The Effect of Heroin Dependence on Resumption of Heroin Self-administration after a Period of Abstinence and Extinction

by

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ABSTRACT

The effect of heroin dependence on resumption of heroin self-administration

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It has been proposed that relapse vulnerability in previously dependent individuals results from augmentation of drug-induced reinforcement due to repeated associations between the interoceptive properties of the drug and reduction of acute withdrawal distress. To test this hypothesis, male Sprague-Dawley rats self-administered 0.05 mg/kg/inf heroin on continuous and progressive ratio (PR) schedules. During this period, they also received injections of vehicle or escalating doses of heroin. Following tests of naloxone-precipitated withdrawal (0.01 or 0.1 mg/kg, SC), as well as abstinence (4 days), and extinction training (9 sessions), they were pre-treated with vehicle or yohimbine (0.5 mg/kg, IV) and tested for resumption of heroin self-administration (0.05 mg/kg/inf) on continuous and PR schedules (Experiments 1 & 2), or tested for reinstatement in extinction conditions. Differences between vehicle- and heroin-injected rats were noted on self-administration on the continuous reinforcement schedule, but not on the PR schedule, in spite of greater signs of withdrawal precipitated by naloxone in the heroin-injected rats. More importantly, there were no group differences in resumption of heroin self-administration, and this was not altered by yohimbine. These results suggest that relapse vulnerability cannot be uniquely ascribed to the altered reinforcing action of drugs; contextual and other conditioning factors must play a role in modulating resumption of drug intake after periods of abstinence.
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General rationale and summary of experiments

Relapse is a major problem in the treatment of drug addiction. There is evidence that withdrawal mediates drug-seeking behaviour during dependence, however less is known about its role in relapse. Wikler suggested that repeated reduction of acute withdrawal allows for the interoceptive effects of morphine to acquire secondary reinforcing properties. During relapse, these interoceptive properties act as secondary reinforcers, to increase the reinforcing effect of the drug, above just the primary reinforcing effects (Miller et al., 1979). The current experiments were designed to test Wikler’s hypothesis using experimenter delivered injections of vehicle or heroin to create different levels of interoceptive conditioning during acquisition of heroin self-administration. Following a period of abstinence and extinction rats were allowed to resume heroin self-administration on a progressive ratio schedule. Additionally, yohimbine (YOH) was used during tests of relapse to mimic some of the physiological symptoms of opiate withdrawal and investigate whether previous operant learning that heroin alleviates withdrawal, increased resumption of heroin self-administration. The experiments revealed no group differences in resumption of heroin on a progressive ratio schedule, and this was not altered by YOH. Incidentally, YOH-induced reinstatement was significantly greater in rats given injections of heroin during self-administration. An additional experiment was carried out to explore the results of Experiment #1. Experiment #2 explored the resumption of heroin self-administration using a combination of fixed and progressive ratio schedules. The experiment revealed that the introduction of a continuous reinforcement session prior to relapse tests on a PR schedule returns responding for heroin on the progressive ratio schedule similar to
levels obtained on the last day of acquisition. Collectively, these results suggest that relapse vulnerability cannot be solely ascribed to the altered reinforcing action of drugs; other factors may play a role in modulating resumption of drug intake after a period of abstinence.

**Relapse**

The risk of relapse during periods of abstinence is a major challenge for the treatment of opiate drug addiction. Clinical studies reveal a high incidence of relapse to opiate use, soon after the completion of addiction treatment programs (Gossop, Green, Phillips, & Bradley, 1989; Unnithan, Gossop, & Strang, 1992). In fact, in 2002, in a follow-up study one month after the treatment for heroin addiction, Gossop et al. found that 60% of the 242 clients relapsed to heroin use. This relapse rate in opiate addiction is similar across other addictive substances, such as cocaine and amphetamine (McKay et al., 1999; Hunt, Barnett & Branch, 1971). While the factors known to precipitate relapse are common across the classes of drugs of abuse i.e., drug-primes, drug cues, and stress (Heather, Stallard, & Tebbutt, 1991; Sinha, 2007; Martin & Jasinski, 1969; O'Brien, 1998), it has been postulated that withdrawal may play a major role in relapse to opiate use.

**Opiate Withdrawal**

Opiate withdrawal is expressed when the administration of a drug is terminated. It can be further classified into *acute* and *protracted withdrawal*. Acute withdrawal can manifest as early as 8 hours after the last injection of an opioid and last for as long as 72 hours (Alper et al., 1999). In animals, it can include symptoms classified as physical and motivational/psychological. Specifically, in rats, physical symptoms can manifest
themselves as wet dog shakes, diarrhea, ptosis etc. (Way et al., 1969). Moreover "psychological" and motivational symptoms are noted as increased anxiety, irritability (Julien, Advokat & Comaty, 2007), depressed brain reward thresholds and anhedonia; all of which may influence operant behaviour (Kenny et al., 2006) and place aversion (Stinus et al., 2000). Additionally, in both humans and animals withdrawal is accompanied by neurobiological changes that include elevated levels of stress hormones and alterations in hypothalamic pituitary adrenal (HPA) axis functioning (Sinha, 2001; Heilig, Egli, Crabbe & Becker, 2010; Koob & LeMoal, 2005). The aforementioned symptoms of acute withdrawal and neurobiological changes are thought to be significant in the motivation towards the maintenance of drug addiction (Koob & LeMoal, 2005). After the symptoms of acute withdrawal subside, there are alterations in blood pressure, heart rate, weight, and basal metabolic rates that can remain up to 6 months after the last exposure to an opioid (Himmelsbach, 1942); this is also known as protracted withdrawal.

Dependence and self-administration

The negative reinforcement theory posits if a behaviour provides relief from an aversive stimulus, this behaviour is likely to occur in future situations where this aversive stimulus is present. In the context of drug dependence, negative reinforcement predicts that continued use of a drug is reinforcing because it allows escape from withdrawal (Frenois et al. 2005; Kenny and Markou 2005; Kenny et al. 2006; Gerak et al.,2009). Spragg (1940) first provided support for this in a study, where chimpanzees were made dependent on injections of morphine. Spragg observed that when the chimpanzees were both hungry and in morphine withdrawal, given the choice between
a morphine syringe and food, they preferred the syringe. However, after being injected with morphine when given the same choice, they selected food. These findings suggest that withdrawal can mediate drug-seeking behaviour in animals during dependence. However, this does not explain why subjects relapse long after signs of opiate withdrawal have dissipated.

**Dependence and Relapse**

Early studies demonstrated that animals previously dependent on opiates are more likely to resume opioid intake after a period of abstinence. For example, Hinson et al (1986) trained rats to consume morphine for 235 days. After an 80-day drug-free period, and in the absence of withdrawal symptoms, when rats were re-introduced to the drug, those previously dependent on morphine consumed more drug than drug-naive controls. Likewise, Wikler & Pescor (1967) induced physical dependence by administering escalating doses of morphine (or saline) until rats were maintained on 200 mg/kg (I.P) morphine a day. Following a drug-free period, rats were given free-choice relapse tests, where the amount of water and etonitazene consumption was measured. During acute withdrawal, the previously dependent rats drank more etonitazene solution than drug-naive controls. These differences were found repeatedly even when tests of relapse were conducted several months after the termination of morphine treatment. This enhanced resumption of opioid drinking in previously dependent rats has been demonstrated repeatedly (Kumar and Stolerman, 1972; Garcin et al., 1976). These findings are also consistent with self-administration studies, where increased drug consumption after abstinence is observed in previously drug dependent rats (Weeks
and Collins, 1968). The role of physical dependence in relapse was further explored by Abraham Wikler.

Wikler (1971) hypothesized that relapse vulnerability in previously dependent individuals results from an interaction between three factors: 1) long-term persistence of low-grade physiological deviations from normal; 2) classical conditioning of the abstinence phenomena to environmental situations repeatedly associated with acute withdrawal distress (i.e., known as conditioned withdrawal; Hellemans, Dickinson, & Everitt, 2006); and 3) augmentation of drug reinforcement due to repeated associations between the interoceptive properties of the drug and reduction of acute withdrawal distress.

(1) Persistence of physiological deviations

Wikler draws on findings presented by Himmelsbach (1942) to suggest that physical recovery from addiction takes approximately 6 months. In 1942, Himmelsbach conducted a longitudinal study in which various physiological observations (i.e., blood pressure, heart rate, weight) were made during the last week an individual was self-administering opiates, on the 15th day following withdrawal, and then every 30 days into the 9th month of abstinence. He observed that physiological measures did not return to normal until the 6th month of complete abstinence. This suggests that certain deviations from baseline persist long after the last exposure to an opioid.

