously extinguished lever pressing behavior. A cue priming model is designed to simulate exposure to drug-associated cues, such as drug paraphernalia, that can lead to renewed drug taking. A stress priming model is designed to simulate renewed drug taking in response to major life stressors such as grief, sorrow, and anger.

In 1971, Rodger Stretch, Gary Gerber, and Susan Wood published a study titled *Factors Affecting Behavior Maintained by Response-Contingent Intravenous Infusions in Squirrel Monkeys* which utilized such a procedure. In daily two hour sessions, subjects developed drug-seeking behavior for intravenous infusions of d-amphetamine on a modified progressive ratio schedule of reinforcement. Responding was then extinguished by replacing infusions of d-

amphetamine with infusions of saline. When pre-session intramuscular injections of d-amphetamine were administered, responding was restored and indistinguishable from that observed when drug infusions were available [37]. Researchers later termed this phenomenon *prime-induced reinstatement*.

In 1976, Marvin Davis and Stanley Smith, using a self-administration procedure, explored the motivational properties of secondary reinforcers derived from the primary reinforcing effects of intravenous morphine injections. Subjects were trained to acquire morphine self-administration with a buzzer presentation during each morphine infusion. By substituting saline for morphine, the lever press behavior was extinguished in the absence of the buzzer, the reinforcing conditioned stimulus. In subsequent sessions, elevated responding occurred during the presentation of the buzzer, confirming the occurrence of secondary reinforcement [38]. Researchers later termed this phenomenon *cue-induced reinstatement*.

In 1995, Yavin Shaham and Jane Stewart tested the effect of footshock stress on relapse to heroin-seeking behavior. Subjects trained on intravenous heroin self-administration were exposed to footshock stress in a reinstatement procedure after extinction. After numerous extinction sessions, and after a prolonged drug-free period, the footshock stress produced responding that mimicked the effect of a non-contingent priming infusion of heroin. Such results suggest that stress is a powerful stimulus for relapse to drug-seeking behavior and is comparable to heroin itself [39]. Researchers later termed this phenomenon *stress-induced reinstatement*.

In summary, these three approaches are commonly utilized to reinstate drug-taking behavior in preclinical models. A non-contingent, pre-session drug injection, referred to as a *drug-priming*; presentation of an exteroceptive cue, or *cue priming* and a noxious stimulus or *stress priming* have all been repeatedly demonstrated to renew expression of extinguished lever pressing behavior even when the emission of that behavior does not result in drug infusion. Overall, the individual approaches to inducing reinstatement in preclinical models have provided

information about the underlying neuroanatomical circuitry and neurochemical mechanisms that drive relapse in humans.

2.5 CURRENT UNDERSTANDING OF REINSTATEMENT TO OPIOID SELF-ADMINISTRATION

A database search for published literature on reinstatement of opioid self-administration yields a number of preclinical studies in which agonists and antagonists are screened as potential pharmacotherapies for relapse prevention. Such approaches are used to elucidate the neurobiological and neurochemical mechanisms that mediate relapse. Our test drug of abuse, oxycodone, has been used in only a limited capacity in preclinical studies on reinstatement of self-administration, compared to other opioids such as heroin and morphine.

In a 2005 study, investigators tested the hypothesis that co-administration of ultra-low-doses of naltrexone, a mu-opioid antagonist, with oxycodone, a mu-opioid agonist, attenuate prime-, cue-, and stress-induced reinstatement [40]. Male Sprague-Dawley rats individually housed under a reverse light-dark cycle with ad libitum access to food and water, acquired oxycodone-reinforced self-administration behavior in combination with naltrexone (1, 10, 100 pcg/kg/infusion) in 10 daily, three-hour sessions under an FR10 schedule of reinforcement. Subjects' active lever-pressing behavior during acquisition and maintenance sessions resulted in an infusion of oxycodone infused over 10 seconds and a 30-second presentation of the light cue. Subjects' active lever-pressing behavior during extinction sessions had no programmed consequences.

In cue- and prime-reinstatement sessions (0.25 mg/kg, SC), subjects that previously self-administered ultra-low doses of naltrexone (1, 10 pcg/kg/infusion) in combination with oxycodone showed attenuated levels of responding. In stress-induced reinstatement sessions, subjects that previously self-administered naltrexone in combination with oxycodone showed a naltrexone dose-dependent attenuation in responding. Such a result is a successful demonstration of the ability to alter reinstatement of lever-pressing behavior following extinction by manipulation of

opioid neurotransmission. It is suggested, then, that patients initially acquiring a drug taking behavior with oxycodone and naltrexone in combination may be less liable to abuse opioids after treatment or abstinence from drug taking.

In 2014 study, investigators tested the hypothesis that ATPM-ET, a kappa-opioid agonist with mixed mu-opioid agonist-antagonist activity, attenuates prime-induced reinstatement in subjects receiving opioids [41]. Male Sprague-Dawley rats individually housed on a reverse light-dark cycle acquired heroin-reinforced nose-poking behavior under a FR2 schedule of reinforcement (50 mcg/kg/infusion) in three-hour operant sessions, limited to 25 injections per session, for 10 consecutive days. In the acquisition phase, subjects were administered saline or ATPM-ET (1.2 or 2.4 mg/kg, IP) 15 minutes prior to session and subjects continued responding for 8 to 10 days until subjects could discriminate between the active and inactive hole for 3 consecutive days. Subjects extinguished responding in daily three-hour extinction sessions for 3 weeks where heroin solution was replaced with saline solution. In reinstatement tests, ATPM-ET (1.2 and 2.4 mg/kg, IP) or saline (1 ml/kg, IP) was injected 15 minutes prior to injection of heroin (0.25 mg/kg, SC) or saline (1 ml/kg, SC), which was injected 10 minutes prior to subject placement in chamber. It is reported that ATPM-ET at high doses attenuated the ability of heroin to reinstate active nose-poking behavior without affecting inactive nose-poking responding. It is suggested, then, that ATPM-ET may prevent heroin priming induced reinstatement of extinguished drug seeking behavior.

In a 1996 study, investigators tested the hypothesis that dopamine antagonists attenuate prime- and stress-induced reinstatement in subjects receiving opioids [42]. Male Long-Evans rats housed on a reverse light-dark cycle, acquired heroin-reinforced lever-pressing behavior under a FR1 schedule of reinforcement (25 mcg/kg/infusion) in four, three-hour operant sessions per day, two sessions per light cycle, for eight to eleven consecutive days. In the extinction phase, subjects received an infusion of saline for pressing the previously reinforced lever for five consecutive days. Subsequently, subjects were tested twice, separated by 48 hours, for reinstatement of self-

administration of heroin under either prime or stress conditions in three-hour sessions where either non-contingent, subcutaneous heroin injection (0.25 mg/kg, 10-minute incubation) or intermittent footshock (0.5 mA, 0.5 s active, 10-70 s inactive, 10-minute session) were given prior to session. As in the extinction phase, subjects received a saline infusion under reinstatement conditions for pressing the previously active lever. Subjects were pretreated with either saline, the opioid antagonist, naltrexone (1 or 10 mg/kg, SC), the D1-like receptor antagonist SCH 23390 (0.05 or 0.1 mg/kg, IP), the D2-like receptor antagonist raclopride (0.25 or 0.5 mg/kg, IP), or the non-selective DA antagonist flupenthixol (3 or 6 mg/kg, IM). In stress conditions, only flupenthixol dose-dependently attenuated reinstatement. In prime conditions, however, naltrexone, raclopride, and flupenthixol dose-dependently attenuated reinstatement.

It is possible to conclude, then, that dopaminergic signaling plays an important role in reinstatement of behavior following exposure to aversive stimuli, or re-exposure to heroin, since flupenthixol, the non-selective dopaminergic antagonist attenuated both stress-induced and prime-induced reinstatement in this procedure. However, these findings also suggest that stress-induced reinstatement and prime-induced reinstatement are also mediated by at least two different neurobiological or neurochemical systems since naltrexone and raclopride, which have different mechanisms of action than flupenthixol, attenuated prime-induced reinstatement, but not stress-induced reinstatement.

In a 2012 study, investigators tested the hypothesis that dopamine antagonists attenuate prime-induced reinstatement in subjects receiving opioids [43]. Food-restricted (20 g daily), male Sprague-Dawley rats housed on a reverse light-dark cycle acquired heroin-reinforced nose-poking behavior under a FR1 schedule of reinforcement (30 mcg/kg/infusion) in daily three-hour operant sessions for 12 to 14 days. In the extinction phase, subjects received an infusion of saline for nose-poking the previously reinforced hole. Subsequently, subjects were tested for prime-induced reinstatement in two-hour sessions, where a non-contingent, subcutaneous heroin injection (0.25 mg/kg) was administered prior to session. In reinstatement sessions, prior to prime

injection, subjects were treated with saline or levotetrahydropalmatine (1.25, 2.5, or 5 mg/kg, IP), a D1/D2/D3 antagonist. In prime conditions, levotetrahydropalmatine dose-dependently attenuated heroin prime-induced reinstatement—that is, that the number of nose-pokes in the active hole was significantly decreasing as a function of increasing treatment dose.

It is common, in reinstatement studies, to include complementary data such as food-reinforced lever press performance or locomotion data that indicate that a particular treatment produces a specific effect, or one that selectively modulates a single system or set of systems, and not a non-specific effect, that non-selectively modulates many or all systems. In this study, investigators elected to include nose-poke performance data on the non-reinforcing, or inactive nose-poke hole. These data suggest that the treatment mechanism is specific to a single system or a set of systems, such as the mesocorticolimbic dopamine system, which mediates behavior in accordance with a multiple-term contingency. Furthermore, data from locomotion assays indicate that only high doses of levotetrahydropalmatine (5 mg/kg, IP) significantly decrease locomotion, which reinforces the notion that the treatment acts directly and not through sedative effect. It is possible to conclude, then, that levotetrahydropalmatine may have therapeutic utility in the prevention of relapse prompted by re-exposure to drug.

In a 2013 study, investigators tested the hypothesis that dopamine antagonists attenuate prime- and cue-induced reinstatement in subjects receiving opioids [44]. Male Sprague-Dawley rats individually housed on a reverse light-dark cycle acquired heroin-reinforced nose-poking behavior under a FR1 schedule of reinforcement (50 mcg/kg/infusion) in daily four-hour operant sessions for 14 days. In extinction sessions, there were no programmed consequences for behavior in daily one-hour sessions for 10 consecutive days. Subsequently, subjects were tested for reinstatement of self-administration of heroin under cue conditions and prime conditions in two-hour sessions. In cue conditions, visual and auditory cues were presented for five seconds at the start of the session and for each nose-poke on the previously reinforced hole thereafter. However, no reinforcement was delivered for behavior. In prime conditions, subjects were

administered heroin (0.1 or 0.25 mg/kg, SC) 10 minutes prior to sessions in which no reinforcement was delivered for behavior. In cue conditions, risperidone (0.01, 0.03, or 0.1 mg/kg, IP), a D2/D3/D4 antagonist, was administered 10 minutes prior to session, and in prime conditions, 10 minutes prior to prime injection.

In cue conditions, risperidone (0.03 and 0.1 mg/kg, IP) pre-treatment dose-dependently attenuated reinstatement of nose-poking behavior on the active hole with no significant change in nose-poking behavior on the inactive hole. In heroin (0.1 or 0.25 mg/kg, SC) prime conditions, risperidone (0.01, 0.03, or 0.1 mg/kg, IP) pre-treatment had no significant effect on reinstatement of nose-poking behavior on either the active or inactive hole. Lai et al, suggest that while risperidone serves as an antagonist for numerous DA receptor subtypes, most notably D2 receptors, they also report that risperidone has a greater affinity for 5HT-2A receptors exerts similar action at adrenergic receptors and histamine receptors. It is possible to suggest, then, that risperidone, and drugs with similar mechanism of action, may have therapeutic utility in the prevention of relapse prompted by cues previously associated with drug-taking behavior.

In a 2014 study, investigators tested the hypothesis that the mixed dopamine agonist-antagonist, L-stepholidine, attenuates cue-induced reinstatement in subjects receiving an opioid agonist [45]. Male Sprague-Dawley rats housed on a reverse light-dark cycle acquired heroin-reinforced nose-poking behavior under a FR1 schedule of reinforcement (30 mcg/kg/infusion) in daily two-hour operant sessions. In the acquisition phase, each infusion was paired with a 5 second presentation of visual (light) and auditory (tone) cue. In the extinction phase, subjects' responding had no programmed consequences for behavior. Subsequently, subjects were tested for cue-induced reinstatement in two-hour operant sessions. In the reinstatement test sessions, subjects were pre-treated with saline or L-stepholidine (2.5, 5.0, 10.0 or mg/kg, IP) 30 minutes prior to session. In these sessions nose poking behavior was not reinforced with heroin. It is reported that L-stepholidine, but not saline, significantly and dose-dependently attenuates cue-

induced reinstatement. Furthermore, L-stepholidine and saline had no significant effect on locomotor activity nor nose-poking behavior on the inactive hole.

In a concurrent 2014 study, performed by the same group, investigators tested the hypothesis that the same mixed dopamine agonist-antagonist, L-stepoholidine, attenuates cue-induced reinstatement in subjects receiving an opioid agonist [46]. Male Sprague-Dawley rats individually housed under a reverse light-dark cycle acquired heroin-reinforced nose-poking behavior under a FR1 schedule of reinforcement (50 mcg/kg/infusion) in daily three-hour operant sessions for 12 consecutive days. In the extinction phase, there were no programmed consequences for behavior in daily two-hour sessions for 12 consecutive days. Subsequently, subjects were tested for heroin prime-induced reinstatement (0.25 mg/kg, SC). In the reinstatement procedure, subjects pre-treated with saline or L-stepholidine (2.5, 5.0 or 10.0 mg/kg, SC) 30 minutes prior to session. It is reported that L-stepholidine, but not saline, significantly and dose-dependently attenuated heroin prime-induced reinstatement. Furthermore, L-stepholidine and saline had no significant effect on locomotor activity nor inactive nose-poking behavior on the inactive hole.

Indeed, L-stepholidine is characterized as a dual D1-receptor agonist and D2-receptor antagonist [47–50] and therefore supports the rationale for use of dopamine agonist-antagonist approaches to relapse control following opioid abuse. Since the dopamine receptor and its subtypes have been major targets of investigation in the relapse to opioid abuse, the use of L-stepholidine is justified, even with mixed action at dopamine receptors. However, others also report significant partial agonistic activity at 5-HT1A receptors [51]. It is possible to conclude then, that, while L-stepholidine may decrease relapse liability, it may not mediate these effects through dopamine receptors alone. Overall, these pharmacological manipulations provide insight into the receptor mechanisms which mediate relapse to opioids, induced by both re-exposure to drug-associated cues and renewed drug taking. Collectively, these studies demonstrate that the

mesocorticolimbic dopamine system is involved in the mediation of prime-, cue-, and stress-induced reinstatement of heroin-reinforced self-administration behavior.

It should be noted that while the authors of each study characterize their experiments with a great degree of detail, no single study reported a complete set of controlled variables, which are necessary to make direct comparisons. For example, some studies explicitly state their acquisition and extinction criteria, while other studies differ in that they report only the number of operant sessions or the number of days required to meet an unspecified set of criteria. Furthermore, no two studies used exactly the same values in their set of controlled variables. Even experiments reported by the same group—seeking to answer nearly identical questions—in two separate publications in the same year, had marked variations in their procedures, including the drug infusion concentration. In addition, while most of the studied pre-treatment ligands are selective for a particular receptor or receptor subtype, these ligands tend to bind, to at least some degree, many different molecular targets. It should be noted, then, that the effects observed in the reviewed reinstatement assays may be due to a combination of ligand-receptor interactions, and not solely due to the interaction between the receptor and ligand with greatest affinity.

In summary, many of these studies have demonstrated that receptors in the mesocorticolimbic dopamine system play a role in the modulation of effects of acute opioid exposure, opioid-associated cues, and stressors on the reinstatement of opioid seeking. Overall, the reinstatement paradigm has been shown to be a viable platform for the investigation of relapse-like behavior and the variables which may impact relapse including pharmacological, genetic and environmental variables. Our study utilized this well-established model to investigate the hypothesis that moderate TBI increases the risk for developing an opioid use disorder as measured by reinstatement of lever-pressing behavior previously reinforced by oxycodone in a self-administration procedure, a proposed model of preoccupation and anticipation leading to relapse.