Koob & LeMoal (2005) suggest, *allostasis*, as an explanation for the lasting changes. The hypothesis assumes that the body adjusts its 'set point' to adapt to the neurobiological changes that occur in drug addiction and withdrawal. These neuroadaptations activate brain stress systems and underactivate brain reward
systems: (1) activation of the reward system in a binge; (2) downregulation of dopamine and opioid peptide systems at the end of a binge; (3) continued downregulation of reward systems during acute withdrawal; and (4) HPA activation and central corticotrophin-releasing factor (CRF) activation during acute withdrawal and persisting into protracted withdrawal. The changes that remain after acute withdrawal, create cravings by the activation of drug-, cue-, and stress-induced neurocircuits (Koob and LeMoal, 2008; Koob and LeMoal, 2005; Koob & Kreek, 2007).

(2) Classical conditioning of the abstinence phenomena.

Wikler (1967) also proposed that environmental stimuli through classical conditioning might evoke withdrawal-like responses because those stimuli were previously associated with drug withdrawal. For example, Wikler & Pescor (1967) induced physical dependence in rats by intraperitoneal (i.p) injections of morphine over 9 weeks. Throughout this period, when rats experienced withdrawal they were put into a distinct environment in order to facilitate conditioning of withdrawal. Animals were then tested for wet-dog shakes in their home cage and in the withdrawal environment. They found that there was a greater number of wet-dog shakes in the place of withdrawal; therefore demonstrating that morphine withdrawal can be conditioned to environmental cues. More recently, Kenny et al. (2006) found that environmental stimuli previously paired with naloxone-precipitated withdrawal, increased heroin consumption and elevated ICSS thresholds, a pattern consistent with withdrawal.

The conditioned withdrawal syndrome observed in animals is similar to that displayed by humans. O'Brien, Ehrman, & Ternes (1984) precipitated withdrawal by repeatedly injecting the opiate antagonist, naloxone, in methadone maintained patients
in the presence of distinctive cues. Later, when a saline injection was administered in the presence of those same cues, symptoms of conditioned withdrawal were evoked. Similarly, long after physical withdrawal symptoms had subsided, dependent individuals reported feelings of elevated anxiety, dysphoria, and strong cravings for opioids when exposed to drug-associated stimuli (Gowing, Farrell, Ali, & White, 2004; Childress, McLellan, & O'Brien, 1986; Heilig, Egli, Crabbe, Becker, 2010). These results are consistent with Wikler's observations that withdrawal associated cues can predict withdrawal like responses. Moreover, he postulates that these conditioned stimuli interact with low-grade physiological deviations in previously dependent rats to increase the vulnerability to relapse.

(3) Conditioning of the suppression of acute withdrawal distress by self-administration of opioids.

Wikler also proposed a role of interoceptive conditioning in relapse to drug-taking and seeking. The hypothesis predicts that the repeated reduction of acute withdrawal allows for the interoceptive effects of morphine (nausea, hypothermia, pruritis) to acquire conditioned reinforcing properties. This conditioned (secondary) reinforcer augments drug reinforcement. Therefore, during the test of relapse, the consumption of an opiate would be more reinforcing because these interoceptive stimuli act as additional reinforcers. Miller et al. (1979) further explored the role of interoceptive conditioning. In their study, they employed two protocols of morphine administration that equated for total amount of drug exposure, but that differed in induction of withdrawal distress. One group of rats received single daily intravenous morphine injections (escalating up to 200 mg/kg) while another group received continuous intravenous
morphine infusions at the same dose. Consistent with the interoceptive conditioning hypothesis, a test of relapse 12 days after termination of treatment revealed greater consumption of etonitazene by rats that repeatedly experienced a reduction of acute withdrawal by morphine (single daily injection group). Therefore, rats that experienced repeated alleviation of withdrawal distress showed enhanced opiate consumption during relapse. This idea of conditioning early drug onset cues has been previously explored in the conditioned tolerance model (Kim, Siegel, Patenall, 1999; Krank, 1987). For example, Kim et al (1999) found that early drug-onset cues paired with later larger drug effects become associated with larger drug effects and elicit conditioned compensatory responses. However, enhanced drug-intake in previously dependent rats caused by interoceptive conditioning has not been further explored in the context of withdrawal and self-administration.

**Animal models of relapse**

One method used to study relapse in animals is drug self-administration. In self-administration, there are two primary models of relapse: reinstatement and resumption. In both models, animals acquire drug self-administration by performing an operant response to obtain an intravenous infusion of a drug. Following training (acquisition), animals undergo extinction of drug-reinforced cues. This is accomplished by no longer reinforcing behaviour with an infusion of the drug (Shaham et al., 2003). Extinction continues until animals reach low levels of responding.

During resumption, animals are re-introduced to the drug and subsequent behaviour is observed after abstinence (Kumar and Stolerman, 1972; Garcin et al., 1976; Weeks and Collins, 1968) and/or extinction (Leri & Stewart, 2002). For
example, Weeks and Collins (1968) induced physical dependence through self-administration of morphine. Following a 4-week abstinence period, rats resumed morphine self-administration on a continuous reinforcement schedule. Results suggest that "post addict" rats show greater morphine self-administration than a control group. In 2002, Leri & Stewart demonstrated that a resumption of heroin self-administration on a continuous reinforcement schedule was affected by the presentation of drug related cues. Additionally, they found that response contingent drug taking after abstinence affected drug-seeking the following day.

In the reinstatement model, drug seeking is assessed in extinction conditions and precipitated by factors such as drug primes, cues, or stress (Shaham et al., 2003). These factors play a significant role in modulating relapse. Stretch & Gerber (1973) discovered that a non-contingent injection of the same drug (drug priming) that had been previously self-administered restored operant responding following extinction. This effect of priming injections on relapse has since been supported by many replications (for example: Stewart, 2003; Stewart & Wise, 1992). Davis and Smith (1976) demonstrated that the reintroduction of the previously drug-associated stimuli (conditioned cues) was sufficient to engender a reinstatement in responding. This is similar to other studies showing that environmental stimuli previously associated with drug use can induce reinstatement (Shalev, Grimm & Shaham, 2002; Kenny et al., 2006). More recently, it has been shown that brief exposure to a stressor is capable of reinstating responding (Shaham & Stewart, 1995).

Clinical studies indicate that relapse can be affected by stress (Sinha, 2001). For example, in humans Higgins & Marlatt (1975) found that drug-taking was enhanced by
stressful situations. Similarly, in rodents Shaham & Stewart (1994) found that animals exposed to footshock stress immediately before self-administration displayed greater motivation for drug taking on a progressive ratio schedule of reinforcement. In animals, many stressors that are capable of reinstating responding vary in nature, i.e., social defeat, restraint, footshock & pharmacological agents (Shaham et al., 2000; Erb & Placenza, 2010). However, social defeat and restraint stress are not reliable in their ability to promote reinstatement of drug-seeking (Shaham, 1996). Conversely, foot shock induces consistent and robust reinstatement; however, it is limited in its translational applicability to humans. Although footshock can be used on animals, it is considered unethical to use on humans. In contrast, pharmacological stressors like yohimbine have been used in both humans and animals. Yohimbine like footshock is also reliable and consistent in its ability to generate stress-induced reinstatement (Banna, Back, Do & See, 2010; Le, Harding, Juzytsch, Funk, & Shaham, 2005; Campbell et al., 2012) and reacquisition of place preference (Campbell et al., 2012).

**Opiate withdrawal, noradrenaline, yohimbine and relapse**

Stress systems are activated by opiate withdrawal. One of the stress systems affected is the noradrenergic system. During acute administration, opioids inhibit noradrenergic (NA) neurons in the locus coeruleus (LC), by hyperpolarizing the neuron, which prevents it from firing. This is supported by in-vitro studies that show acute administration of morphine decreases the release of NA on slice preparations from many brain areas like the cortex, thalamus, hippocampus, and cerebellum (Maldonada, 1997). However, with chronic opiate use, the NA in the brain and adrenals return to pre-opioid levels, which suggests an adaptation to chronic opioid exposure. It is proposed
that as a result of the constant inhibition, compensatory mechanisms upregulate the number of NA neurons in the LC. Subsequently, this causes LC firing to return closely to pre-opioid treatment levels. Microdialysis studies, confirm that when tolerance is developed to morphine’s inhibitory effects during chronic morphine treatment, further administration of morphine has no effect on the NA output in dependent animals (Maldonada, 1997). However, when there is an abrupt cessation of opioid use (i.e., withdrawal), an individual has an upregulated NA system with nothing inhibiting LC firing. As a result, this causes an increase in the amount of NA which is correlated with physiological symptoms of withdrawal (Maldonada, 1997).

Yohimbine (YOH), an α-2-adrenergic antagonist, can be used to characterize the hyperactivity of the noradrenergic locus coeruleus (LC) neurons following withdrawal from chronic opiates, because similar patterns in NA activity are seen after the administration of YOH (Nakai et al., 2002; Akbarian et al., 2002). YOH occupies the α2 adrenergic receptor sites, and prevents inhibition by these receptors, causing an increase in NA cell firing (Nakai et al., 2002). This hyperactivity of NA neurons makes YOH useful in studying opiate withdrawal as it mimics the previously mentioned physiological symptoms of opioid withdrawal (Nakai et al., 2002). For instance, a study in humans found that an injection of YOH in methadone maintained patients significantly increased objective and subjective withdrawal symptoms (Stine et al., 2002).

**Startle reactivity**

The acoustic startle response has been used to study the anxiety-like effects of opiate withdrawal (Schulteis et al., 1998). Previous studies show an elevated acoustic startle response in animals during withdrawal from opiates (Rothwell, Gewirtz &
Thomas, 2010; Harris & Gewirtz, 2004). This elevated startle response during opioid withdrawal is thought to be mediated by the anxiety-like effects of withdrawal (Harris & Gewirtz, 2004). It is a valid index of anxiety and is sensitive to drugs that alter anxiety in humans (Davis, Redmond, Baraban, 1979). For example, in humans, after an injection of YOH, both control and methadone maintained patients showed an elevation in measures of anxiety (Stine et al., 2002). In laboratory animals, YOH has been found to enhance anxiety in tests that assess the anxiogenic properties of drugs, i.e., the elevated plus maze (Pellow et al., 1985; Davis, Redmond, & Baraban, 1979). This startle response can be potentiated with the administration of YOH (Davis, et al., 1979).

**Progressive ratio schedule**

The research reported in this thesis employed the progressive ratio (PR) schedule of reinforcement. PR schedules have been used to study the reinforcing strength of a particular drug. Under these schedules, the number of responses required to obtain an infusion of a drug progressively increases within a single session. At the end of a session, a breakpoint (BP) is calculated based on the point at which responding ceases and is used as an index that reflects the maximum effort a rat will exert to obtain an infusion of the drug (Richardson & Robert, 1996).

The PR schedule is an alternative to the commonly used continuous reinforcement (CR) schedule. The CR schedule is useful as it allows animals to learn more readily, provides information on whether a drug will support self-administration and whether the drug can act as a reinforcing stimulus (Arnold & Roberts, 1997). However, there are several disadvantages of using the CR schedule, which are overcome by a PR
schedule of reinforcement. A PR schedule limits the interference that a drug self-administered on a low-ratio schedule can have on self-administration; since a PR schedule restricts heroin intake, it minimizes the carry over effects of a drug on a rat's performance (see review by Stafford, LeSage, & Glowa, 1998). Additionally, drug intake on a CR schedule is inversely related to the drug dose/drug potency (Xi et al., 2004; Sim-Selley et al., 2000). The tolerance caused by opioids make it difficult to distinguish whether the pattern of self-administration on a CR schedule is due to the increased reinforcing effect of the drug, or to a decrease in heroin potency caused by the development of tolerance. For example, Cummins et al. (2008) found that increased responding on a CR schedule was predicted by a decrease in the depressant action of heroin on locomotion, a sign of opioid tolerance (Kalivas & Duffy, 1987). Moreover, it was found that an increase in unreinforced responding during self-administration predicted an increase in BP for heroin on PR schedule (Cummins et al., 2008), suggesting that a PR schedule is able to reflect a shift in the animal's motivation to obtain the drug.

Previous studies have found the PR schedule to be useful in studying opioid relapse. For example, Zhang et al (2004) conducted a study where rats were exposed to different amounts of heroin on a PR schedule. They found that the there was a positive relationship between the amount of heroin intake during self-administration and the magnitude of cue-induced reinstatement. Additionally, prior morphine dependence has been found to increase drug-seeking on a PR schedule (Yanagita, 1973).
Hypothesis and predictions

According to Wikler, the repeated alleviation of opiate withdrawal will allow for the development of secondary conditioning to the interoceptive effects of heroin. The current study employed intravenous (IV) self-administration to investigate the effect of dependence on the resumption of heroin self-administration following abstinence and extinction. Rats were administered experimenter-delivered injections of heroin (or vehicle) to induce different amounts of interoceptive conditioning. Based on the interoceptive hypothesis, there are three predictions. First, additional injections of heroin should alter opiate self-administration, because of augmented reinforcing properties of the drug as hypothesized by Wikler. Second, these augmented reinforcing properties of the drug will be learned and reveal themselves in the resumption of opiate self-administration. Lastly, previous operant learning that withdrawal distress is relieved by the self-administration of opioids, should enhance the incentive for the drug when these symptoms are induced by injections of YOH; and this effect should be greater in the Heroin Injection group. To assess the motivational properties of the drug, a PR schedule was used. The PR tests in Experiment 1 yielded unexpected results. That is, irrespective of vehicle or heroin injections, the BPs maintained by 0.05 mg/kg heroin on PR Test I (prior to extinction) were much higher than BPs on PR Test II (following a drug-free period and extinction). It was proposed that perhaps this decrease in BP may be a result of prolonged extinction training and testing on a PR schedule. Based on the results of Experiment 1, two additional experiments were added. Experiment 2 explored resumption of heroin self-administration using a combination of continuous and progressive ratio schedules to investigate whether extinction produced the same
suppression of responding on different schedules of reinforcement. Experiment 3 employed startle reactivity to investigate whether the anxiogenic effect of yohimbine (Davis et al., 1979) was enhanced by repeated injections of escalating heroin doses.

This thesis includes a manuscript submitted to *Drug and Alcohol Dependence* that describes the effect of heroin dependence on the resumption of heroin self-administration.
THE EFFECT OF HEROIN DEPENDENCE ON RESUMPTION OF HEROIN SELF-ADMINISTRATION IN RATS

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Abstract

**Background:** It has been proposed that relapse vulnerability in previously dependent individuals results from augmentation of drug-induced reinforcement due to repeated associations between the interoceptive properties of the drug and reduction of acute withdrawal distress. **Methods:** To test this hypothesis, male Sprague-Dawley rats self-administered 0.05 mg/kg/inf heroin on continuous reinforcement (CR) and progressive ratio (PR) schedules. During this period, they also received injections of vehicle or escalating doses of heroin. Following tests of naloxone-precipitated withdrawal (0.1 mg/kg, SC), as well as a drug-free period (4 days), and extinction (9 sessions), they were pre-treated with vehicle or yohimbine (0.5 mg/kg, IV) and tested for resumption of heroin self-administration (0.05 mg/kg/inf) on CR and PR schedules, or tested for reinstatement in extinction conditions. **Results:** Increased self-administration on the CR schedule was observed in the heroin-injected rats, but no group differences were observed on the PR schedule, in spite of greater signs of withdrawal precipitated by naloxone in the heroin-injected rats. More importantly, there were no group differences in resumption of heroin self-administration, and this was not altered by yohimbine. **Conclusions:** These results suggest that relapse vulnerability cannot be uniquely ascribed to enhanced reinforcing action of drugs; contextual and other conditioning factors must play a role in modulating resumption of drug intake after abstinence.

**Key words**

Heroin; withdrawal; progressive ratio; reinstatement; resumption; yohimbine
1. Introduction

An interesting topic in drug addiction research is the role of physical dependence in relapse to drug use after the dissipation of clear signs of withdrawal. Early studies demonstrated that animals previously dependent on opiates are more likely to resume opioid intake after periods of abstinence (Hinson et al. 1986; Wikler and Pescor 1967; Kumar and Stolerman, 1972; Garcin et al., 1976; Weeks and Collins, 1968). Wikler (1971) hypothesized that relapse vulnerability in previously dependent individuals results from augmentation of drug-induced reinforcement due to repeated associations between the interoceptive properties of the drug and reduction acute of withdrawal distress. To test this hypothesis, Miller et al. (1979) employed two protocols of morphine administration that equated for total amount of drug exposure, but that differed in induction of withdrawal distress. One group of rats received single daily intravenous morphine injections (escalating up to 200 mg/kg) while another group received continuous intravenous morphine infusions at the same dose. A test of relapse 12 days after termination of treatment revealed greater consumption of etonitazene in rats given the single daily injections. It was reasoned that, in this group, the interoceptive effects of morphine (nausea, hypothermia, pruritis) acquired conditioned reinforcing properties because they were followed by alleviation of withdrawal (Wikler and Pescor, 1967) experienced every day, prior to the morphine injection. Therefore, during the test of relapse, consumption of the opiate was more reinforcing because these interoceptive stimuli acted as conditioned (secondary) reinforcers.