CHAPTER 2: PART 2

2.6 CURRENT UNDERSTANDING OF PHYSICAL DEPENDENCE TO OPIOIDS

In the development of drug addiction, drug taking often begins in a social setting and is compounded by acute reinforcement. Escalation of drug taking can lead to a transition from compulsive use to physical dependence and withdrawal symptoms following abstinence. It is proposed that dysphoria, a result of cessation of drug use in dependent individuals, may be a sufficient motivating factor in the reinitiation of drug taking, also known as relapse. According to Goodman & Gilman's, *The Pharmacological Basis of Therapeutics*, physical dependence refers to an "altered physiological state produced by the repeated administration of drug, which necessitates the continued administration of the drug to prevent the appearance of a stereotypical syndrome, the withdrawal or abstinence syndrome, characteristic for a particular drug" [52]. The development of physical dependence is not predicated on the motivating factors for drug taking such as misuse, abuse, and supervised medical use, rather it is the repeated drug administration alone that results in altered physiology [53,54].

It is proposed that while these reinforcing effects serve to promote the initial development of drug taking through positive reinforcement, physical dependence is important in the continuation and maintenance of drug taking which serve to alleviate an aversive withdrawal syndrome, a form of negative reinforcement [55–59]. It follows then, that a withdrawal syndrome, as occurs with opioids, can be a major determinant of continued use and abuse of a drug [60]. In both preclinical and clinical studies, withdrawal will occur after abrupt cessation of chronic opioid intake, or spontaneous withdrawal [61], and through the administration of an opioid antagonist, or precipitated withdrawal [62]. Our study used this well-established approach to test the hypothesis that moderate TBI increases the risk for developing a physiological dependence to oxycodone as measured by changes in food-reinforced lever-pressing and other withdrawal behaviors during both precipitated and spontaneous withdrawal.

It is reported that a strong correlation exists between the species specific withdrawal signs and symptoms of both humans and rodents with respect to physical dependence induced by repeated administration of opioids [63–66]. In humans, signs and symptoms of withdrawal include dysphoric mood, nausea or vomiting, muscle aches, cramps, lacrimation, rhinorrhea, pupillary dilation, piloerection, sweating, chills, diarrhea, yawning, fever, insomnia, craving for opioid drug, sneezing, tachycardia, and hypertension (see table for qualifying criteria) [67–70]. In rodents, signs and symptoms of withdrawal include diarrhea, rhinorrhea, piloerection, teeth chattering, “wet dog shakes,” genital grooming and penile erection and decreased food consumption (anorexia) [71].

Operant responding has been shown as a sensitive measure in the detection of withdrawal signs and symptoms. For example, in a model of physical dependence, food-reinforced operant responding is disrupted in a precipitated withdrawal procedure by the administration of an opioid antagonist in opioid-dependent subjects [72,73]. Moreover, doses of an opioid antagonist that are sufficiently small to not result in observable withdrawal in morphine-dependent rats will disrupt food-reinforced responding [74]. Collectively, these studies suggest that the development of physical dependence as an adaptive, homeostatic response to the acute and chronic administration of opioids is well established in rodent models and can be quantified through gross observation of unlearned behavior and learned behavior as in schedule controlled responding [72,74,75].

Qualification Criteria for Withdrawal Syndrome	
[DSM 5][1] Opioid Withdrawal Diagnostic Criteria 292.0 (F11.23)	**[ICD-10][2] Opioid Withdrawal State Criteria (F11.3)**
A. Presence of either of the following: 1. Cessation of (or reduction in) opioid use that has been heavy and prolonged (i.e., several weeks or longer). 2. Administration of an opioid antagonist after a period of opioid use. B. Three (or more) of the following developing within minutes to several days after Criterion A: 1. Dysphoric mood 2. Nausea or vomiting 3. Muscle Aches 4. Lacrimation or rhinorrhea 5. Pupillary dilation, piloerection, or sweating 6. Diarrhea 7. Yawning 8. Fever 9. Insomnia C. The signs or symptoms in Criterion B cause clinically significant distress or impairment in social, occupational, or other important areas of functioning D. The signs or symptoms are not attributable to another medical condition and are not better explained by another mental disorder, including intoxication or withdrawal from another substance	A. The general criteria for withdrawal state (F1x.3) must be met. (Note that an opioid withdrawal state may also be induced by administration of an opioid antagonist after a brief period of opioid use.) B. F1x.3 Withdrawal State Criteria: 1. There must be clear evidence of recent cessation or reduction of substance use after repeated, and usually prolonged and/or high-dose, use of that substance. 2. Symptoms and signs are compatible with the known features of a withdrawal state from the particular substance or substances (see below). 3. Symptoms and signs are not accounted for by a medical disorder unrelated to substance use, and not better accounted for by another mental or behavioral disorder. C. Any three of the following signs must be present: 1. Craving for an opioid drug 2. Rhinorrhoea or sneezing 3. Lacrimation 4. Muscle aches or cramps 5. Abdominal cramps 6. Nausea or vomiting; 7. Diarrhea 8. Pupillary dilatation 9. Piloerection, or recurrent chills 10. Tachycardia or hypertension 11. Yawning 12. Restless sleep
[1] American Psychiatric Association., (2013). Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5). [2] World Health Organization., (2010). International Classification of Diseases (ICD).	

CHAPTER 3: MATERIALS AND METHODS

3.1 SUBJECTS

Subjects were adult male Sprague-Dawley rats individually housed under a reverse light-dark cycle (light 1800 to 0600; dark 0600 to 1800) with ad libitum access to food and water in a vivarium approved by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). All procedures were reviewed and approved by the Virginia Commonwealth University (VCU) Institutional Animal Care and Use Committee (IACUC) and the Animal Care and Use Review Office (ACURO) of the United States Army Medical Research and Materiel Command (USAMRMC) Office of Research Protections (ORP).

3.2 FLUID PERCUSSION INJURY PROCEDURE

Subjects underwent a fluid percussion procedure to induce moderate, closed-head, intracranial injury (Day 0) following handling, operant training, and when appropriate intravenous catheterization for subsequent behavioral procedures. Subjects were anesthetized with 3 percent isoflurane vapor and transferred to a stereotaxic device for craniectomy and maintained on 4 percent isoflurane for the duration of the procedure. Subjects were divided approximately evenly into two groups: 1) a control group that underwent all procedures with the exception of the fluid percussion injury (referred to as control, non-injured, or sham subjects) and 2) a treatment group that underwent all procedures including the fluid percussion injury (referred to as injured, brain-injured, or TBI subjects).

One longitudinal incision of 9 mm in length was made in the scalp and the underlying fascia was displaced to expose the dorsal surface of the exterior skull. A craneictomy of 4.8 mm in diameter was then performed by hand with a trephine over the right motor cortex at the midpoint between bregma and lambda and the central fissure and lateral ridge. A cannula fabricated from the hub of a female leur-lock compatible 20-gauge needle was affixed to the skull at the craniectomy site and secured to the skull with dental acrylic. Once the dental acrylic hardened, the cannula was filled with sterile saline and the intracranial injury was induced with a fluid

percussion device (Custom Design and Fabrication, VCU, Richmond, VA). Subsequently, a visual observation to confirm the integrity of the dura mater was performed. A piezoelectric sensor in the fluid percussion device measured the profile of the pressure pulse and an oscilloscope recorded and reported the resultant amplitude and duration of the pressure pulse.

Subjects' transient loss of consciousness was assessed immediately after the procedure by measurement of the *righting reflex* assessed by the time each subject required to reorient itself to the prone position when placed in the on its back. It reported by numerous sources that the time to return of the righting reflex, is a valid measure of the extent and severity of tissue damage [76–82]. After these procedures, the scalp was closed with a polydioxanone suture and the subject was returned to a clean, warmed, home chamber for recovery.

Statistical analysis of the recorded time to return of righting reflex between the brain-injured and non-injured subjects was performed using both a Student's T-Test and a Levene's Test for Equality of Variances. Subjects whose recorded time to return to right reflex was greater than two standard deviations from the mean did not qualify for inclusion in experiments and were excluded from calculations in the final data analysis.

3.3 CATHETERIZATION PROCEDURE

Subjects for testing in the self-administration procedure underwent surgical implantation of an indwelling, polyurethane catheter five days prior (Day -5) to the fluid percussion procedure (Day 0). Subjects were pre-medicated with 2 mg/kg morphine and anesthetized with 4 percent isoflurane vapor and maintained for the duration of the procedure. One longitudinal incision of approximately 20 mm in length was made in the skin on the ventral surface of the neck, right lateral and parallel to the trachea in line with the point of the mandible. A blunt dissection technique was performed to locate and isolate a 10 mm segment of the right external jugular vein. Once the vein was located and isolated, the cranial end of the segment was ligated with a braided nylon suture. Subsequently, a latitudinal incision was made on the ventral surface of the vein. A polyurethane catheter was inserted into the vein and adjusted such that the final position of the

catheter was at a level near, but not obstructing the right atrium. Once the catheter positioning was complete and catheter patency verified, braided nylon sutures were placed proximal and distal to the catheter cuff to secure the catheter to the vessel and these ties were used to anchor the catheter to the surrounding fascia.

Subsequently, the subject was placed ventral side down on the surgical surface and one longitudinal incision of 20 mm in length was made 10 mm right lateral to the mid-scapular point. A second longitudinal incision of 3 mm in length was made at the mid-scapular point and the cannula pedestal was inserted subcutaneously through the dorsal incision of 20 mm in length and the upper post exposed through the dorsal incision of 3 mm in length. After, the distal end of the catheter was passed subcutaneously from the ventral incision to the dorsal incision and secured to the cannula pedestal and catheter patency verified again with sterile saline. Subsequently, all incisions were closed with Michel wound clips. Catheter maintenance included a daily flush with a sterile solution of 20 mg/kg amoxicillin, 10 mg/kg sublactam, 250 units/ml of heparin sodium in a solution of 75 percent saline, 25 percent glycerol by volume. Catheter patency was verified at periodic intervals with an intravenous administration of 7.5 mg/kg ketamine solution and confirmed by the presence immediate onset sedation.

3.4 SELF-ADMINISTRATION PROCEDURE

On Day 5, subjects began self-administration testing conducted in standard operant chambers housed within isolated and ventilated enclosures (Med Associates, Saint Albans, VT). Each chamber was equipped with two response levers, a white stimulus light above each lever, and a five-watt chamber light. Before each session, infusion tubing, protected by a stainless steel spring tether (Plastics One, Roanoke, VA), was connected to the upper post of the implanted cannula pedestal and the tether secured. Subsequently, infusions were delivered via a peristaltic pump located outside of each enclosure. Control of the schedule parameters was performed with MED-PC IV software and hardware (Med Associates, Saint Albans, VT). Subjects were transported from the vivarium to the laboratory each day and allowed to acclimate for 15 to 30

minutes prior to testing. In acquisition and maintenance sessions, each lever press on the designated active lever (right lever), resulted in a 3-second infusion of 0.1-ml of 0.03 mg/kg oxycodone solution and activation of the white stimulus light above the right lever. In this regard, the white stimulus light paired with the delivery of oxycodone served as a conditioned cue. Furthermore, the chamber light served as a discriminative stimulus signaling the availability of the reinforcer. A 60-second timeout was imposed following each infusion during which the chamber light was inactivated, and depression of the active lever was recorded but did not result in infusion delivery. In these sessions, all lever-pressing behavior on the inactive lever (left lever) was recorded, but had no programmed consequences. Subjects' self-administration behavior met acquisition criteria when the number of responses emitted was greater than or equal to 15 on the active lever, and the number of responses on the active lever was greater than on the inactive lever for three consecutive sessions. Once a subject met acquisition criteria, they continued testing in self-administration sessions until they met stable maintenance criteria. Stable performance was defined as a period of three consecutive days during which the daily mean number of infusions did not differ from the mean number of infusions by more than 25 percent and no trends of increasing or decreasing behavioral performance were evident.

Once subjects' behavior met stable maintenance criteria, subjects were tested in extinction sessions during which responses on the active lever had no programmed consequences. In other words, responding did not result in the delivery of oxycodone or presentation of oxycodone-associated cues, such as the white stimulus light. Subjects continued testing in extinction sessions until self-administration behavior met extinction criteria. Extinction criteria was defined as three consecutive days with response levels less than 50 percent of the level of responses during stable maintenance performance.

Once subjects' behavior met extinction criteria, subjects were tested in reinstatement sessions for oxycodone prime-induced reinstatement (0.3, 1 mg/kg) or oxycodone-associated cue-induced reinstatement. In prime-induced reinstatement sessions, conditions were identical to

those conditions in the extinction sessions, however, 15 minutes prior to the session, a single non-contingent injection of 0.3 or 1 mg/kg, subcutaneous (SC) oxycodone was delivered by the experimenter. In cue-induced reinstatement sessions, conditions were similar to those conditions in extinction sessions, however, depression of the designated active lever resulted in the illumination of the white stimulus light above the designated active lever. Subjects' testing for reinstatement of lever-pressing behavior was counterbalanced for order, and subjects resumed daily extinction sessions for at least three consecutive days and until performance met extinction criteria between reinstatement tests.

Statistical analysis was performed on data collected including responses on the designated active lever, responses on the designated inactive lever, and responses during the timeout period. Significant main effects were determined with a Two-Way ANOVA with between subject factors of fluid percussion condition (injured or non-injured) and schedule parameter (maintenance, extinction, cue-induced reinstatement, or prime-induced reinstatement).

3.5 PHYSICAL DEPENDENCE PROCEDURE

3.5.1 PRECIPITATED WITHDRAWAL ASSAY

Two weeks prior to fluid percussion procedure on Day 0, subjects began training to emit lever-pressing behavior for food pellet reinforcement under a FR5 schedule in daily 100-minute sessions. Each session was comprised of five identical 20-minute trials. Each trial consisted of three components presented in the following order: a 15-minute time-out period in which the house-light was not illuminated and both left and right lever were absent; a 2-minute response period in which the house-light was illuminated, the right lever was present and completion of a FR5 on the right lever resulted in the presentation of a food pellet reinforcer; a 3-minute observation period in which the house-light remained on, but both levers were absent. Once food-reinforced lever-pressing behavior was reliably established, subjects underwent the fluid percussion injury procedure as described above, designated Day 0. On Day 6, dose effect curves using cumulative doses of naltrexone (0, 1, 3, 10, 20 mg/kg, SC) were determined for suppression

of food-reinforced lever-pressing behavior and production of other withdrawal signs. Each dose was administered at the beginning of every 15-minute time-out period. In the 3-minute observation period, precipitated withdrawal signs were scored as present or absent in three 1-minute intervals. Opioid withdrawal signs measured included jumping, teeth chattering, salivation, face rubbing, abdominal stretches, erection/genital grooming, wet dog shakes, ptosis, diarrhea, and lethargy.

On Day 6 at approximately 1800, following the determination of the baseline naltrexone dose response curve, subjects were surgically implantated with sterile osmotic pumps (2ML2, Alzet, Cupertino, CA). Subjects were anesthetized with 3 percent isoflurane vapor and one longitudinal incision of approximately 20 mm in length was made on the back of each subject. Blunt dissection of the fascia was performed to create sufficient space for accommodation of an osmotic pump, which was then implanted subcutaneously and the surgical site closed with Michel would clips. Subjects were divided approximately evenly into two groups: 1) a control group that received osmotic pumps charged with a solution of sterile saline, and 2) a treatment group that received osmotic pumps charged with a solution of oxycodone. A solution of oxycodone was made to a concentration that allowed for the continuous and non-contingent delivery of 12 mg/kg/day (6x the ED80 value determined in an acute model of antinociception using a tail immersion assay) at a rate of 5 microliters per hour for a total of 14 days. On Day 11, dose effect curves using cumulative doses of naltrexone (0, 0.03, 0.1, 0.3, 1, 3, 10, 20 mg/kg, SC) were re-determined to assess changes in food-reinforced responding and withdrawal scores following continuous treatment with saline or oxycodone.

Statistical analysis was performed on data collected including responses on the active lever for food pellet reinforcer and mean composite withdrawal scores as assessed by the experimenter. Significant main effects were determined with a Two-Way ANOVA with between subject factors of fluid percussion condition (injured or non-injured) and osmotic pump treatment condition (continuous oxycodone or continuous saline).

3.5.2 SPONTANEOUS WITHDRAWAL ASSAY

On Day 12, subjects began training to emit lever-pressing behavior for food pellet reinforcement under an FR5 schedule in three daily 30-minute, single-trial operant sessions. Each session consisted of three components presented in the following order: a 15-minute time-out period in which the house-light was not illuminated and both levers were absent; a 5-minute response period in which the house-light was illuminated, the right lever was present, and completion of a FR5 on the right lever resulted in the presentation of a food pellet reinforcer; and a 10-minute observation period in which the house-light was on, but both levers were absent. On Day 15 or 16, once food-reinforced behavior was reliably established, changes in food-reinforced behavior and other withdrawal signs were assessed as previously described at approximately 0600, 1200, and 1800.

On Day 16 at approximately 2400, subjects were anesthetized with 4 percent isoflurane vapor and one latitudinal incision of approximately 20 mm in length was made on the back of each subject. Subsequently, the implanted osmotic pumps were removed and the surgical site closed with Michel surgical clips. On Day 17, changes in food-reinforced responding and other withdrawal signs were again assessed as previously described three times per day at approximately 0600, 1200, and 1800 for 60 hours.

Statistical analysis was performed on data collected including responses on the designated active lever for food pellet reinforcer, and mean composite withdrawal scores as assessed by the experimenter. Significant main effects were determined with a Three-Way ANOVA with between subject factors of fluid percussion condition (injured or non-injured) and osmotic pump treatment condition (continuous oxycodone or continuous saline), and time (18, 12, 6 hours prior to pump removal; 6, 12, 18, 30, 36, 42, 54, and 60 hours post pump removal).

3.5.2.1 LOCOMOTOR ACTIVITY ASSESSMENT

On Day 15 or 16, subjects were placed in an open field chamber (41cm X 41 cm X 20 cm) equipped with 16 photobeam cells (ENV15, Med Associates, Saint Albans, VT) and allowed to

ambulate freely for 30 minute, twice per day at approximately 0900 and 1500. On Day 17, subjects were assessed for changes in locomotion during 30-minute sessions at approximately 0900 and 1500 and testing was repeated throughout the 60-hour spontaneous withdrawal assessment. Distance traveled was determined based on photobeam breaks and was recorded and analyzed using MedPC software (Med Associates, Saint Albans, VT).

Statistical analysis was performed on data collected including the total distance traveled by each subject. Significant main effects were determined with a Three-Way ANOVA with between subject factors of fluid percussion condition (injured or non-injured) and osmotic pump treatment condition (continuous oxycodone or continuous saline), and time (18, 12, 6 hours prior to pump removal; 6, 12, 18, 30, 36, 42, 54, and 60 hours post pump removal).

CHAPTER 4: RESULTS

4.1 SELF-ADMINISTRATION PROCEDURE

For those subjects tested in the self-administration procedure, brain-injured subjects (n = 14; 606.73 ± 26.31), relative to non-injured subjects (n = 9; 288.22 ± 18.61), required significantly more time to restore the righting reflex; $t(22) = 8.586$, $p < 0.001$. (**FIGURE 1**). In oxycodone-reinforced sessions, brain-injured subjects (10.93 ± 0.78) showed no significant difference in the mean number of sessions required to meet criteria for stable maintenance for lever-pressing behavior relative to non-injured subjects (16.44 ± 3.14); $t(9.010) = -1.705$, $p = 0.122$ (**FIGURE 2**). In non-reinforced extinction sessions, brain-injured subjects (10.67 ± 1.38) showed no significant difference in the mean number of sessions required to meet criteria for extinction of lever-pressing behavior relative to non-injured subjects (6.56 ± 1.14); $t(22) = 2.059$, $p = 0.052$ (**FIGURE 3**). During stable maintenance of oxycodone-reinforced lever-pressing behavior, brain-injured subjects (22.29 ± 1.50) emitted fewer oxycodone-reinforced lever presses than did non-injured subjects (28.19 ± 8.66) [$F(1, 155) = 20.102$, $P < 0.001$] (**FIGURE 4**). During extinction of lever-pressing behavior, there were no significant differences in active lever responding between brain-injured subjects and non-injured subjects (**FIGURE 4**).

Once extinction criteria for lever-pressing behavior were met, all subjects were tested for oxycodone-associated cue- and oxycodone prime-induced reinstatement (1 mg/kg, SC). A subset of all subjects (n = 4, brain-injured; n = 6, non-injured), were also tested for oxycodone prime-induced reinstatement with 0.3 mg/kg, SC oxycodone administered prior to session start. In reinstatement test sessions, brain-injured subjects lever-pressing behavior under oxycodone-associated cue- and oxycodone prime-induced conditions showed no significant differences relative to lever-pressing behavior during extinction sessions (**FIGURE 4**). However, non-injured subjects' lever-pressing behavior (18.22 ± 3.96) showed significant increases relative to lever-pressing behavior in extinction sessions (10.30 ± 3.64) under oxycodone-associated cue-induced reinstatement conditions, but not oxycodone prime-induced reinstatement conditions [$F(1, 155)$

= 16.627, $p < 0.001$] (**FIGURE 4**). In these same tests, while brain-injured subjects showed no significant differences in inactive lever responding from non-injured subjects during acquisition sessions, brain-injured subjects emitted significantly fewer inactive lever presses during extinction sessions (7.57 ± 1.55) than did the non-injured subjects (11.04 ± 2.65) [$F (1, 155) = 5.261$, $p < 0.05$] (**FIGURE 5**). However, both brain-injured subjects (1.93 ± 0.67) and non-injured subjects (6.00 ± 2.74) showed decreases in inactive lever-pressing behavior during tests for oxycodone prime-induced reinstatement (1 mg/kg, SC) relative to lever-pressing behavior during extinction sessions [$F (1, 155) = 3.744$, $p < 0.05$] (**FIGURE 5**). During timeouts, there were no significant differences in lever-pressing behavior between brain-injured subjects and non-injured subjects in acquisition sessions, extinction sessions, or oxycodone prime-induced reinstatement sessions (**FIGURE 6**). However, in test sessions for oxycodone-associated cue-induced reinstatement, non-injured subjects showed an increase in lever-pressing behavior (52.67 ± 38.89) emitted during timeouts which was significantly greater than their timeout responding in acquisition sessions (37.93 ± 13.10) and extinction sessions (13.89 ± 4.85) [$F (2, 155) = 9.308$, $p < 0.001$] (**FIGURE 6**).

In the subset of subjects tested for oxycodone prime-induced reinstatement (0.3 mg/kg, SC), during oxycodone maintenance, brain-injured subjects' lever-pressing behavior on the active lever (20.83 ± 1.52) was significantly less than lever-pressing behavior of non-injured subjects on the active lever (29.22 ± 3.36) [$F (1, 64) = 3.744$, $p < 0.05$] (**FIGURE 7**). However, during extinction sessions, there were no significant differences in active lever responding between brain-injured and non-injured subjects (**FIGURE 7**). In the reinstatement test sessions, brain-injured subjects showed lever-pressing behavior on the active lever (9.25 ± 1.31) that was similar to lever-pressing behavior during extinction sessions (7.75 ± 0.61), while non-injured subjects showed a significant increase in lever-pressing behavior on the active lever (19.83 ± 6.32) relative to lever-pressing behavior during extinction sessions (10.94 ± 1.21) [$F (2, 64) = 36.48$, $p < 0.001$] (**FIGURE 7**). In these same tests, there was no significant difference in inactive lever-pressing behavior

regardless of the schedule parameters or injury condition (**FIGURE 8**). During timeouts, brain-injured subjects showed decreases in lever-pressing behavior relative to lever-pressing behavior by non-injured subjects regardless of the schedule parameters, however this difference between brain-injured subjects (15 ± 3.97) and non-injured subjects (34.94 ± 17.27) was only significant during acquisition when oxycodone served to reinforce lever-pressing behavior [$F(1, 64) = 4.860$, $p < 0.05$] (**FIGURE 9**).

4.2 PHYSICAL DEPENDENCE PROCEDURE

4.2.1 PRECIPITATED WITHDRAWAL ASSAY

For those subjects tested in the physical dependence procedure, brain-injured subjects (n = 22; 693.00 ± 39.38), relative to non-injured subjects (n = 15; 279.13 ± 11.32) required significantly more time to restore the righting reflex; $t(24.367) = 10.100$, $p < 0.001$ (**FIGURE 10**). On Day 6, prior to pump implantation, the mean withdrawal scores observed in response to challenge with naltrexone approached a value of zero for both brain-injured subjects and non-injured subjects with no significant differences observed across injury condition (**FIGURE 11, 12, 13, 14**). Similarly, there were no significant differences in mean withdrawal scores observed in response to challenge with naltrexone between brain-injured and non-injured subjects after treatment with continuously delivered oxycodone (**FIGURE 11**). However, the mean withdrawal scores observed for subjects of both injury conditions were significantly elevated when challenged with naltrexone after treatment with continuously delivered oxycodone relative to when challenged with vehicle [$F(4,65) = 23.300$, $p < 0.001$] (**FIGURES 11, 13, 14**). In brain injured and non-injured subjects treated with continuously delivered saline, there were no significant and biologically relevant differences in mean withdrawal scores when challenged with naltrexone (**FIGURE 12, 13, 14**). Overall, there were no significant differences in naltrexone-generated mean withdrawal scores between brain-injured subjects and non-injured subjects before or after treatment with continuously delivered oxycodone (**FIGURE 11**) or saline (**FIGURE 12**).

In subjects treated with continuously delivered oxycodone, there was a significant difference in the mean number of food-reinforced lever presses before treatment for brain-injured subjects and non-injured subjects when challenged with a dose of 3 mg/kg, SC naltrexone [$F(1, 75) = 5.449$, $p < 0.05$] and after treatment when challenged with the dose of 0.03 mg/kg, SC naltrexone [$F(1, 60) = 3.976$, $p < 0.05$] (**FIGURES 15, 17, 18**). However, there was no significant difference in the mean number food-reinforce lever presses between brain-injured and non-injured subjects when challenged with vehicle either before or after treatment with continuously delivered oxycodone (**FIGURE 15**). Conversely, in subjects treated with continuously delivered saline, brain-injured subjects and non-injured subjects showed significant baseline differences in the mean number of lever presses both before [$F(1, 80) = 24.530$, $p < 0.001$] and after treatment with continuously delivered oxycodone [$F(1, 80) = 7.967$, $p < 0.05$] with the non-injured subjects showing greater lever-pressing behavior (**FIGURE 16**). Moreover, non-injured subjects demonstrated greater lever-pressing behavior when challenged with naltrexone across all but the highest dose tested after treatment with continuous saline (**FIGURE 16**). In both brain-injured subjects [$F(7, 87) = 8.379$, $p < 0.001$] (**FIGURE 17**) and non-injured subjects [$F(7, 53) = 13.726$, $p < 0.001$] (**FIGURE 18**) treated with continuously delivered oxycodone, a challenge with naltrexone after treatment produced a dose-dependent (0.03, 0.1, 0.3 mg/kg, SC) attenuation in the mean number of food-reinforced lever presses relative to the mean number of food-reinforced lever presses after treatment with continuously delivered saline.

4.2.2 SPONTANEOUS WITHDRAWAL ASSAY

In the spontaneous withdrawal assay, there were no significant differences in mean withdrawal scores across time between brain-injured subjects and non-injured subjects after treatment with continuously delivered oxycodone (**FIGURE 19**) or saline (**FIGURE 20**). In brain-injured subjects treated with continuous delivered oxycodone, mean withdrawal scores were elevated at the 6 [$F(1, 207) = 31.772$, $p < 0.001$], 12 [$F(1, 207) = 75.316$, $p < 0.001$], 18 [$F(1, 207) = 32.161$, $p < 0.001$], and 36 [$F(1, 207) = 4.255$, $p < 0.05$] hour time points relative to brain-

injured subjects treated with continuously delivered saline (**FIGURE 21**). In non-injured subjects treated with continuously delivered oxycodone, mean withdrawal scores were significantly elevated at the 6 [$F(1, 143) = 23.209$, $p < 0.001$], 12 [$F(1, 143) = 21.809$, $p < 0.001$], 18 [$F(1, 143) = 41.629$, $p < 0.001$], 30 [$F(1, 143) = 6.472$), $p < 0.05$], 36 [$F(1, 143) = 4.520$, $p < 0.05$], and 42 [$F(1, 143) = 9.591$, $p < 0.05$] hour time points relative to non-injured subjects treated with continuously delivered saline (**FIGURE 22**).

In subjects treated with continuously delivered oxycodone, there were no significant differences in the mean number of food pellets earned between brain-injured or non-injured subjects at any of the time points before or after pump removal (**FIGURE 23**). Similarly, in subjects treated with continuously delivered saline, there were no significant and biologically relevant differences in the mean number of food pellets earned between brain-injured and non-injured subjects at any of the time points before or after pump removal (**FIGURE 24**). In brain-injured subjects, those treated with continuously delivered oxycodone showed significant decreases in the mean number of food pellets earned at the 6 [$F(1, 209) = 4.283$, $p < 0.05$)], 12 [$F(1, 209) = 8.558$, $p < 0.01$), and 18 [$F(1, 209) = 3.852$, $p < 0.05$] hour time points relative to those subjects treated with continuous saline (**FIGURE 25**). In non-injured subjects, those treated with continuously delivered oxycodone showed significant decreases in the mean number of food pellets earned at all time points [$F(10, 142) = 2.363$, $p < 0.05$] (**FIGURE 26**).

4.2.2.1 LOCOMOTOR ACTIVITY ASSESSMENT

In the assessment of locomotor activity, brain-injured subjects that were treated with continuously delivered oxycodone showed significant decreases in distance traveled at the 9 [$F(1, 198) = 17.431$, $p < 0.001$], 33 [$F(1, 198) = 7.543$, $p < 0.01$], and 57 [$F(1, 198) = 4.905$, $p < 0.05$] hour time points, relative to brain-injured subjects that were treated with continuously delivered saline (**FIGURE 27**). Subjects that were non-injured and treated with continuously delivered oxycodone showed significant decreases in distance traveled at the 9 [$F(1, 198) = 8.679$, $p < 0.01$], 15 [$F(1, 198) = 4.418$, $p < 0.05$], and 33 [$F(1, 198) = 10.466$, $p < 0.001$] hour

time points, relative to non-injured subjects that were treated with continuously delivered saline (**FIGURE 27**). Overall, while subjects treated with continuously delivered saline traveled significantly greater total distances relative to subjects treated with continuously delivered oxycodone, there were no significant differences in total distance traveled within treatment groups based on injury condition.

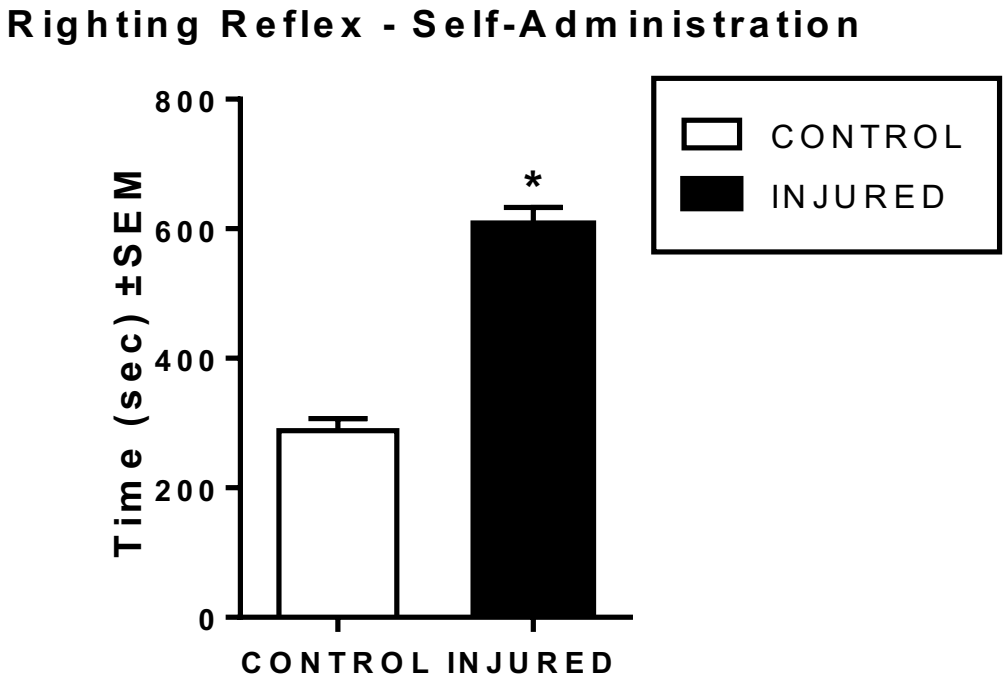

FIGURE 1. Shown are the mean times required for the return of the righting reflex in seconds (± standard error) for subjects tested in the self-administration procedure (n = 9, control; n = 14, injured). * significantly different from control, $p < 0.05$.