As far as the authors know, this hypothesis has never been explored in the context of drug self-administration and modern models of relapse. Therefore,
Experiment 1 was designed to test this putative mechanism of relapse vulnerability using a protocol in which all animals were trained to lever press for intravenous heroin infusions on a continuous reinforcement schedule (CR), prior to extinction and resumption of heroin self-administration testing. During acquisition, four hours after each self-administration session, some rats received additional injections (SC) of heroin to increase total level of heroin exposure and induce a state of withdrawal prior to the self-administration of heroin the following day. Prior to the test of resumption, some rats were also pre-treated with yohimbine, an alpha-2 adrenergic antagonist, to mimic some interoceptive features of opiate withdrawal (Stine et al., 2002). All tests of resumption were performed using a progressive ratio (PR) schedule of self-administration (Roberts et al., 1989). To control for the effects of yohimbine on heroin seeking, a test of reinstatement (i.e., lever pressing in extinction conditions) was also performed (Shaham et al., 2003). On the basis of the results of Experiment 1, an additional experiment was carried out. Experiment 2 specifically explored the interaction between CR and PR schedules during acquisition and resumption of heroin self-administration.

2. Materials and Methods

2.1. Subjects

Subjects were adult male Sprague-Dawley rats (Charles River, QC) weighing 250–300 g at the beginning of the experiments. Rats were individually housed, maintained on a reverse light/dark cycle (7:00 AM lights off; 7:00 PM lights on) and behavioral testing occurred during their active cycle. All rats had free access to food and water except during testing. All procedures were approved by the Animal Care
Committee of the University of Guelph and were carried out in accordance with the recommendations of the Canadian Council on Animal Care.

2.2. Intravenous surgery and self-administration and activity chambers

Details of surgical procedures and apparatus have been described in Leri et al (2009).

2.3. Procedures experiment 1

The experimental design, final sample size and conditions/tests are represented in Figure 1. The design included 4 phases: self-administration, tests of precipitated withdrawal, extinction, and tests of resumption and reinstatement.

2.3.1. Self-administration

A total of 110 rats were trained to self-administer heroin (0.05 mg/kg/inf) on a continuous reinforcement (CR) schedule for 10 consecutive days. Each day, self-administration sessions began at 8:00 AM and ended at 11:00 AM. Each session was initiated by the activation of the house light, the entry of the levers, and the illumination of the stimulus light located above the active lever for 30 seconds. Subsequently, each press on the active lever resulted in the delivery of a 150 µl infusion and illumination of the stimulus light for 5 sec.

The study included two groups of rats: Vehicle Injection (initial sample size = 52) and Heroin Injection (initial sample size = 58). After each self-administration session, the groups received subcutaneous injections of vehicle (saline) or heroin at 3 PM, 5 PM and 7 PM. The drug doses employed in the Heroin Injection group escalated across the 10 days of self-administration according to the regimen: 1, 2, 3, 4, 4, 6, 6, 8, 8, and 8 mg/kg. Therefore, by the last day of operant training, rats in this group self-
administered 0.05 mg/kg/inf heroin in the AM, and received 3 injections of 8 mg/kg heroin in the PM.

On day 11, rats self-administered 0.05 mg/kg/inf heroin between 8:00 AM and 11:00 AM on a progressive ratio (PR Test I) schedule of reinforcement. On this schedule, the number of responses required for each infusion progressively increased according to the ratio: 1, 5, 11, 19, 31, 49, 76, 117, 177, 267, 401, 602, 900 (adapted from Roberts and Bennett 1993). The breakpoint (BP) was calculated by adding the number of responses made for each infusion obtained in the 3 h test session (Roberts and Bennett, 1993).

2.3.2. Tests of precipitated withdrawal

Within an hour following PR Test I on day 11 of self-administration, different groups of animals were injected with 0.1 mg/kg naloxone prior to assessment of three indices of heroin withdrawal. The assignment of specific animals to specific tests of withdrawal was done randomly, but no rat received more than one injection of naloxone.

2.3.2.1. Locomotion

Vehicle and the Heroin Injection groups were injected with 0.1 mg/kg naloxone and placed in activity chambers. Horizontal and vertical activities were recorded. It should be noted that 10 and 8 rats in the Vehicle and the Heroin Injection groups, respectively, received a lower test dose of naloxone (0.01 mg/kg) prior to locomotion testing. However, because on this measure of withdrawal, the effect of 0.01 mg/kg did not differ statistically from that of 0.1 mg/kg, the data from the animals were combined. At the conclusion of the experiment, it was confirmed that rats injected with 0.01 or 0.1 mg/kg naloxone did not differ on tests of extinction and resumption.
2.3.2.2. Loss of body weight

A subset of rats in the Vehicle (n = 5) and the Heroin (n = 8) Injection groups tested on locomotion were weighed prior to, and 2 hours following, the injection of 0.1 mg/kg naloxone. Percent weight loss was calculated.

2.3.2.3. Wet dog shakes

A subset of rats in the Vehicle (n = 14) and the Heroin (n = 13) Injection groups tested on locomotion were videotaped for 2 hours after the injection with 0.1 mg/kg naloxone. Subsequently, an observer blind to treatment scored instances of wet dog shakes.

2.3.3. Extinction

Following a 4-day drug free period in home cages, rats received 9 sessions of extinction over 9 days (3 hours each) during which responding on the active lever was not reinforced by heroin.

2.3.4. Tests of resumption and reinstatement (PR Test II)

Following extinction, different rats in the Vehicle and Heroin Injection groups were randomly assigned to two different tests during which responding was assessed using the same PR schedule employed on day 11 of self-administration. For the test of resumption, animals were tested for 3 hours in self-administration conditions; that is, each completed ratio resulted in an intravenous infusion of 0.05 mg/kg/inf heroin. Five minutes prior to this test, rats were injected with either 0 or 0.5 mg/kg yohimbine (see Figure 1). In contrast, for the test of reinstatement, animals were tested for 3 hours in extinction conditions; that is, each completed ratio resulted in an intravenous infusion of
vehicle. Five minutes prior to this test, rats were injected with either 0 or 0.5 mg/kg yohimbine (see Figure 1).

2.4. Procedures experiment 2

The PR tests in Experiment 1 yielded unexpected findings. In fact, irrespective of vehicle or heroin injections, the BPs maintained by 0.05 mg/kg heroin on PR Test II (assessed following a drug-free period and extinction) were much lower than BPs on PR Test I (assessed on day 11 of heroin self-administration). This observation generated three research questions that were explored in Experiment 2. First, it was asked whether the BPs observed on PR Test II were within the range of BPs that would be observed in rats that never self-administered heroin. Second, it was verified whether acquisition of heroin self-administration would increase BPs on the particular PR schedule used in these experiments. Finally, and more importantly, it was asked whether the decrease in BPs observed from acquisition to tests of resumption was a by-product of having tested the rats on the PR schedule.

Therefore, animals in this experiment (n = 17) did not receive injections of heroin (or vehicle) during acquisition of heroin self-administration. The first session of self-administration involved a test of responding for 0.05 mg/kg/infusion heroin on the PR schedule (i.e., baseline test; PR Test BL). Animals then self-administered the same heroin dose on a CR schedule, for 3 hours, over 10 consecutive sessions. On day 12, a second PR test was given (i.e., PR Test I), followed by a 4-day drug-free period and 9 sessions of extinction. In this experiment, the first test of resumption was performed using the CR schedule (0.05 mg/kg/inf, 3 hours), and the second test (24h later) was performed using the PR schedule (PR Test II).
2.5. Drugs

Heroin (3,6-diacetylmorphine HCl, Almat Pharmachem, Concord, ON) was dissolved in 0.9% physiological saline self-administered intravenously at a dose of 0.05 mg/kg/infusion (Levy, Choleris, and Leri, 2009; Cummins and Leri, 2008). In Experiment 1, heroin was also injected subcutaneously, and the rate of dose escalation was selected on the basis of toxicity pilots performed in the laboratory. Yohimbine was dissolved in distilled water, and administered IV at a dose of 0.5 mg/kg (Moreau et al., 1995; Penning and Jhamandas, 1991). Naloxone was dissolved in 0.9% physiological saline, and administered SC at doses of 0.1 mg/kg (Schulteis et al., 1994; Espejo et al., 1995; Swerdlow et al., 1985; Azar et al., 2003).