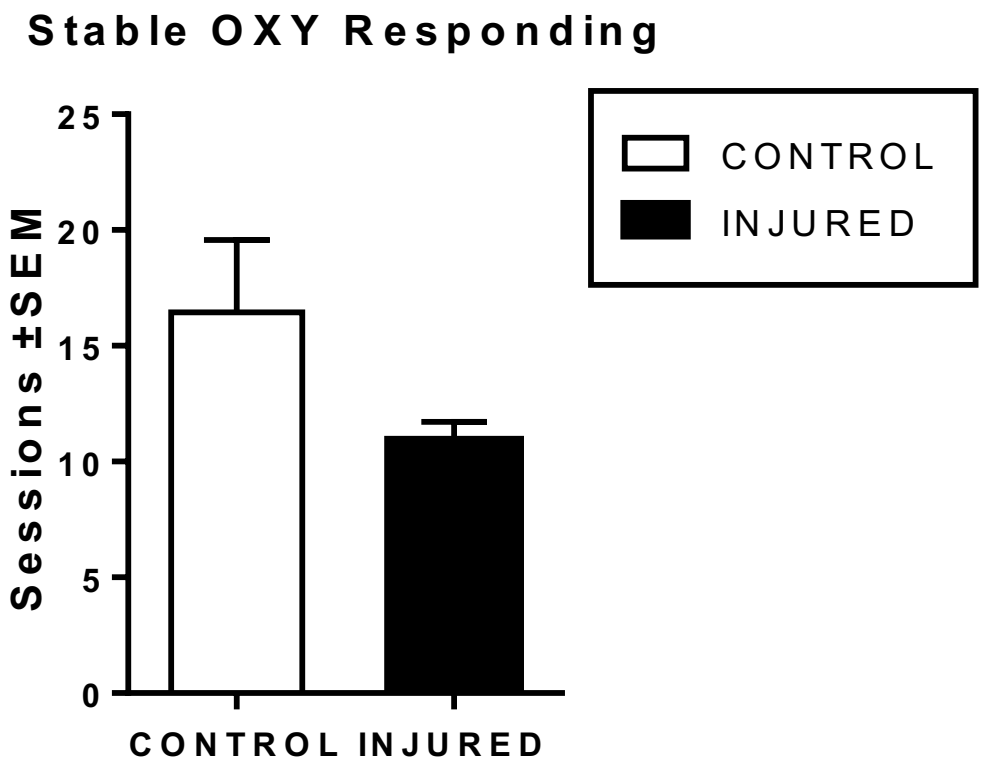

FIGURE 2. Shown are the mean number of sessions with oxycodone available (± standard error) required by subjects to meet stable maintenance criteria (n = 9, control; n = 14, injured). * significantly different from control at $p < 0.05$.

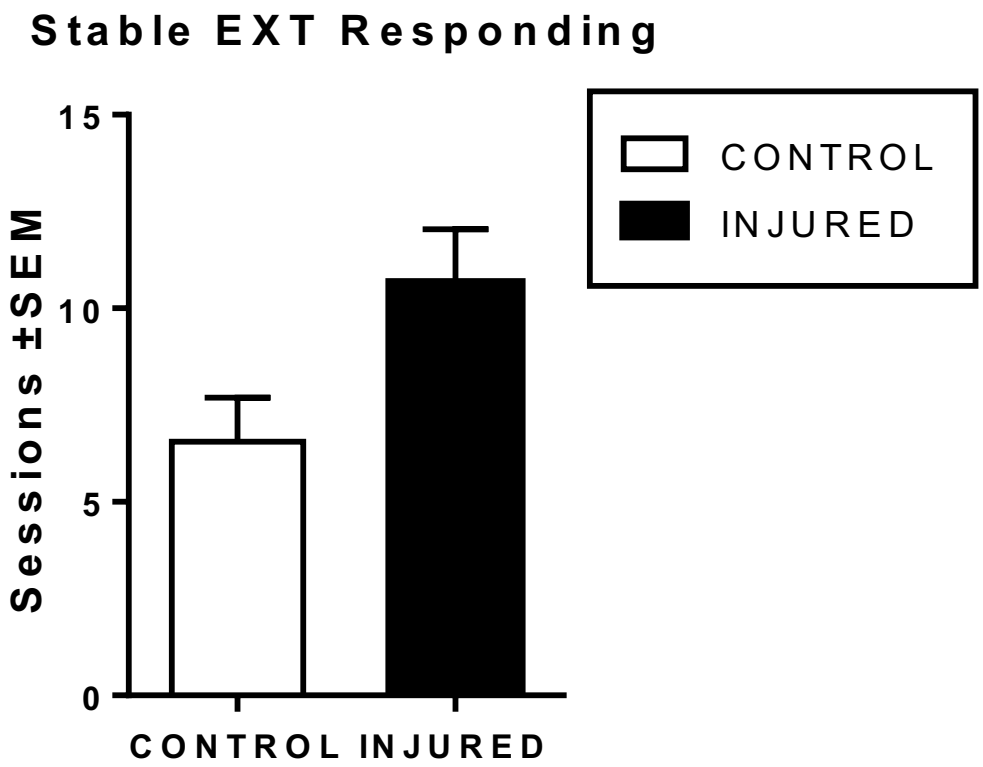

FIGURE 3. Shown are the mean number of sessions (± standard error) without oxycodone available required by subjects to meet extinction criteria (n = 9, control; n = 14, injured). * significantly different from control at $p < 0.05$.

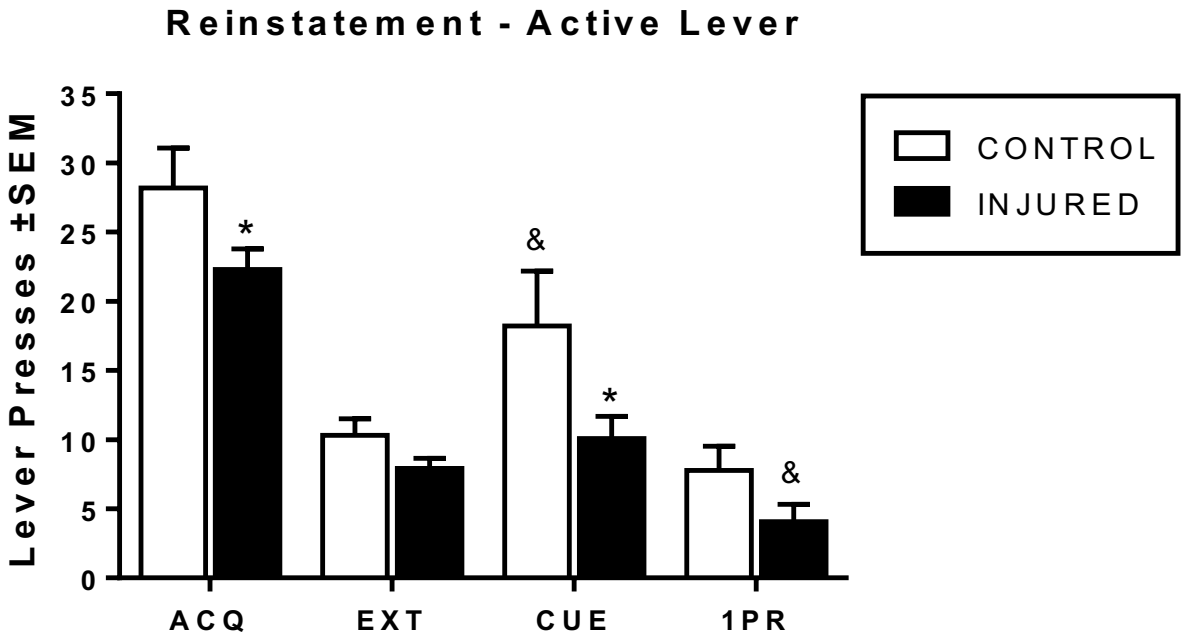

FIGURE 4. Shown are the mean number of responses on the active lever (± standard error) during FR1 oxycodone reinforced sessions (ACQ), following extinction training (EXT), during cue- induced (CUE) and 1 mg/kg SC oxycodone prime-induced reinstatement (1PR) (n = 9, control; n = 14, injured). * significantly different from control, $p < 0.05$. & significantly different from extinction baseline within injury condition, $p < 0.05$.

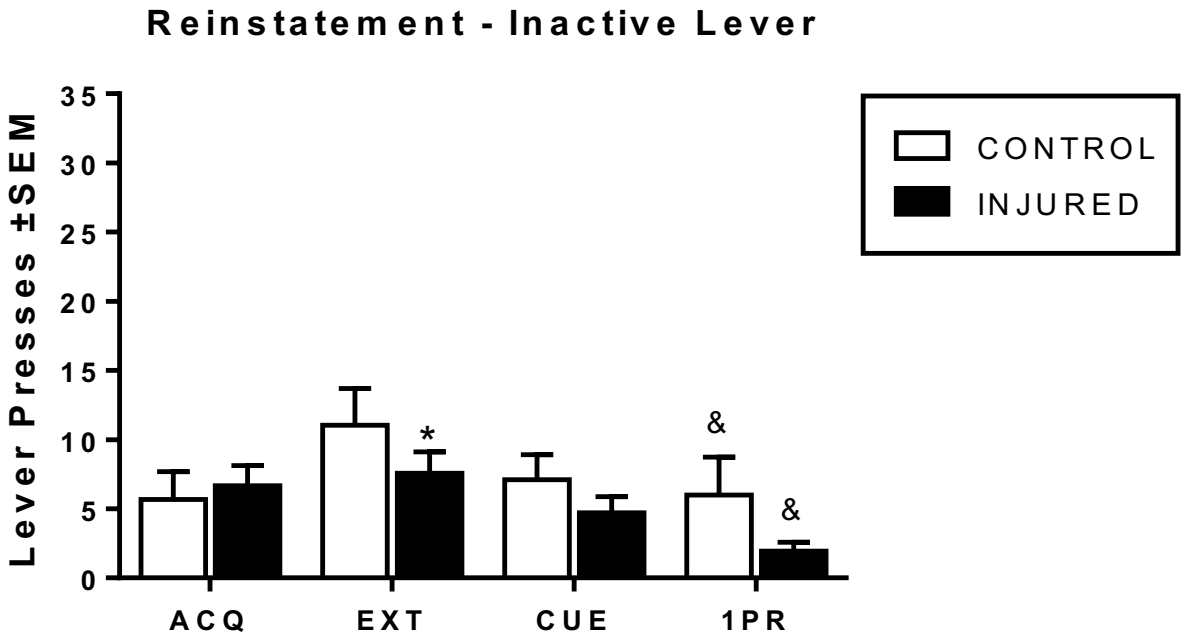

FIGURE 5. Shown are the mean number of responses on the inactive lever (± standard error) during FR1 oxycodone reinforced sessions (ACQ), following extinction training (EXT), during cue- induced (CUE) and 1 mg/kg SC oxycodone prime-induced reinstatement (1PR) (n = 9, control; n = 14, injured). * significantly different from control injury, $p < 0.05$. & significantly different from extinction baseline within injury condition, $p < 0.05$.

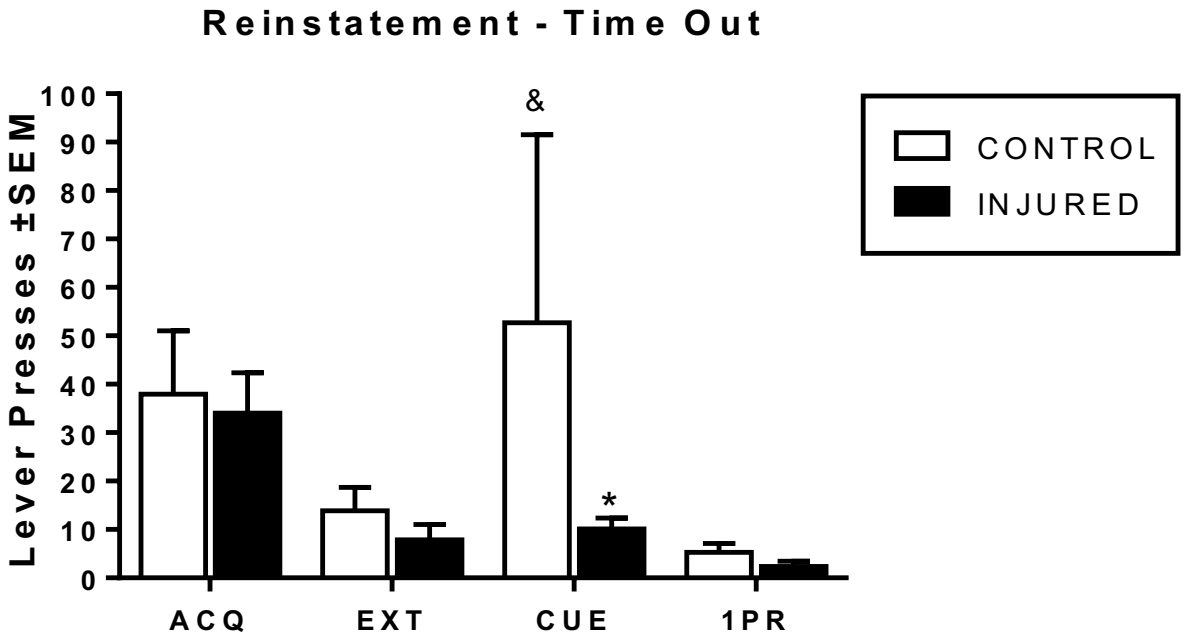

FIGURE 6. Shown are the mean number of responses in the time out (± standard error) during FR1 oxycodone reinforced sessions (ACQ), following extinction training (EXT), during cue-induced (CUE) and 1 mg/kg SC oxycodone prime-induced reinstatement (1PR) (n = 9, control; n = 14, injured). * significantly different from control injury, $p < 0.05$. & significantly different from extinction baseline within injury condition, $p < 0.05$.

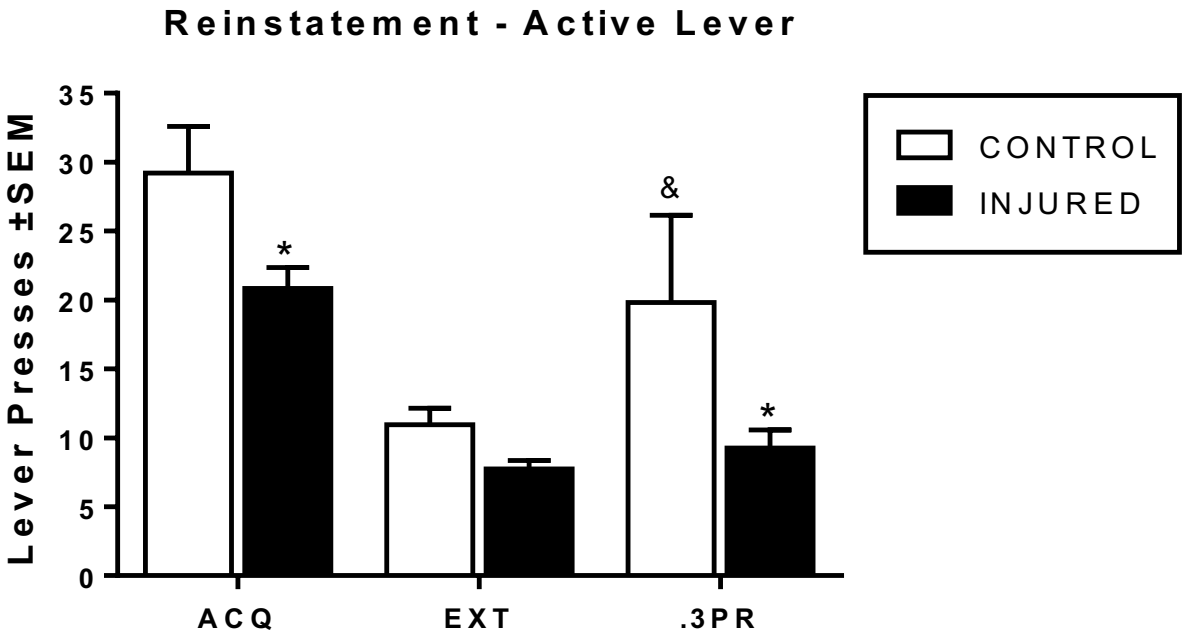

FIGURE 7. Shown are the mean number of responses on the active lever (± standard error) during FR1 oxycodone reinforced sessions (ACQ), following extinction training (EXT), and 0.3 mg/kg SC oxycodone prime-induced reinstatement (.3PR) (n = 6, control; n = 4, injured). * significantly different from control injury, $p < 0.05$. & significantly different from extinction baseline within injury condition, $p < 0.05$.

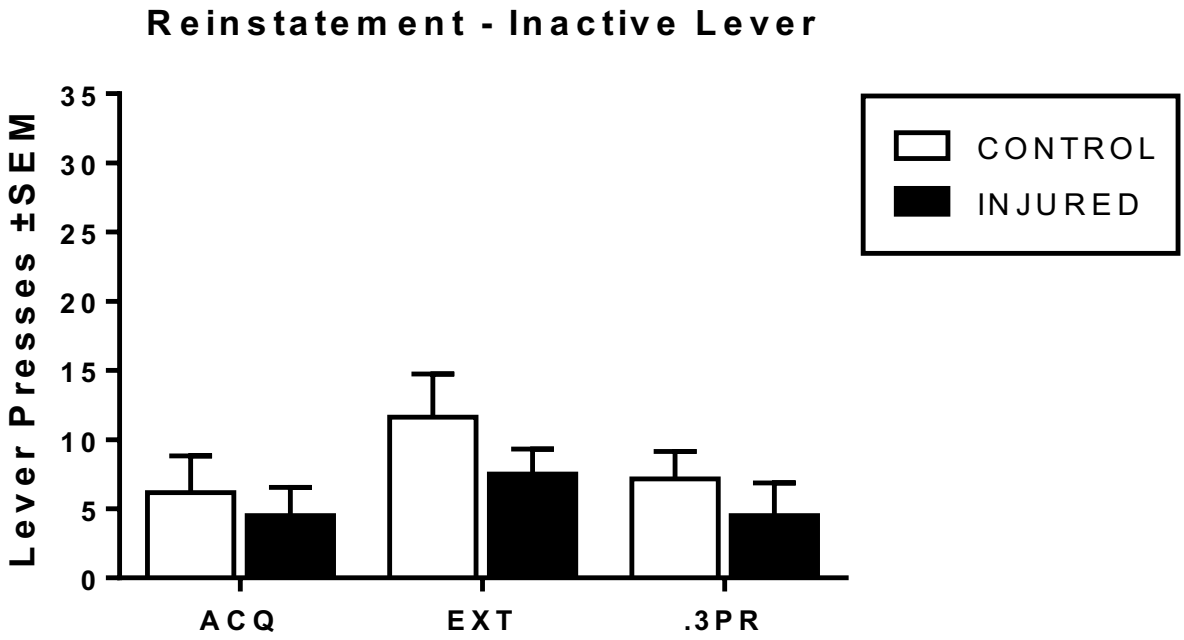

FIGURE 8. Shown are the mean number of responses on the inactive lever (± standard error) during FR1 oxycodone reinforced sessions (ACQ), following extinction training (EXT), and 0.3 mg/kg SC oxycodone prime-induced reinstatement (.3PR) for sham controls (n = 6) and brain-injured (n = 4) subjects. * significantly different from control injury, $p < 0.05$. & significantly different from extinction baseline within injury condition, $p < 0.05$.

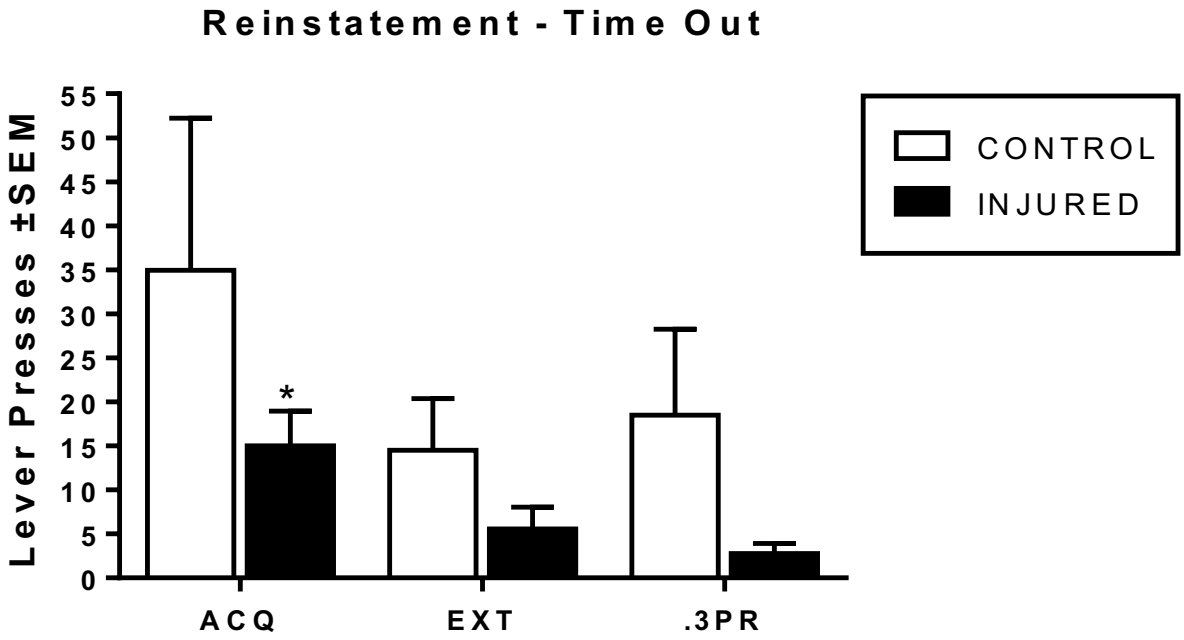

FIGURE 9. Shown are the mean number of responses in the time out (± standard error) during FR1 oxycodone reinforced sessions (ACQ), following extinction training (EXT), and 0.3 mg/kg SC oxycodone prime-induced reinstatement (.3PR) for sham controls (n = 6) and brain-injured (n = 4) subjects. * significantly different from control injury, $p < 0.05$. & significantly different from extinction baseline within injury condition, $p < 0.05$.

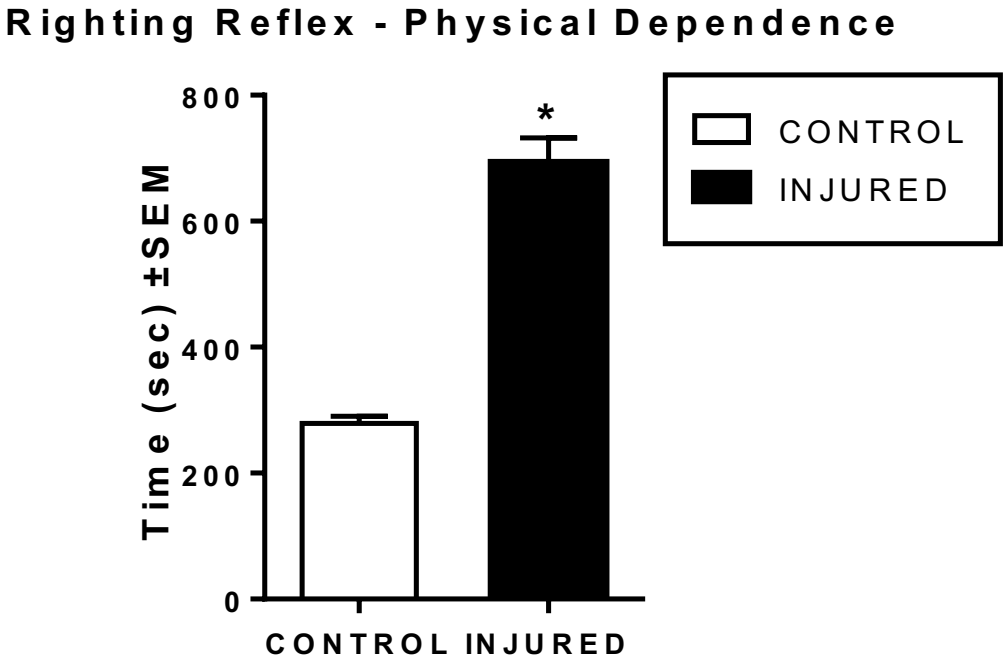

FIGURE 10. Shown are the mean times required for the return of the righting reflex righting in seconds (± standard error) for subjects tested in the physical dependence procedure (n = 15, control; n = 22, injured). * significantly different from control, $p < 0.05$.

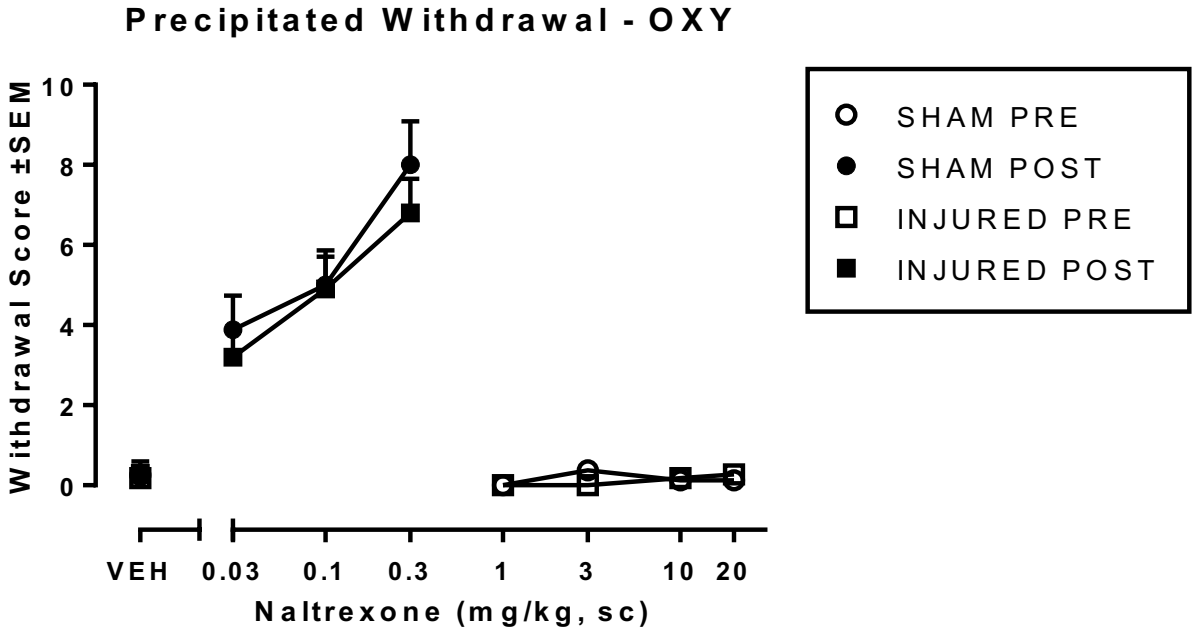

FIGURE 11. Shown are the mean withdrawal scores (± standard error) during baseline (PRE dosing curves) and precipitated withdrawal sessions (POST dosing curves) induced by 0.03, 0.1, 0.3, 1, 3, 10, and 20 mg/kg SC naltrexone for sham control (n = 7) and brain-injured subjects (n = 11) continuously delivered oxycodone 12 mg/kg/day for 5 days.

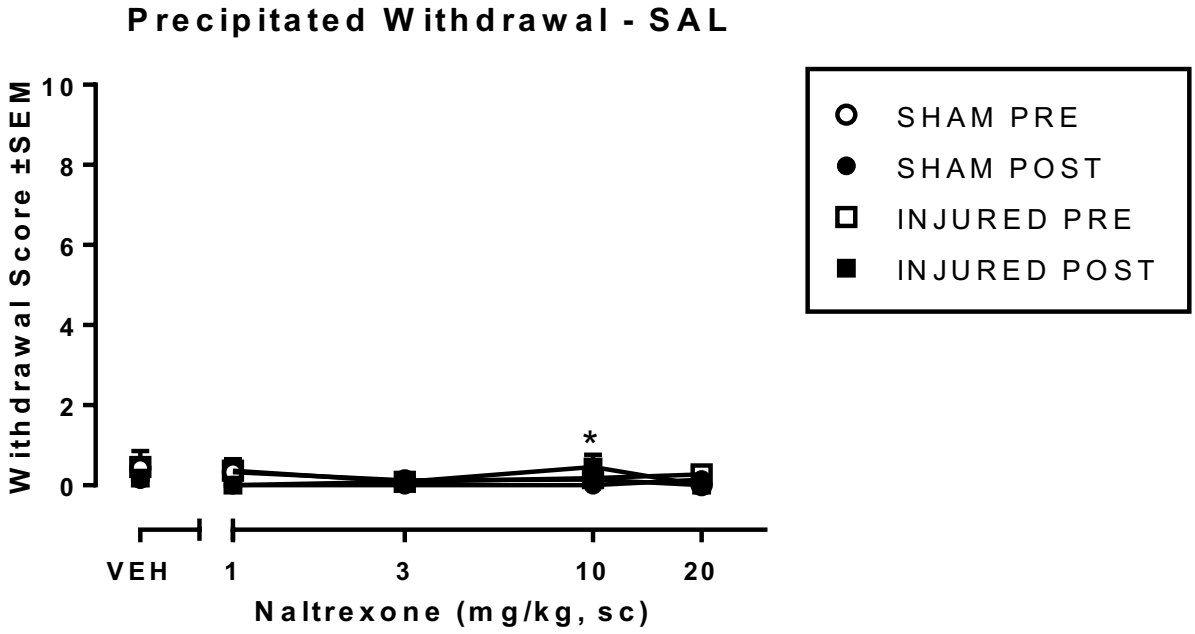

FIGURE 12. Shown are the mean withdrawal scores (± standard error) during baseline (PRE dosing curves) and precipitated withdrawal sessions (POST dosing curves) induced by 1, 3, 10, and 20 mg/kg SC naltrexone for sham control (n = 7) and brain-injured subjects (n = 11) continuously delivered saline for 5 days. * significantly different from injury control, $p < 0.05$.

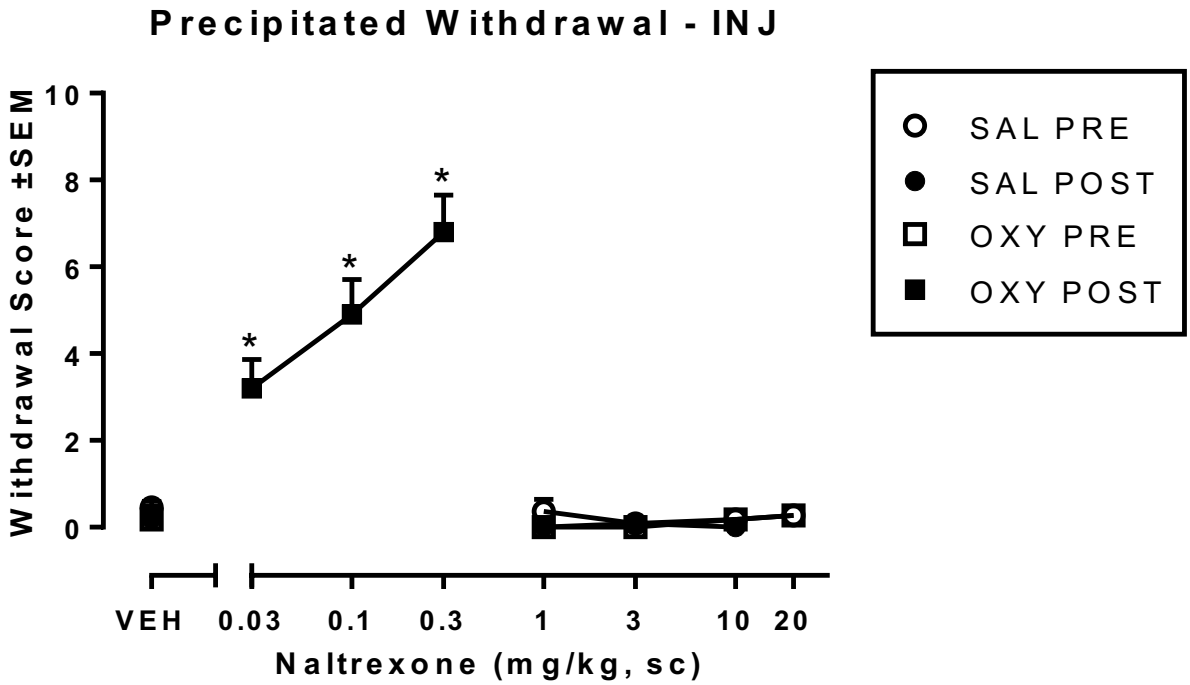

FIGURE 13. Shown are the mean withdrawal scores (± standard error) during baseline (PRE dosing curves) and precipitated withdrawal sessions (POST dosing curves) induced by 0.03, 0.1, 0.3, 1, 3, 10, and 20 mg/kg SC naltrexone for brain-injured subjects continuously delivered saline (n = 11) or oxycodone 12 mg/kg/day (n = 11) for 5 days. * significantly different from vehicle $p < 0.05$.