2.6. Statistical Analyses

Two and three factors Analyses of Variance (ANOVAs) were used as appropriate. In case of significant interactions or main effects, individual mean differences were identified by multiple comparisons using the Student-Newman-Keuls methods ($\alpha = 0.05$). To test specific predictions, planned comparisons were performed using t-tests. The exact values of non-significant analyses are not reported. Analyses were performed using SigmaStat (3.5 for Windows, Systat Software, Inc) and GB-Stat School Pak (Dynamic Microsystems, Inc).

3. Results

3.1. Experiment 1

This experiment was performed in several replications over the course of about 12 months. In each replication, animals were included in both Vehicle and Heroin Injection groups. And, in each replication, animals were assigned to particular tests of
naloxone-precipitated withdrawal, and to particular tests of relapse, to achieve similar group sizes. However, at the conclusion of data collection, it was noted that not all rats acquired heroin self-administration. In approximately 50% of cases, questionable catheter patency (could not withdraw blood, or leaks around the cannula) could have explained the absence of acquisition. In the other cases, however, the reason(s) was/were less clear. Regardless, because it was deemed inappropriate to include animals that never acquired self-administration in tests of relapse, a criterion for exclusion from data analysis had to be established. Therefore, operant responding was assessed in a different group of rats (n = 6) that was tested as described above, but that did not receive IV surgery, and that did not self-administer heroin. Total number of responses made by these rats on the active lever during the last 5 days of testing was calculated, and used as exclusion threshold: any rat in the Vehicle and Heroin Injection group that did not exceed this threshold by at least 1 response was excluded from data analysis. Using this criterion, 5 and 18 rats in the Vehicle and Heroin Injection groups were excluded, respectively. In these rats, there was no increase of responding on the active lever during acquisition, and no evidence of discrimination between the active and the inactive levers. Furthermore, a statistical comparison of responding of removed Vehicle and Heroin Injection rats (i.e., acquisition, extinction and relapse) revealed no differences at the different experimental stages. Therefore, it is appears that the difference in the ratio of included/excluded subjects in the two experimental groups was fortuitous. Figure 1 represents sample sizes included in final data analysis.
3.1.1. Self-administration

The ANOVA comparing infusions (Figure 2A) identified significant main effects of Injection Group [F(1, 85) = 6.11, p < 0.05] and of Session [F(9, 765) = 9.45 p < 0.001]. Multiple comparisons revealed significant differences between groups during acquisition days 4-9, suggesting that animals injected with heroin self-administered more heroin on the CR schedule.

An ANOVA comparing responses on the active and inactive levers (Figures 2B and 2C) identified a significant Session by Lever Interaction [F(9, 803) = 4.99, p < 0.001], as well as significant main effects of Injection Group [F(1, 85) = 6.05, p < 0.05], Session [F(9, 765) = 8.32, p < 0.001] and Lever [F(1, 85) = 54.42, p < 0.001]. Multiple comparisons indicated that responding on the active lever, but not on the inactive lever, increased significantly in both groups from sessions 1 to 10. Furthermore, animals in the Heroin Injection group emitted more responses on the active lever than animals in the Vehicle Injection group, but statistical significance was only observed on session 8. No significant group differences were noted on responding on the inactive lever.

The results of PR Test I are represented in Figure 3. The t-test comparing breakpoints (Figure 3A) failed to identify group differences. Similarly, the ANOVA comparing total responses on the active and inactive levers (Figures 3B and 3C, respectively) identified a significant main effect of Lever [F(1, 85) = 31.78, p < 0.001], but no significant group differences.

3.1.2. Tests of precipitated withdrawal - Locomotion

In a 15 minutes test, the ANOVA revealed significant main effects of Injection Group [F(1, 85) = 7.26, p < 0.001] and of Session Time [F(2, 170) = 27.26, p < 0.001].
Multiple comparisons indicated significantly reduced motor activity in the Heroin Injection animals at 5 and 10 minutes of the session (Figure 4A).

3.1.3. Tests of precipitated withdrawal - Loss of body weight

A t-test comparing total % weight loss during a 2 hour period following an injection of 0.1 mg/kg naloxone identified a significantly greater loss in the Heroin Injection group (Figure 4B; [t(11) = 4.67, p < 0.001]).

3.1.4. Tests of precipitated withdrawal - Wet dog shakes

A t-test comparing incidence of wet dog shakes indicated that animals in the Heroin Injection group displayed a greater number of wet dog shakes than animals in the Vehicle Injection group (Figure 4C; [t(25) = 4.22, p < 0.01]).

3.1.5. Extinction

The ANOVA comparing responses on the active and inactive lever in the Vehicle and Heroin Injections groups during the entire extinction period (data not shown) identified a significant Session by Lever interaction [F(8, 672) = 42.62, p < 0.001], as well as main effects of Session [F(8, 672) = 87.46, p < 0.001] and of Lever [F(1, 85) = 96.11, p < 0.001]. Multiple comparisons indicated that responding on the active lever and inactive lever decreased significantly in both groups from sessions 1 to 9. On session 9, there were no group differences between the Vehicle Injection and Heroin Injection groups on the active lever (Mean ± SEM: 16.1 ± 1.8 and 20.6 ± 2.1, respectively) and on the inactive lever (Mean ± SEM: 10.5 ± 1.7; 12.4 ± 1.6, respectively).
3.1.6. Tests of resumption and reinstatement (PR test II)

The results of the resumption test are represented in Figure 5. The ANOVAs performed on BPs and on responses on active and inactive levers identified no differences between Injection Groups, and no effect of YOH pre-treatment.

The results of the reinstatement test are represented in Figure 6. The ANOVA comparing BPs (Figure 6A) identified a significant main effect of YOH pre-treatment \( [F(1, 37) = 6.65, p < 0.05 ] \) and a significant main effect of Injection Group \( [F(1, 37) = 4.06, p = 0.05] \). Multiple comparisons revealed a significant difference in BPs between Heroin and Vehicle Injection groups after pre-treatment with 0.5 mg/kg YOH. The ANOVA comparing responses on the active and inactive levers in the Vehicle and Heroin Injections groups (Figures 6B and 6C) revealed a significant Lever by YOH pre-treatment interaction \( [F(1, 42) = 4.81, p < 0.05] \), as well as significant main effects of Lever \( [F(1, 42) = 14.28, p < 0.001] \), YOH pre-treatment \( [F(1, 42) = 11.31, p < 0.01] \), and Injection Group \( [F(1, 42) = 4.48, p < 0.05] \). Multiple comparisons revealed that 0.5 mg/kg YOH elevated responding over 0 mg/kg YOH only in the Heroin Injection group. Furthermore, after pre-treatment with 0.5 mg/kg YOH, animals in Heroin Injection group responded significantly more on the active lever than rats in the Vehicle Injection group.

3.2. Experiment 2

In this experiment, rats were tested for acquisition (Figure 7A) and resumption of heroin self-administration (Figure 7B) using the CR schedule. Differently from Experiment 2, rats did not receive vehicle or heroin injections after self-administration. The ANOVA comparing infusions identified only a significant main effect of Session \( [F(10, 160) = 10.29 p < 0.001] \). That is, multiple comparisons revealed the expected
increase of infusions over the period of acquisition, but no difference between self-administration session 10 and the resumption test. The ANOVA comparing responses on the active and inactive levers also identified the expected significant Session by Lever interaction [F(10, 160) = 2.46, p < 0.01], as well as significant main effects of Session [F(10, 160) = 6.61, p < 0.001] and Lever [F(1, 85) = 19.78, p < 0.001]. Importantly, operant responding reinforced by heroin was statistically indistinguishable between session 10 of self-administration and the resumption test.

PR responding is represented in Figures 7C, 7D, and 7E. The ANOVA on BPs identified a significant main effect of PR Test [F(2, 32) = 6.75, p < 0.01], and multiple comparisons revealed significant differences between PR Test BL and PR Tests I and II. The ANOVA comparing responses on the active and inactive levers identified a significant Lever by PR Test interaction [F(2, 32) = 4.66, p < 0.05], as well as significant main effects of Lever [F(1, 32) = 16.69, p < 0.001] and of PR Test [F(2, 32) = 9.08, p < 0.001]. Multiple comparisons revealed significant differences in active lever responses between PR Test BL and PR Tests I and II.