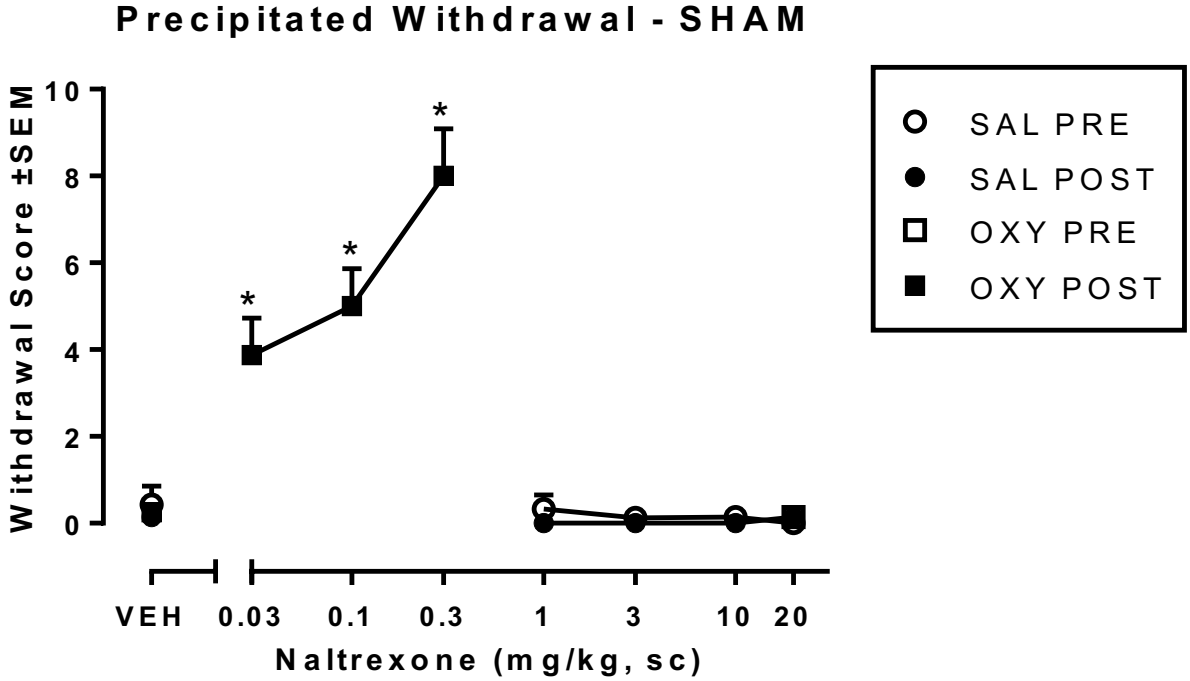

FIGURE 14. Shown are the mean withdrawal scores (± standard error) during baseline (PRE dosing curves) and precipitated withdrawal sessions (POST dosing curves) induced by 0.03, 0.1, 0.3, 1, 3, 10, and 20 mg/kg SC naltrexone for sham control subjects continuously delivered saline (n = 7) or oxycodone 12 mg/kg/day (n = 7) for 5 days. *significantly different from vehicle $p < 0.05$.

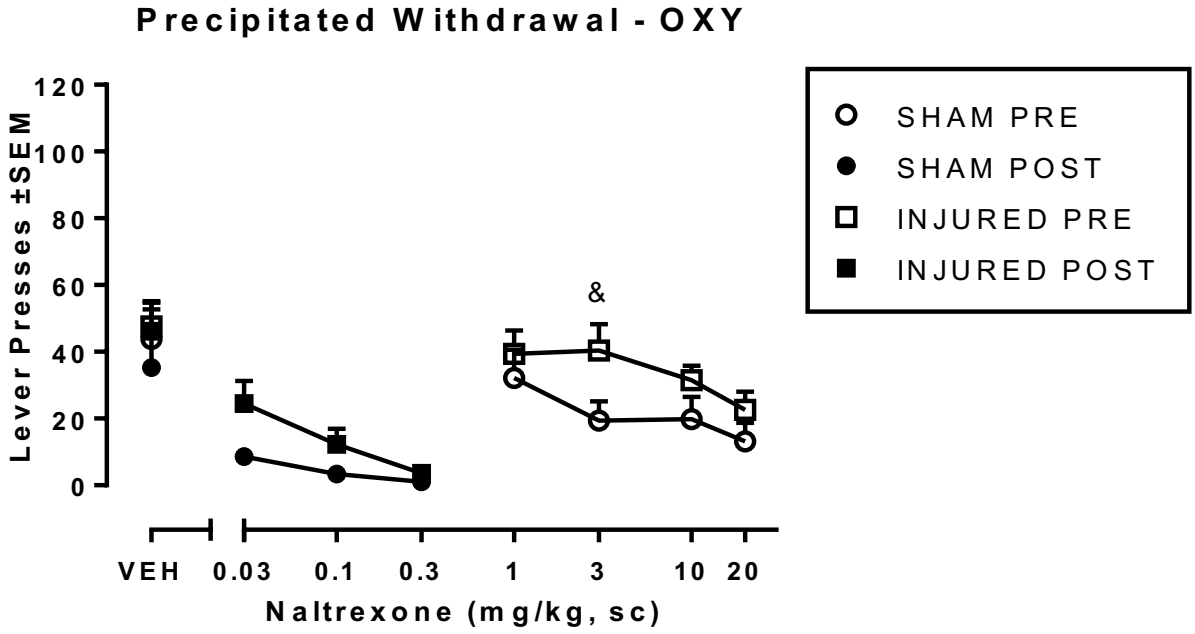

FIGURE 15. Shown are the mean number of food-reinforced lever presses emitted (± standard error) during baseline (PRE dosing curves) and precipitated withdrawal sessions (POST dosing curves) induced by 0.03, 0.1, 0.3, 1, 3, 10, and 20 mg/kg SC naltrexone for sham control (n = 7) and brain-injured (n = 11) subjects continuously delivered oxycodone 12 mg/kg/day for 5 days. * significantly different from injury control post-treatment. $p < 0.05$; & significantly different from injury control pre-treatment, $p < 0.05$.

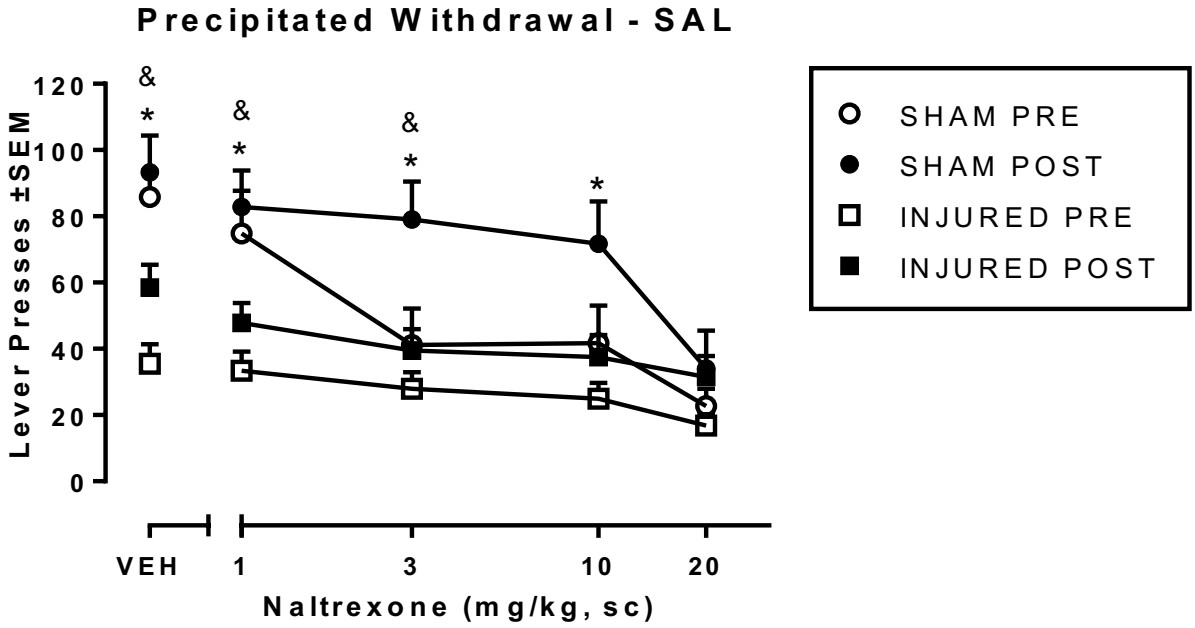

FIGURE 16. Shown are the mean number of food-reinforced lever presses emitted (± standard error) during baseline (PRE dosing curves) and precipitated withdrawal sessions (POST dosing curves) induced by 1, 3, 10, and 20 mg/kg SC naltrexone for sham control (n = 7) and brain-injured (n = 11) subjects continuously delivered saline for 5 days. * significantly different from injury control post-treatment. $p < 0.05$; & significantly different from injury control pre-treatment, $p < 0.05$.

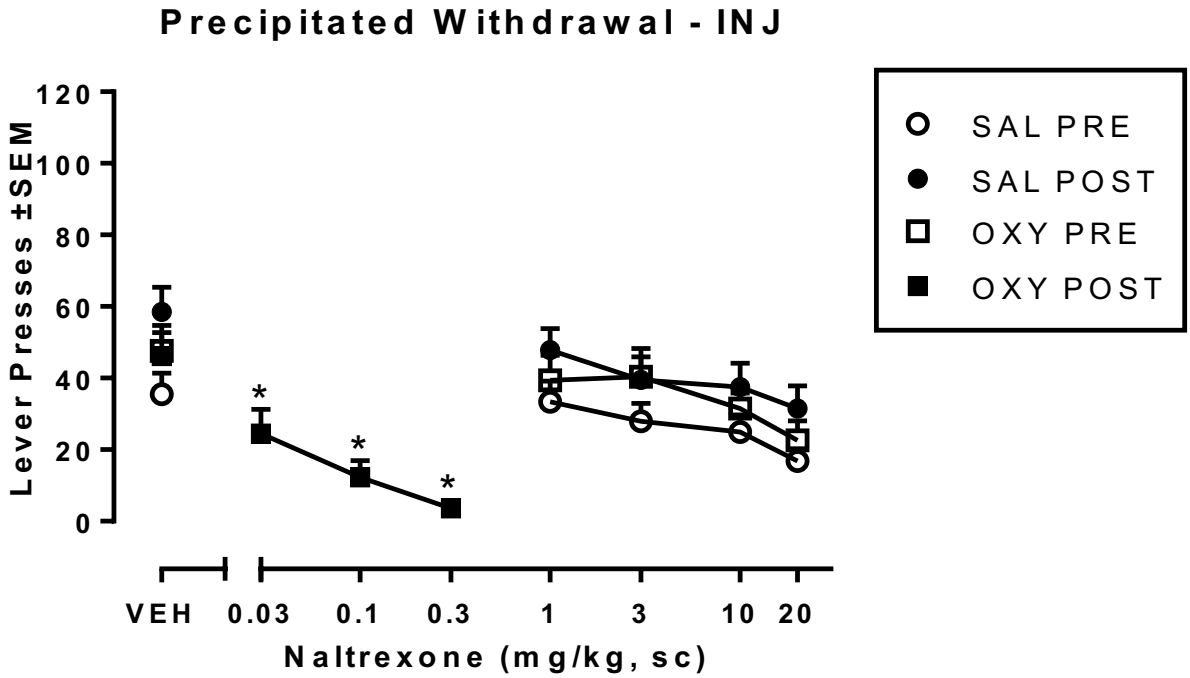

FIGURE 17. Shown are the mean number of food-reinforced lever presses emitted (± standard error) during baseline (PRE dosing curves) and precipitated withdrawal sessions (POST dosing curves) induced by 0.03, 0.1, 0.3, 1, 3, 10, and 20 mg/kg SC naltrexone both pre- and post-treatement for brain-injured subjects continuously delivered saline (n = 11) or oxycodone 12 mg/kg/day (n = 11) for 5 days. * significantly different from vehicle $p < 0.05$.

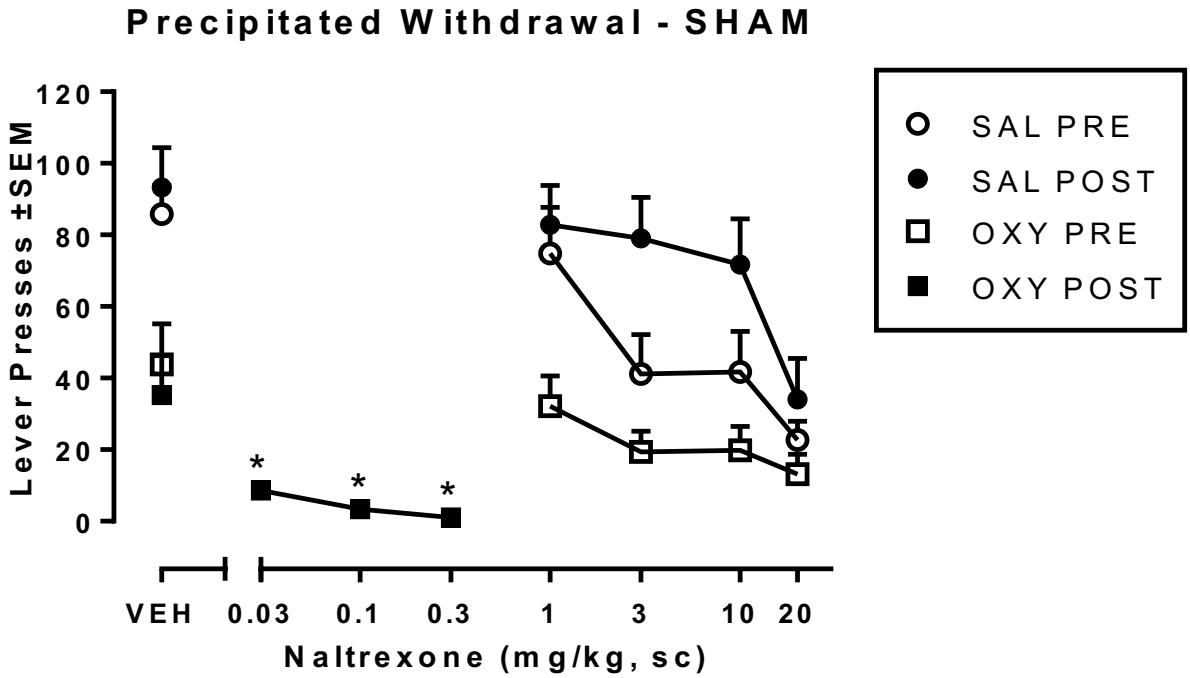

FIGURE 18. Shown are the mean number of food-reinforced lever presses emitted (± standard error) during baseline (PRE dosing curves) and precipitated withdrawal sessions (POST dosing curves) induced by 0.03, 0.1, 0.3, 1, 3, 10, and 20 mg/kg SC naltrexone both pre- and post-treatement for sham control subjects continuously delivered saline (n = 7) or oxycodone 12 mg/kg/day (n = 7) for 5 days. * significantly different from vehicle $p < 0.05$.

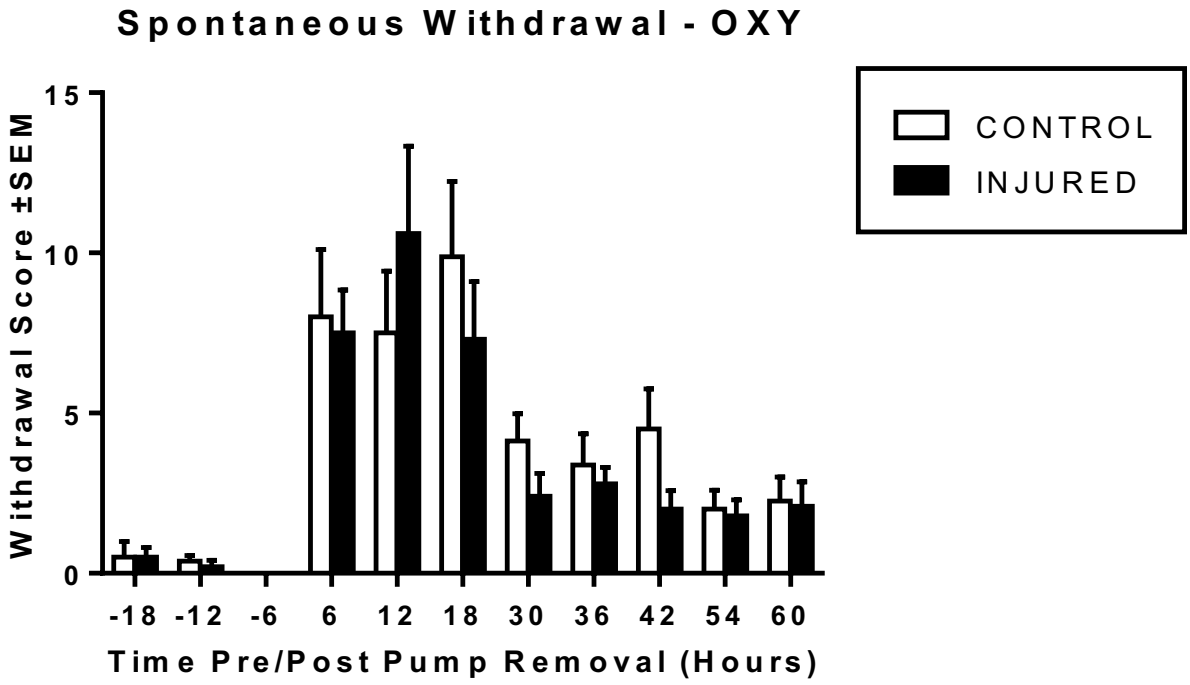

FIGURE 19. Shown are the mean withdrawal scores (± standard error) across time both pre- and post-pump removal for sham control (n = 8) and brain-injured (n = 10) subjects continuously delivered oxycodone 12 mg/kg/day for 10 days.