4. Discussion

In Experiment 1, animals were trained to self-administer heroin (single dose: 0.05 mg/kg/infusion) on a continuous reinforcement (CR) schedule for 10 days. Four hours after each self-administration session, they received injections of vehicle or escalating doses of heroin (Vehicle and Heroin Injection groups, respectively). The goal was to create different levels of withdrawal prior to each self-administration session (delay between last heroin injection and heroin self-administration was 12 hours) and hence more prominent interoceptive conditioning in the Heroin Injection group.
During self-administration, the Heroin Injection group obtained progressively more heroin infusions, and emitted more responses on the active lever (see Figures 2A and 2B). Although this effect was smaller than anticipated, the finding is consistent with previous studies indicating that non-contingent opiate exposure increases opiate self-administration (Walker et al., 2003; Vendruscolo et al., 2011). But, it is known that intake on a CR schedule is inversely related to the drug dose/drug potency (Xi et al., 2004; Sim-Selley et al., 2000). Therefore, it is possible that enhanced self-administration in the Heroin Injection group was not due to increased reinforcing effect of heroin, but rather due to a decrease in heroin potency caused by tolerance.

To disambiguate the direction of the effect observed on the CR schedule, rats were also tested for heroin self-administration on a PR schedule, the morning after the last set of heroin injections. Responding on this schedule, and BPs achieved, are directly related to drug dose (Roberts and Bennet, 1993) and considered indicative of the reinforcing effect of a drug (for review see Stafford, LeSage and Glowa, 1998; Markou et al., 1993). Surprisingly, when BPs achieved on PR Test I by the Heroin and Vehicle Injection groups were compared, no differences emerged (see Figure 3A). There may be three possible reasons for this lack of group difference. First, it is possible that the supplemental heroin injections did not augment withdrawal over and above self-administration of heroin alone. However, this is unlikely because low doses of naloxone had larger effects on locomotion, wet dog shakes, and body weight in the Heroin Injection group. Second, it is possible that the ratio of escalation employed in the PR schedule was too steep, and therefore not sensitive to changes in reinforcing effect of heroin. However, rats in Experiment 2 displayed a clear increase in BPs from PR Test
BL to PR Test I, and this is consistent with studies that used a PR schedule with a more gradual rate of escalation (Cummins et al., 2008). Finally, it is possible that the interoceptive conditioning hypothesis may fail to predict self-administration behaviour on PR schedule during dependence because alleviation of withdrawal would be experienced after the first heroin self-infusion of the session (Roberts and Bennett, 1993).

The hypothesis outlined by Wikler (1971) also predicts that the augmented reinforcing effect of heroin “learned” during self-administration in a dependent state would be evident during resumption of self-administration after a period of abstinence. However, in Experiment 1, Heroin and Vehicle Injection groups did not differ on BPs achieved on the PR Test II used for the test of resumption. Although this could be viewed as evidence against the hypothesis, it should also be noted that BPs on PR Test II were much lower than the BPs achieved on PR Test I (compare Figures 3A and 5A). This finding cannot be explained simply by the “difficulty” of the schedule, because the schedule was identical. It is also unlikely that extinction diminished the reinforcing effect of heroin because it is known that extinction does not “erase” what has been learned during conditioning (for review see Bouton, 2000). But, there is evidence that prolonged extinction training can slow down reacquisition of operant responding (Ricker and Bouton, 1996; Bouton, 1986), and that rapid reacquisition is observed primarily when the context of conditioning is renewed over the context of extinction (Bouton, 2002; Bouton and Swartzentruber, 1989). Therefore, group differences may not have been observed on PR Test II because the schedule was not different enough from extinction. Supporting this context-based interpretation, when resumption was explored using the
CR schedule in Experiment 2, responding was identical to the last day of acquisition on the same schedule (Figures 7A and 7B), and the BPs on the subsequent test were identical to the BPs observed before a drug-free period and extinction (Figure 7C). This latter finding is notable because it indicates that one session of resumption of self-administration can be sufficient to increase BPs to levels that were observed prior to abstinence and extinction. This is consistent with the findings that reacquisition of heroin self-administration can be very rapid (Leri and Stewart 2002).

Lastly, it was predicted that renewed self-administration of opiates after abstinence should be directly affected by the induction of a withdrawal state. Again, however, the prediction was not supported: after YOH pre-treatment, Heroin and Vehicle Injection groups did not differ on BPs achieved on the PR Test II during the test of resumption. Although it is possible that the dose of YOH used was not appropriate to reveal group differences, the same dose was effective in inducing reinstatement of operant responding in rats that self-administered vehicle on the PR Test II. Incidentally, YOH-induced reinstatement was significantly greater in the Heroin Injection group, a finding consistent with observations that the amount of heroin intake during self-administration is associated with enhanced reinstatement (Ahmed et al., 2000). Why this effect was not observed in the resumption groups is not clear, although it is possible that heroin gained control on responding on the PR schedule in spite of the YOH pre-treatment. Also, it should be mentioned that yohimbine is often used as a pharmacological stressor to precipitate reinstatement of operant responding previously reinforced by cocaine (Feltenstein and See, 2006), alcohol (Lê et al., 2005), methamphetamine (Shepard et al., 2004) or nicotine (Feltenstein et al., 2012).
Therefore, the interpretation of the present reinstatement data within a context of precipitation of a withdrawal-like state remains tentative.

These tests of the interoceptive hypothesis proposed by Wikler are not without limitations. Roberts and Bennett (1993) demonstrated that, on PR schedules, once animals receive the first infusion of heroin, motivation for further infusions is diminished. Therefore, tests of resumption using multiple PR tests may have produced different results. Also, testing additional doses of heroin and YOH would be required to better characterize the pharmacological aspects of the primary findings. Finally, because active and passive drug exposure have different biological effects (see review Jacobs et al., 2003), it is possible that long access self-administration models (Ahmed et al., 2000; Chen et al., 2006) may have been better suited to study how dependence influences relapse vulnerability. Regardless of these limitations, the results of the current study suggest that relapse vulnerability cannot be uniquely ascribed to altered reinforcing action of drugs; contextual and other conditioning factors must play a role in modulating resumption of drug intake after periods abstinence.

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6. References

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7. Figure Legends

**Figure 1.** Experimental design, sample size and conditions/tests employed in Experiment 1.

**Figure 2.** Mean (SEM) infusions (A), active (B) and inactive (C) lever responses on a continuous reinforcement schedule of reinforcement during the acquisition of 0.05 mg/kg/inf heroin self-administration. The Vehicle (n = 47) and the Heroin (n = 40) Injection groups received subcutaneous injections of vehicle (saline) or heroin at 3 PM, 5 PM and 7 PM. The drug doses employed in the Heroin Injection group escalated across the 10 days of self-administration according to the regimen: 1, 2, 3, 4, 4, 6, 6, 8, 8, and 8 mg/kg. The * indicates significant differences within session between Vehicle and Heroin Injection groups.

**Figure 3.** Mean (SEM) breakpoints (A), active (B) and inactive (C) lever responses on a progressive ratio schedule of reinforcement for 0.05 mg/kg/inf heroin on day 11 in Vehicle (n = 47) and Heroin Injection (n = 40) animals.

**Figure 4.** (A) Mean (SEM) total distance moved (cm) during the initial 15 minutes of a 2 hour test of motor behaviour after 0.1 mg/kg NAL in Vehicle (n = 39) and Heroin (n = 28) Injection groups. (B) Mean (SEM) % weight loss and (C) mean (SEM) number of wet dog shakes in a 2- hour period after 0.1 mg/kg NAL injection. The * indicates a significant difference between Vehicle and Heroin Injection groups.

**Figure 5.** Mean (SEM) breakpoints (A), active (B) and inactive (C) lever responses during the test of resumption of self-administration of 0.05 mg/kg/inf heroin on a PR schedule in Vehicle (n = 26) and Heroin (n = 22) Injection groups after pre-treatment with 0 or 0.5 mg/kg yohimbine (YOH).
**Figure 6.** Mean (SEM) breakpoints (A), active (B) and inactive (C) lever responses during the test of reinstatement of lever pressing in extinction conditions on a PR schedule in Vehicle (n = 21) and Heroin (n = 18) Injection groups after pre-treatment with 0 or 0.5 mg/kg YOH. The * indicates a significant difference between Vehicle and Heroin Injection groups. The # indicates a significant difference from 0 mg/kg YOH.