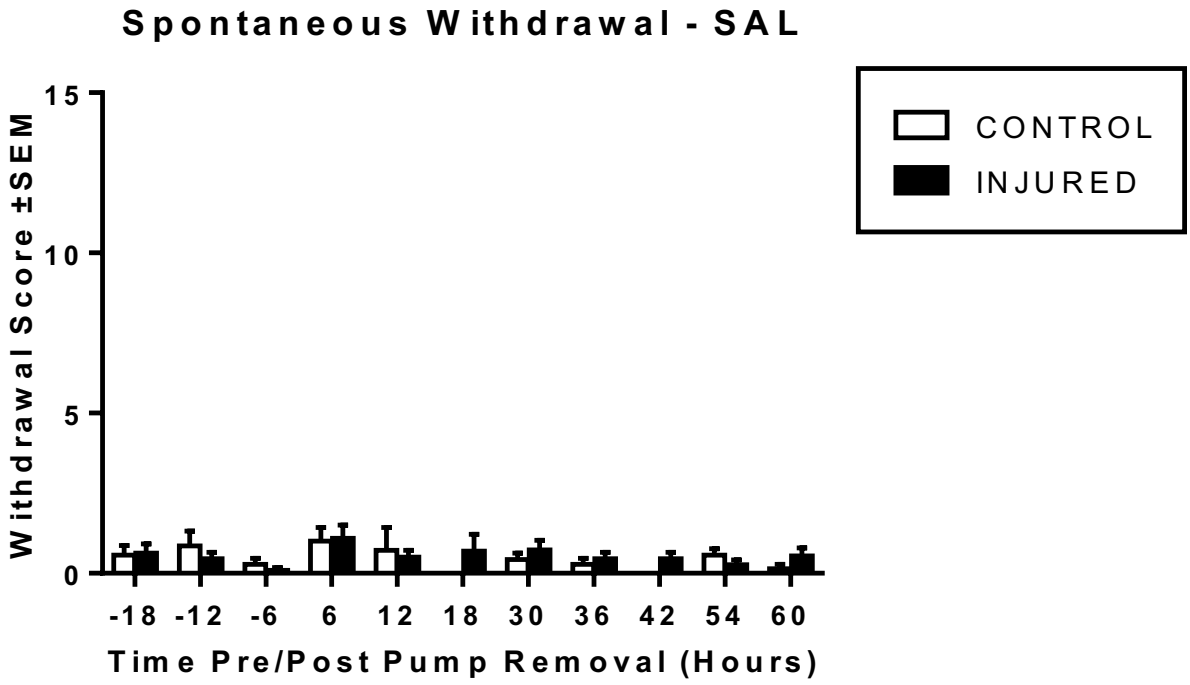

FIGURE 20. Shown are the mean withdrawal scores (± standard error) across time both pre- and post-pump removal for sham control (n = 7) and brain-injured (n = 11) subjects continuously delivered saline for 10 days.

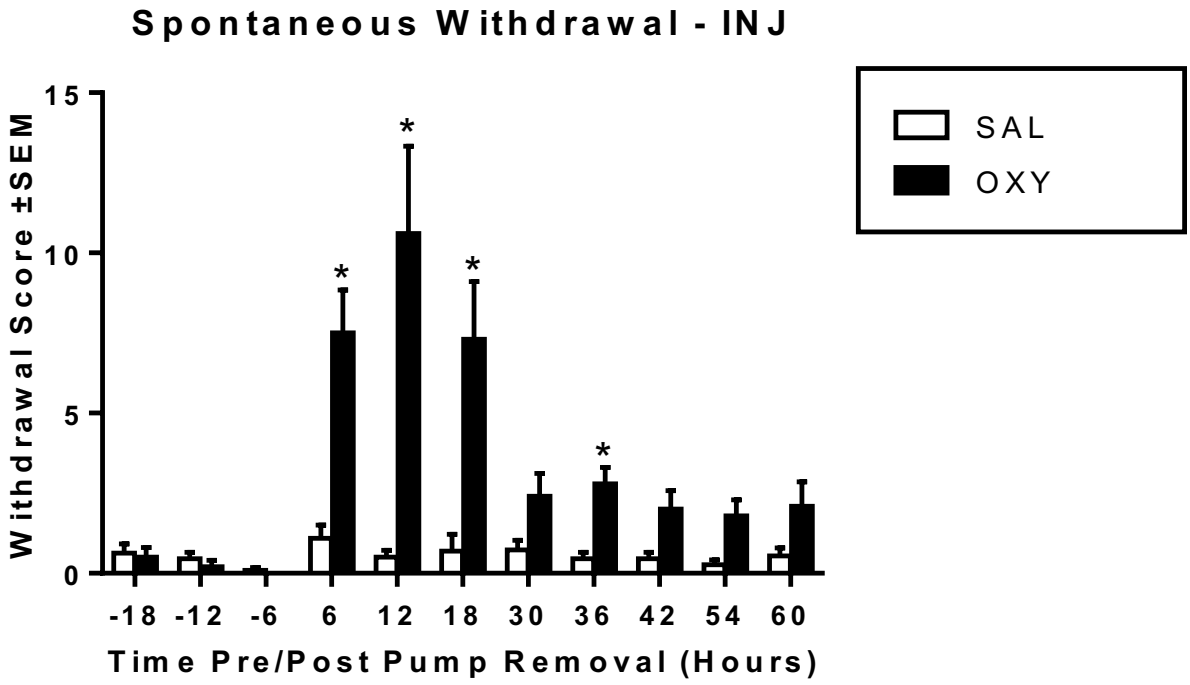

FIGURE 21. Shown are the mean withdrawal scores (± standard error) across time both pre- and post-pump removal for sham control and brain-injured subjects continuously delivered saline (n = 11) or oxycodone 12 mg/kg/day (n = 10) for 10 days. * significantly different from control $p < 0.05$.

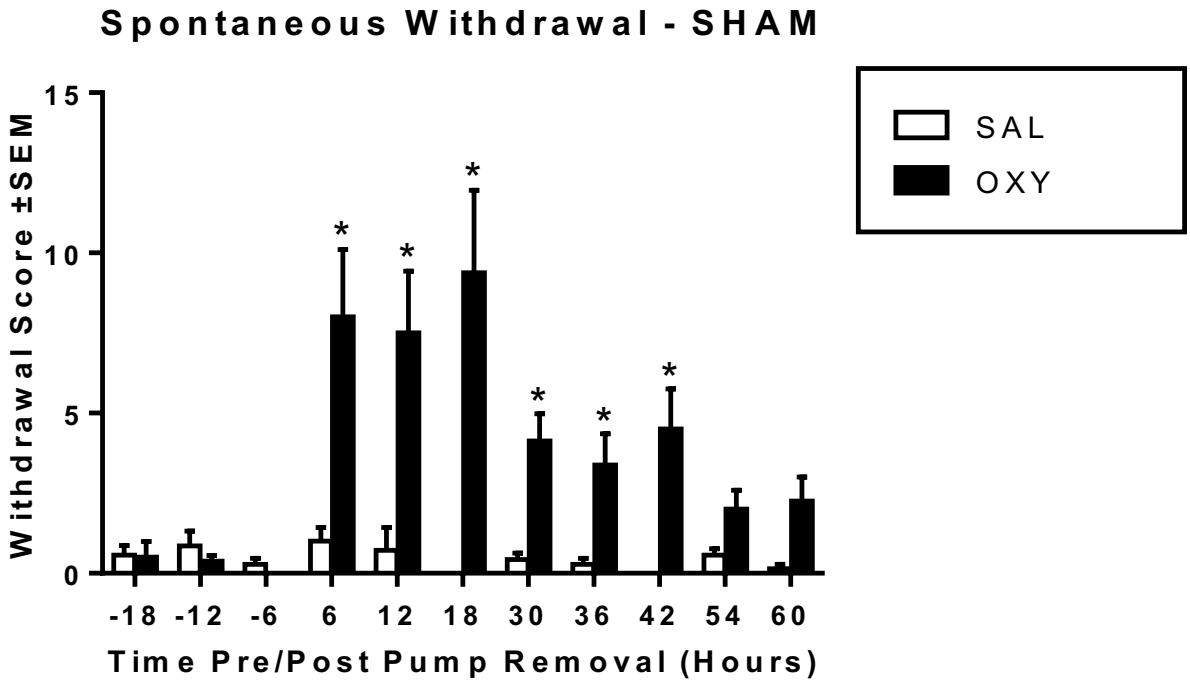

FIGURE 22. Shown are the mean withdrawal scores (± standard error) across time both pre- and post-pump removal for sham control subjects continuously delivered saline (n = 7) or oxycodone 12 mg/kg/day (n = 8) for 10 days. * significantly different from control $p < 0.05$.

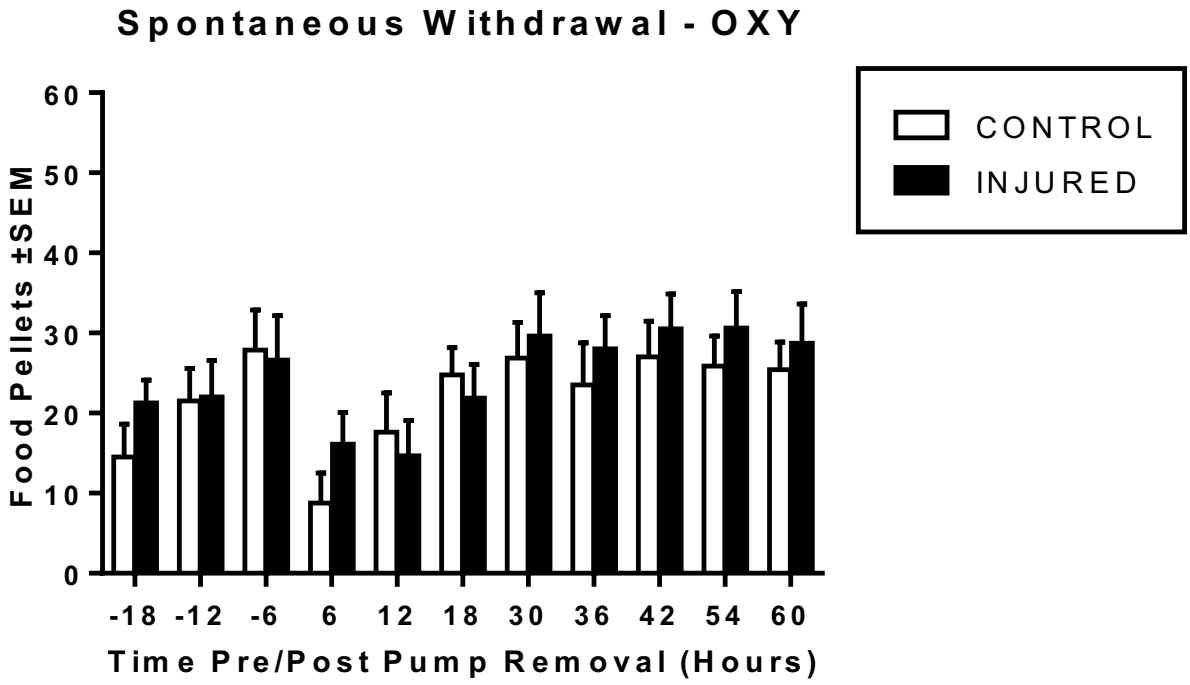

FIGURE 23. Shown are the mean number of food pellets earned (± standard error) across time both pre- and post-pump removal for sham control (n = 8) and brain-injured (n = 10) subjects continuously delivered oxycodone 12 mg/kg/day for 10 days.

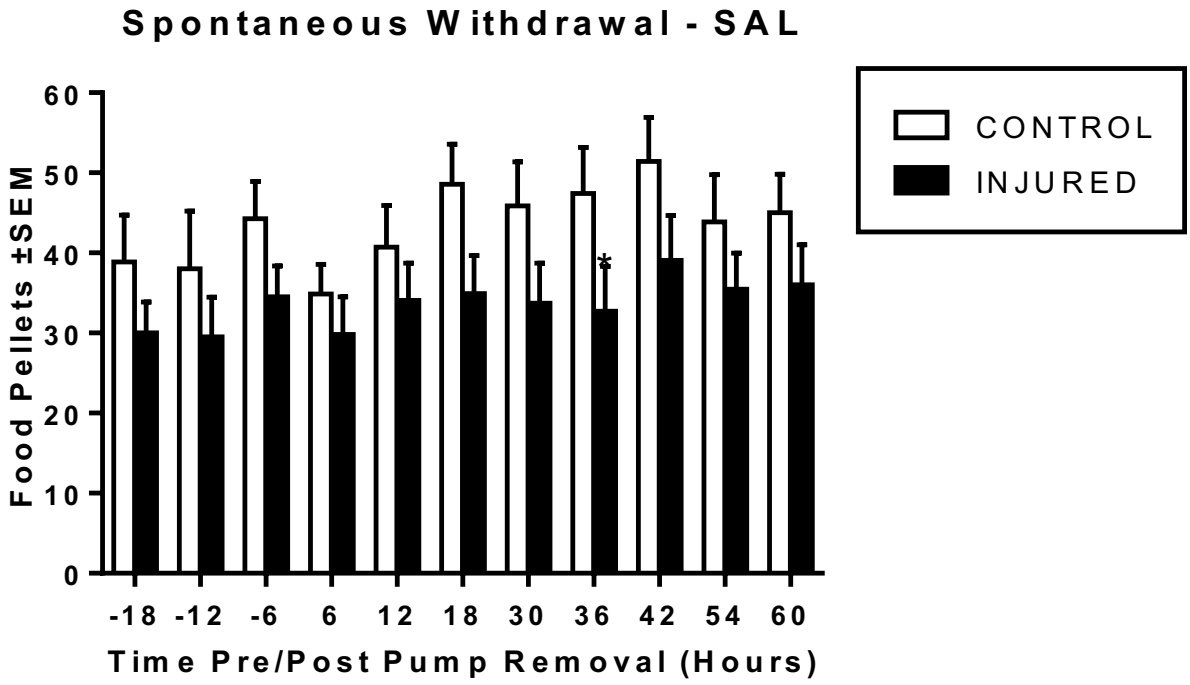

FIGURE 24. Shown are the mean number of food pellets earned (± standard error) from reinforced lever presses across time both pre- and post-pump removal for both sham control (n = 7) and brain injured (n = 11) subjects continuously delivered saline for 10 days. * significantly different from control $p < 0.05$.