**Figure 7.** Mean (SEM) infusions, and responses on the active and inactive levers (A) during self-administration of 0.05 mg/kg/inf heroin on a CR schedule. Animals in this experiment (n=17) did not receive injections of heroin (or vehicle) during this period. (B) Mean (SEM) infusions, and responses on the active and inactive levers during resumption of self-administration of 0.05 mg/kg/inf heroin on a CR schedule after extinction. The * indicates a significant difference between active and inactive responses within session. Mean (SEM) breakpoints (C), active (D) and inactive (E) lever responses on the PR schedule for 0.05 mg/kg/inf heroin on days 1 (PR Test BL) and 12 (PR Test I) during acquisition, and day 22 (PR Test II) during resumption. The * indicates significant differences in comparison to the PR Test BL.
Figure 1

Heroin self-administration
Saline injections
n = 47

Precipitated withdrawal
n = 30

Conditioned place avoidance
n = 17

Extinction
n = 47

Resumption
0 YOH 0.5 YOH 0 YOH 0.5 YOH 0 YOH 0.5 YOH 0 YOH 0.5 YOH
n = 10 n = 11 n = 13 n = 13 n = 6 n = 12 n = 9 n = 13

Heroin self-administration
Heroin injections
n = 40

Precipitated withdrawal
n = 27

Conditioned place avoidance
n = 13

Extinction
n = 40

Resumption
0 YOH 0.5 YOH 0 YOH 0.5 YOH 0 YOH 0.5 YOH 0 YOH 0.5 YOH
n = 9 n = 13
Figure 3

(a) Breakpoint

(b) Responses-active

(c) Responses-inactive

- Vehicle Injection
- Heroin Injection

Mean (SEM)
Figure 4

(A) Mean (SEM) distance moved (cm) over session time (min) for Vehicle Injection and Heroin Injection groups.

(B) Mean (SEM) % body weight loss for Vehicle Injection and Heroin Injection groups.

(C) Mean (SEM) wet dog shakes for Vehicle Injection and Heroin Injection groups.
Figure 6

(A) Breakpoint

(B) Responses-active

(C) Responses-inactive

Mean (SEM)

Vehicle Injection
Heroin Injection

YOH dose (mg/kg)

0.0 0.5

0 40 80 120 160
Figure 7

(A) Infusions, Active responses, Inactive responses

(B) Resumption

(C) Breakpoint

(D) Responses (active)

(E) Responses (inactive)

Progressive Ratio Test

Mean (SEM)
**Additional Experiment**

An additional experiment not included in the above manuscript explored the effect of YOH-induced startle reactivity in previously dependent rats. The objective of this experiment was to explore whether the anxiogenic effects of YOH were enhanced by previous exposure to repeated injections of heroin. It was predicted that repeated injections of heroin during dependence would activate stress systems, which would remain active through protracted withdrawal (Koob & LeMoal, 2000). Therefore, the Heroin Injection group should be more sensitive to the anxiogenic effects of YOH compared to the Vehicle Injection group.

**Startle chambers**

Four SR-LAB startle chambers/isolation cabinets were purchased from San Diego Instruments (San Diego, CA). A high frequency loud speaker produced a background white noise of 65 dB and startle pulse stimuli of 120 dB. Whole-body startle was digitized by piezoelectric accelerometers. Startle responses were recorded by SR-LAB software.

**Methods**

Animals in this experiment did not receive intravenous surgery and were not trained to self-administer heroin. Two groups of rats were included, Vehicle Injection (n = 15) and Heroin Injection (n = 15), treated as described in Experiment 1. Following a 13-day drug-free period animals received two tests of startle (Geyer and Swerdlow, 2001) separated by 24 hours: one test following an injection of vehicle (Startle Test I) and the other following an injection of vehicle or yohimbine (2.5 mg/kg, IP; Startle Test II). Fifteen minutes after the injections, rats received a 5-min acclimation period to a 65-
dB background noise in the chamber, followed by 11 presentations of the startle stimulus at 120 dB. Startle stimuli were 40 ms long and were separated by a variable interval of 15 s (range, 8–23 s). The output of the piezoelectric accelerometer produced a score for each millisecond of a 200-ms period, which began with the onset of the startle-eliciting stimulus. A measure of startle response amplitude was derived from the mean millivolt of 100 data points collected from stimulus onset at a rate of 1 kHz. The primary dependent measure was mean difference in startle magnitude from Startle Test I to II.

**Results**

The ANOVA comparing the mean difference in startle response from Startle Test I to II (Figure 8), revealed a significant main effect of YOH dose \( [F(1, 26) = 13.72, p = 0.001] \), but no group effect. Thus, YOH caused an amplification of startle magnitude at 120 dB that was equivalent in Vehicle and Heroin Injection groups.

**Summary**

Prior heroin dependence did not alter startle reactivity after an injection with YOH.
**Figure 8.** Mean (SEM) difference in startle magnitude from habituation to test of startle in Vehicle and Heroin Injection groups after pre-treatment with 0 or 2.5 mg/kg (I.P) YOH.
General Discussion

Summary of results

Wikler and Pescor’s (1970) interoceptive conditioning hypothesis proposes that the interoceptive effects of opiates acquire conditioned reinforcing properties when a dependent subject repeatedly experiences the alleviation of withdrawal by drug self-administration. This hypothesis leads to three predictions. First, opiate dependence should alter opiate self-administration because of the augmented reinforcing properties of the drug. Second, these augmented reinforcing properties will be learned, and therefore will also be evident during the resumption of opiate self-administration after a period of abstinence. Third, resumption of self-administration after abstinence should be directly affected by the induction of a withdrawal state.

In the current study, animals were trained to self-administer heroin on continuous reinforcement (CR) schedule. Four hours after the end of each session, rats received three injections (SC) of vehicle or heroin (Vehicle and Heroin Injection groups, respectively). The experiment yielded five findings: (1) the Heroin Injection group obtained more infusions and emitted more responses during acquisition of heroin self-administration; (2) there were no group differences between Vehicle and Heroin Injection groups on responding for heroin on a PR schedule either before or after abstinence and extinction; (3) the induction of withdrawal by YOH had no effect on the resumption of heroin self-administration. Although unexpected, the experiment revealed that the Heroin Injection group emitted (4) more responses on the active lever during the initial extinction session, and displayed (5) greater reinstatement of responding after a challenge with YOH. Based on the results from Experiment #1, two additional
experiments were carried out. Experiment #2 explored the resumption of heroin self-administration using a combination of CR and PR schedules. The results of Experiment #2 indicate that the introduction of a CR session prior to testing on a PR schedule renews responding for heroin. Experiment #3 explored whether prior heroin dependence altered the anxiogenic properties of YOH on a test of startle reactivity. Findings suggest that prior heroin dependence did not alter startle reactivity after pre-treatment with YOH. Taken together, these results indicate that relapse vulnerability cannot be solely ascribed to altered reinforcing action of drugs; other factors like contextual cues, may play a role in modulating resumption of drug intake after a period of abstinence.

**Acquisition of heroin self-administration**

During acquisition of heroin self-administration, the Heroin Injection group displayed greater drug intake and responded more for heroin on a CR schedule than the Vehicle Injection group. This finding was consistent with previous studies that found non-contingent opiate exposure increased opiate self-administration (Walker et al., 2003; Chen et al., 2006). For example, Walker et al (2003) implanted rats with morphine or placebo pellets. Rats then self-administered heroin over 9 days. Results suggest that an implantation of morphine pellets lead to greater escalation of drug-intake during 8-hour self-administration sessions.

This escalation of drug intake could be mediated by withdrawal or tolerance. It was reasoned that, since, opiate withdrawal symptoms are maximal at 12 to 24 hours after last exposure to heroin (Wikler & Pescor, 1967), self-administration sessions the next day worked to alleviate withdrawal. However, since greater opiate exposure leads
to greater withdrawal (Espejo, Cador, & Stinus, 1995) the Heroin Injection group may require more heroin to alleviate the withdrawal symptoms. This is consistent with a study by Gerak et al (2009) where monkeys self-administered heroin until the disappearance of withdrawal symptoms. These results also support Wikler’s prediction that opiate dependence should enhance self-administration in the Heroin Injection group, because of augmented reinforcing properties of the drug due to interoceptive conditioning.

Alternatively, tolerance could also mediate the difference in responding between groups. As tolerance to heroin develops, there would be a decrease in reward sensitivity and therefore a subsequent escalation of heroin intake to experience the same rewarding effects (Kenny et al., 2006). Hence, enhanced self-administration in the Heroin Injection group could be driven by a decrease in heroin potency, rather than an increase in the reinforcement for heroin. A pilot study (unpublished) explored the development of tolerance to the locomotor effects of heroin using the schedule of heroin injections in the current experiment (see stats in Appendix). A comparison of day 8 and day 10 of heroin injections revealed that tolerance developed to the inhibitory action of heroin on locomotor activity (Figure 11). This result is consistent with previous studies that show repeated exposure to opioids leads to a sensitization of locomotor activity (Timar, Gyarmati, & Furst, 2005; Babbini & Davis, 1972).