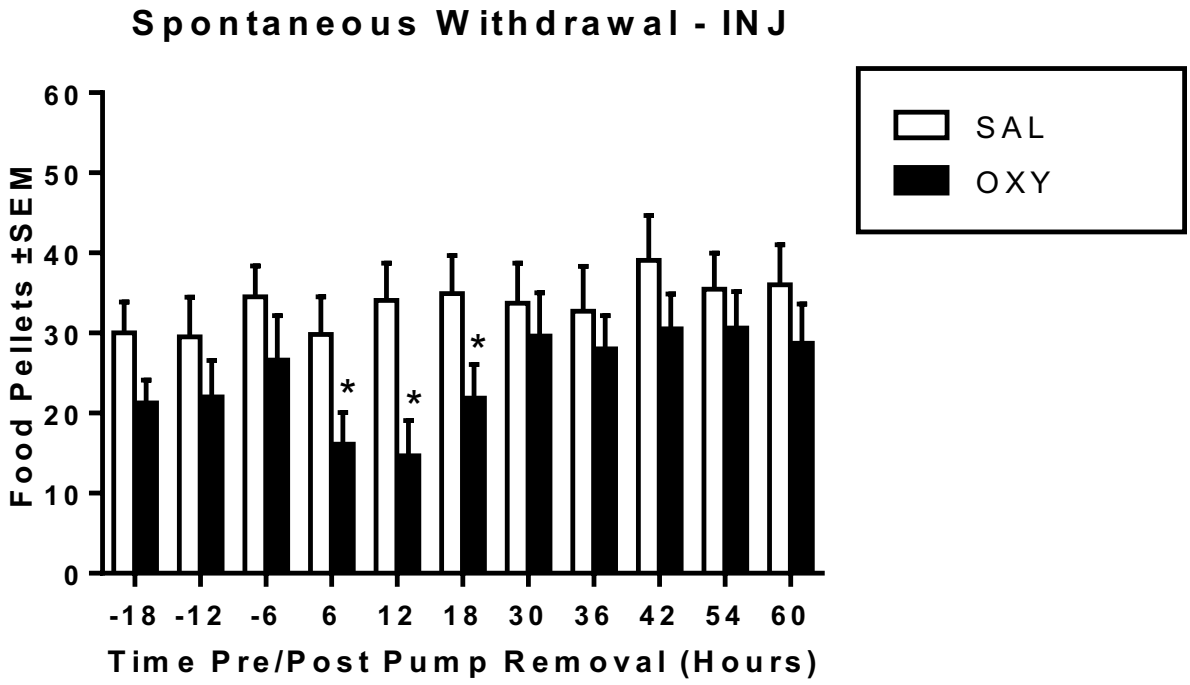

FIGURE 25. Shown are the mean number of food pellets earned (± standard error) from reinforced lever presses across time both pre- and post-pump removal for brain-injured subjects continuously delivered saline (n = 11) or oxycodone 12 mg/kg/day (n = 10) for 10 days. * significantly different from control $p < 0.05$.

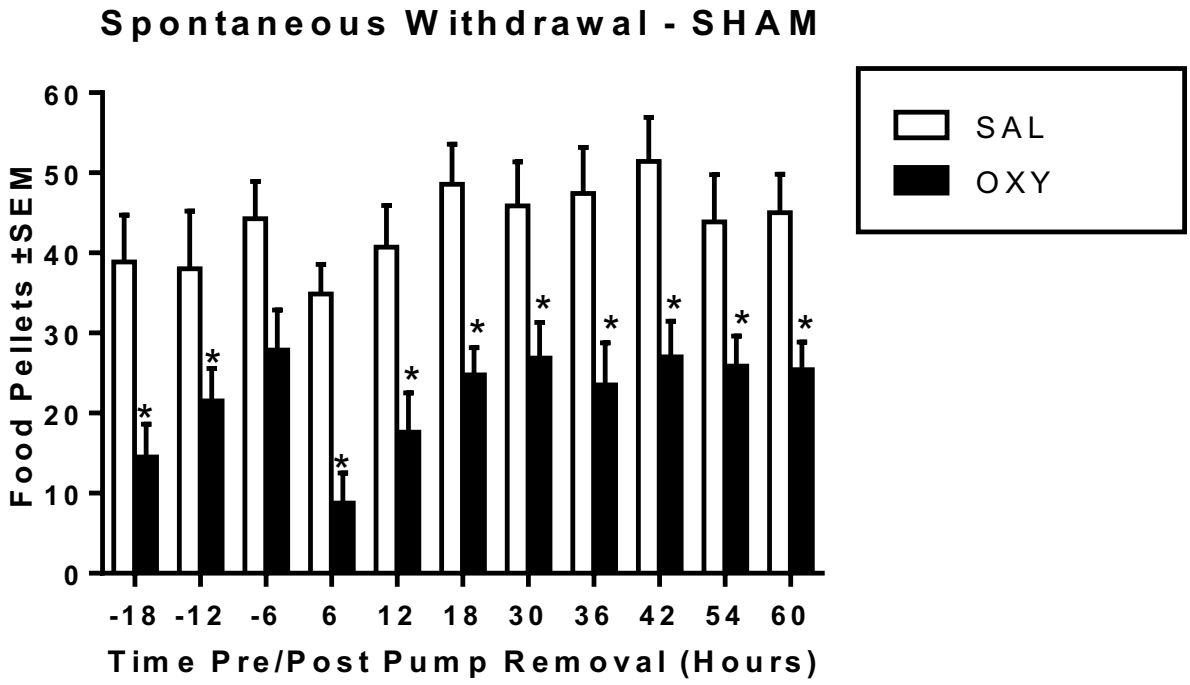

FIGURE 26. Shown are the mean number of food pellets earned (± standard error) from reinforced lever presses across time both pre- and post-pump removal for sham control subjects continuously delivered saline (n = 7) or oxycodone 12 mg/kg/day (n = 8) for 10 days. * significantly different from control $p < 0.05$.

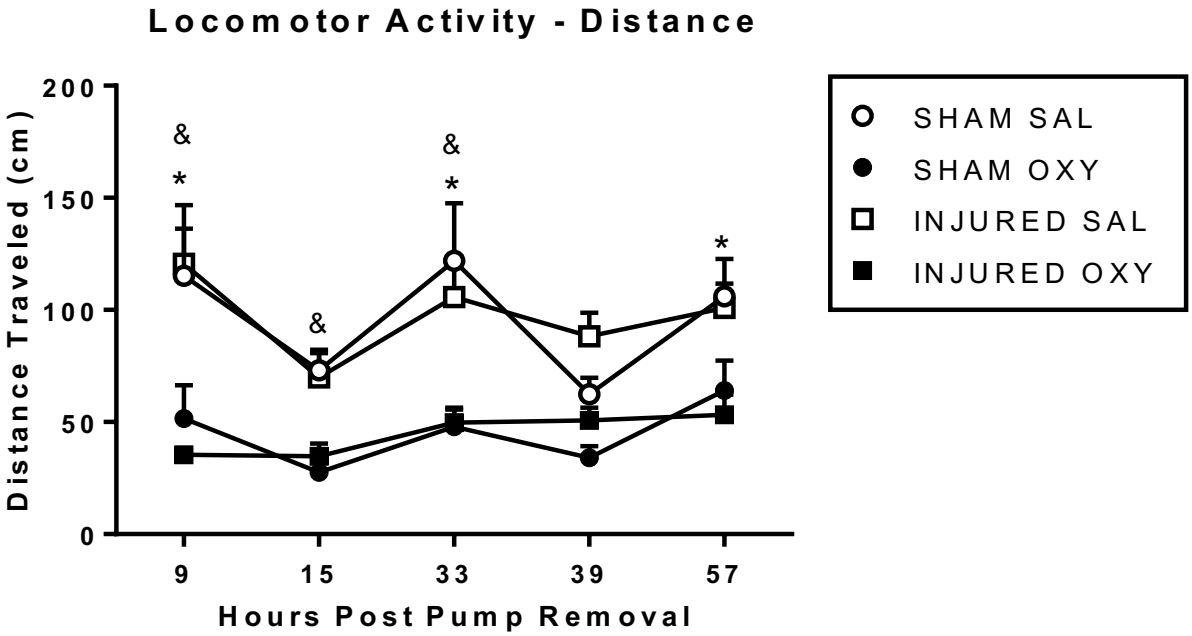

FIGURE 27. Shown is the mean distance traveled (± standard error) in the open field across time post-pump removal expressed as a percent control of the pre-pump removal baseline both sham control and brain-injured subjects continuously delivered saline or oxycodone 12 mg/kg/day for 10 days. SHAM SAL (n = 14); SHAM OXY (n = 8); INJURED SAL (n = 12); INJURED OXY (n = 11). * significant difference between INJURED SAL subjects and INJUIRED OXY subjects, $p < 0.05$; & significant different between SHAM SAL subjects and SHAM OXY subjects, $p < 0.05$.

CHAPTER 5: DISCUSSION

In the first aim, we tested the hypothesis that moderate TBI increases the risk for relapse to an opioid use disorder as measured by reinstatement of lever-pressing behavior following extinction in an intravenous oxycodone self-administration procedure. Subjects sustaining a moderate pressure pulse exhibited damage of central nervous tissue as indicated by elevated latencies in the return of the righting reflex, a correlate of injury severity (**FIGURE 1**) [76–82]. Our data, consistent with prior studies, show that oxycodone is an effective reinforcer of lever-pressing behavior as indicated by preference for the oxycodone-reinforced lever relative to the non-reinforced lever (**FIGURES 4, 7**) [83–86]. In oxycodone-reinforced sessions, brain-injured subjects emitted fewer active lever presses after meeting stable maintenance criteria than did non-injured subjects (**FIGURES 4, 7**), but showed no significant differences in inactive lever presses (**FIGURES 5, 8**). A possible explanation for the lower level of oxycodone self-administration could be differences in the sensitivity to the reinforcing effects of oxycodone following injury. A downward or leftward shift in the oxycodone dose-effect curve, a potency shift, may reflect an increase in the sensitivity to the reinforcing effects of oxycodone [87–89]. It follows that brain-injured subjects, relative to non-injured subjects, would require fewer infusions (read: a lower cumulative intake) to achieve a comparable hedonic state or to reduce motivation for drug-taking. Moreover, changes in sensitivity to the reinforcing effects of a drug of abuse have been correlated with relative risk for developing a substance use disorder [90]. Subjects that are more sensitive to a drug, require less drug to achieve the desired effect, and subjects less sensitive to the drug, administer more drug to achieve the desired effect [90]. Data suggest that subjects that are exposed to more drug are more likely to develop a substance use disorder [90]. It follows, then, that brain-injured subjects may be less likely develop an opioid use disorder. However, further testing must be completed with additional doses of oxycodone and additional schedule parameters to validate this hypothesis.

In non-reinforced extinction tests, brain-injured subjects, relative to non-injured subjects showed a trend to require a greater mean number of sessions to meet extinction criteria for lever-pressing behavior (**FIGURE 3**). In these tests, all subjects met extinction criteria, but showed no significant differences in mean lever presses during extinction sessions across injury condition (**FIGURE 4**). In oxycodone prime-induced reinstatement tests (1 mg/kg, SC), brain-injured and non-injured subjects showed decreases in lever-pressing behavior on the active lever, inactive lever, and during timeouts relative to lever-pressing behavior during extinction tests (**FIGURES 4–6).** A decrease in lever-pressing behavior under these conditions, may represent a non-specific depression of behavior due to the sedative effects of oxycodone [91]. When tested for oxycodone prime-induced reinstatement at a lower dose (0.3 mg/kg, SC), non-injured subjects showed significant increases in lever-pressing behavior relative to lever-pressing behavior during extinction sessions (**FIGURE 7**). Our results, consistent with prior studies, suggest that oxycodone priming injection is sufficient to reinstate previously extinguished lever-pressing behavior [37,40]. However, brain-injured subjects still failed reinstate to lever-pressing behavior under these conditions (**FIGURES 4, 7**).

In tests for oxycodone-associated cue-induced reinstatement, non-injured subjects showed significant increases in lever-pressing behavior relative to lever-pressing during extinction sessions (**FIGURE 4**). Our results, consistent with prior studies, suggest that exposure to oxycodone-associated cues following extinction are sufficient to reinstate lever-pressing behavior [38,40,92–95]. In these tests, however, brain-injured subjects, relative to non-injured subjects, showed no changes in lever-pressing behavior on the previously reinforced lever relative to lever-pressing behavior during extinction tests (**FIGURE 4**).

A histological profile of the fluid percussion injury is well-established and indicates that neurocircuits which mediate reinstatement to opioid-associated cues and opioid priming injection may be disrupted [96–101]. Since injured regions, such as the hippocampus, cortex, and corpus callosum, are among the discrete structures involved in these known circuits, it is logical to

conclude that the reinstatement of behavior following extinction would be affected. It is also established that opioid-associated cue and opioid-prime induced reinstatement are blocked by temporary, bilateral inactivation of the basolateral amygdala with tetrodotoxin [102]. While the histological injury profile suggests that the basolateral amygdala does not sustain damage either ipsilateral or contralateral to the site of injury [97] it is known that reciprocal projections between the basolateral amygdala, hippocampus, and cortex exist suggesting that disruption of these discrete structures, are of importance [57,58,103,104]. It follows, then, that injury to the hippocampus and cortex, by proxy of the basolateral amygdala, may be sufficient to attenuate the salience of both exteroceptive and interoceptive stimuli [57]. In future studies, assays sensitive to changes in the hippocampus, such as a novel object recognition assay or a self-administration procedure with a renewal design, may aid in confirming injury to discrete structures involved in reinstatement pathways by presence of behavioral disruption [105–111].

In the second aim, we tested the hypothesis that moderate TBI increases physiological dependence to oxycodone as measured by decreases in food-reinforced lever-pressing behavior and increases in other withdrawal behaviors in both precipitated withdrawal and spontaneous withdrawal. Subjects sustaining a moderate pressure pulse exhibited damage of central nervous tissue as indicated by elevated latencies in the return of the righting reflex, a correlate of injury severity (**FIGURES 10**) [76–82]. In physiological dependence tests, brain-injured subjects, relative to non-injured subjects showed no meaningful differences in experimenter assessed withdrawal scores or food-reinforced lever-pressing behavior after treatment with continuous oxycodone (**FIGURES 11, 15**). Our results, however, consistent with the results of other studies, showed that continuous, non-contingent delivery of oxycodone leads to the development of physical dependence for both brain-injured and non-injured subjects as evidenced by increases in withdrawal scores (**FIGURE 13, 14**) and decreases in food-reinforced lever-pressing behavior (**FIGURE 17, 18**) [71–75]. The consistent level of withdrawal across injury condition does not support a change in the sensitivity to the effects of oxycodone as suggested by levels of

oxycodone self-administration in the reinstatement study. An increase in the sensitivity to oxycodone would be marked by upward and leftward shifts in the naltrexone dose-effects curves for withdrawal scores, and downward and leftward shifts in the curves for food-reinforced lever-pressing behavior. However, no meaningful differences in withdrawal scores or food-reinforced lever-pressing behavior were observed in tests for precipitated withdrawal or spontaneous withdrawal in subjects delivered continuous oxycodone.

In tests for food-reinforced lever-pressing behavior for subjects treated with continuous saline, there were significant differences in baseline behavior which complicated the interpretation of results. It is possible that this is due to failure to eliminate bias by counterbalancing subjects with high baseline lever pressing behavior and low baseline lever pressing behavior across treatment groups. However, expression of these data as a percent of vehicle control behavior (data not shown) revealed no significant differences in food-reinforced lever pressing behavior across the injury condition, except at the highest naltrexone dose tested. Moreover, there were no differences in the mean composite withdrawal scores between brain-injured and non-injured subjects, the primary comparison of interest.

These results indicate that there are no differences in the somatic signs of withdrawal, a correlate for the development of physical dependence, between brain-injured subjects and non-injured subjects. Somatic signs of withdrawal may be mediated by the bed nucleus of the stria terminalis, central nucleus of the amygdala, and hypothalamus [57,58,103]. Since the injury profile does not indicate damage to either neuroanatomical pathways or discrete structures implicated in the production of somatic signs of withdrawal, it follows that differences between brain-injured subjects and non-injured subjects would not be expected. Other anatomical substrates mediating expression of aversive opioid withdrawal behaviors include the ventral noradrenergic bundle, a major source of noradrenergic projections to the bed nucleus of the stria terminalis [112] are not believed to be disrupted as a result of the lateral fluid percussion injury [113,114]. Collectively,

these data may suggest that physical dependence and withdrawal does not contribute to an increase in opioid use disorders in TBI patients.

Overall, the results of this study add to the collective knowledge of our understanding of the relationship between brain injury and substance abuse through preclinical models of relapse and physical dependence. Our relevant findings are summarized by several principal points. One, that brain-injured subjects did not reinstate lever-pressing behavior under oxycodone-associated cue or oxycodone prime-induced conditions, suggesting that brain-injured patients, with no significant pre-morbid history, are at lesser risk of relapse to opioid abuse. Two, that brain-injured subjects were not significantly different from non-injured subjects with regards to their mean withdrawal scores or food-reinforced lever-pressing behavior, suggesting that the characteristic withdrawal syndrome in opioid-dependent patients does not contribute to continued substance use to greater degree in brain-injured patients versus non-injured patients. Contrary to the epidemiological data about the relationship between brain injury and substance abuse, these results suggest that brain injury appears to have no impact on oxycodone's effects and may actually decrease the motivation to take drug as well as the risk of relapse.

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