A PR schedule was implemented to disambiguate whether the pattern of responding and intake on a CR schedule was due to the increased reinforcing effect of the drug, or rather due to a decrease in heroin potency caused by the development of tolerance. Following acquisition of heroin self-administration on a CR schedule, rats
were administered a test of heroin reinforcement on a PR schedule (PR Test I). It was predicted that there would be an increase in the motivation to obtain heroin in the Heroin Injection group, because of the enhanced incentive for the drug. This difference should manifest itself on the PR schedule; however, contrary to the prediction there was a lack of differences in BP. This lack of difference can be attributed to various reasons. First, it is possible that the additional heroin injections in the evening did not increase dependence or withdrawal over the self-administration of heroin alone. However, this is unlikely because low doses of naloxone had larger effects on locomotion, wet dog shakes, body weight, and conditioned place avoidance in the Heroin Injection group. Second, it is possible that although rats in this group were more sensitive to naloxone-precipitated withdrawal, they did not display spontaneous withdrawal in the morning prior to the self-administration session. However, it has been demonstrated that spontaneous heroin/morphine withdrawal in rats emerges within approximately 8 hours after the last administration (Yoburn et al., 1985; Wikler & Pescor, 1969), and the beginning of self-administration on day 11 occurred approximately 12 hours after the last heroin injection.

Alternatively, it is possible that the interoceptive conditioning hypothesis failed to predict self-administration behaviour during dependence because alleviation of withdrawal was experienced as a result of the first heroin self-infusion of the day, and therefore any subsequent infusion would not necessarily be more reinforcing to the rat (Roberts & Bennett, 1993).
Precipitated withdrawal

It has been demonstrated that symptoms of withdrawal are indicative of a dependent state in rats (Koob & LeMoal, 2006). Measures of body weight loss, wet dog shakes, and locomotion have found to be reliable indicators of withdrawal (Espejo et al., 1995) and hence were used in the current to study to infer a state of dependence. Following PR Test I, indexes of physiological withdrawal were assessed after a 0.1 mg/kg NAL injection. The investigation revealed reduced locomotor activity in the Heroin Injection group after pre-treatment with 0.1 mg/kg NAL. Additionally, the Heroin Injection group scored higher on the number of wet dog shakes and showed a greater total % body weight loss in a 2 hour period. These results are consistent with previous studies where increased opioid exposure was correlated with greater signs of withdrawal (Espejo et al., 1995).

Additionally, the termination of heroin injections revealed weight loss in the Heroin Injection group, however weight gain in the Vehicle Injection group (Figure 12) within the first 24 hours (Abstinence day #1). These differences disappear on Abstinence Day #2. Taken together these results suggest that the manipulation was effective; the Heroin Injection group was in a greater state of withdrawal.

PR Test II: Resumption of heroin self-administration

In accordance with the interoceptive conditioning hypothesis proposed by Wikler & Pescor (1970) the schedule of heroin injections in the evening should create withdrawal symptoms, that are suppressed when heroin self-administration is resumed the following morning. These repeated cycles (10 sessions) of withdrawal at night and suppression the following morning should allow for the sensorial effects of heroin to
acquire properties of a secondary reinforcer. As a result, it is expected that there should be an augmentation of the reinforcing properties of heroin in the Heroin Injection group. Consequently, after abstinence they should self-administer heroin over what might be found with just the primary reinforcing effects. However, this prediction by the interoceptive conditioning hypothesis was not supported; there were no group differences in BPs achieved on PR Test II.

Although, the data presents evidence contrary to the interoceptive conditioning hypothesis proposed by Wikler and Pescor (1970), it may not necessarily suggest a revision of the hypothesis. It may be possible that extinction training interfered with the retrieval of learning during previous conditioning (acquisition). It should be noted that extinction does not destroy original learning but instead reflects new learning (Bouton, 2002). This has been demonstrated in studies that show original learning can be restored through four mechanisms of relapse: reinstatement, renewal, spontaneous recovery, and reacquisition. However, there is evidence to suggest that factors like prolonged extinction training slow down the reacquisition of operant responding (Ricker & Bouton, 1996; Bouton, 1986). Bouton (2002) suggests that following extinction a conditioned stimulus (CS) is left with two meanings: (1) CS is associated with the drug and (2) CS is not associated with the drug. Following extinction, behaviour depends on the context. For example, Bouton and Swartzentuber (1989) found that when a context promotes retrieval of extinction, there is a slower reacquisition; however this effect is eliminated when cues that retrieve extinction are removed. Additionally if a cue present during initial conditioning is present during re-conditioning, re-conditioning is rapid (Bouton and Swartzentuber, 1989).
In the current study, it is possible that following extinction the presentation of the drug on a PR schedule did not have enough information value to signal that heroin was available again (lack of contextual cues). This is consistent with studies that show a rapid reacquisition when the context of conditioning is renewed over the context of extinction (Bouton & Swartzentruber, 1989). The results of Experiment 2 seem to support this context-based interpretation. In Experiment 2, when the first resumption session was performed on a CR schedule, responding was identical to the last day of acquisition on the same schedule, and the BPs on the subsequent test using the PR schedule were identical to the BPs observed before abstinence and extinction. It may be possible that the number of US-CS pairings in the CR session returned the animal to the conditioning context. Therefore, taken together, the results of Experiments 1 and 2 indicate that during resumption, the rate of self-administration could be influenced by an interaction between extinction training, and the schedule of self-administration employed.

**PR Test II: Yohimbine induced resumption**

Lastly, it was predicted that internal cues of withdrawal brought back by YOH after abstinence would renew self-administration for heroin. Previous studies have shown that experience with drug-taking during withdrawal may modulate incentive learning for that drug and drug-related cues (Dickinson & Balleine, 1994; Hutcheson et al., 2001). For example, Hutcheson et al. (2001) found that simply precipitating withdrawal did not modulate heroin-seeking if the animals had only experienced taking drug in the maintained state. However, experience with heroin-taking during withdrawal increased heroin seeking, when withdrawal was later precipitated. Consequently, in the
current experiment it was expected that the resumption of heroin self-administration should be enhanced in the Heroin Injection group, because of previous state dependent learning enhancing the incentive for heroin. Again, the prediction was not supported. After YOH pre-treatment, the Heroin and Vehicle Injection groups did not differ on BPs achieved on PR Test II. Although it is possible that the dose of YOH used was not appropriate to reveal differences in resumption of heroin self-administration, the same dose was effective in inducing reinstatement of operant responding in rats that self-administered vehicle on PR Test II.

**PR Test II: Yohimbine induced reinstatement**

The effect of YOH in reinstating lever pressing is consistent with previous studies that have found that non-contingent exposure to a stressor can interrupt the expression of extinction learning and reinstate drug-seeking behaviour (Erb, 2006; Banna et al., 2010; Le et al., 2005). Although unexpected, in addition to a reinstatement of lever pressing, there was evidence to show that prior heroin dependence increased reinstatement to YOH; the Heroin Injection group attained higher BPs than the Vehicle Injection group. The finding that the amount of heroin exposure during training is related to the magnitude of reinstatement has been previously been shown. For example in a study by Zhang et al. (2004) rats were trained to self-administer different doses of heroin over 4-h daily sessions. They found that rats trained with higher doses of heroin showed greater cue-induced reinstatement (Zhang et al., 2004). Similarly, Ahmed et al. (2000) showed that rats trained 11 h/day for heroin showed a greater reinstatement in responding after footshock compared to rats trained for 1 h/day. The current experiment
provides evidence that the effect of prior heroin dependence on the magnitude of reinstatement can also applied to stressors like YOH.

An additional experiment (Experiment #3) employed startle reactivity to investigate whether the anxiogenic effects of YOH (Davis et al., 1979) were enhanced by repeated injections of escalating heroin doses. Experiment #3 revealed that although yohimbine amplified startle reactivity, there was no effect of prior heroin dependence. It could be reasoned that the YOH dose was too high, therefore creating a ceiling effect and interfering with the ability to detect changes in reactivity. However, it could also be possible that prior heroin dependence did not alter the anxiety-like responses to stress, but altered the salience of drug cues as a response to stress. In other words, drug cues become salient after stress and prior heroin dependence alters this salience of drug cues, which is not a simple pharmacological effect caused by YOH.

However, it is perplexing that YOH affected instrumental behaviour of lever pressing but had no effect on the consummatory behaviour for heroin. There is a possibility that heroin gained control over responding, however further experiments are needed to explore this effect.

Prior heroin dependence and incubation

Previous studies have shown an increase in cue-induced drug seeking as a function of time, also known as "incubation of craving" (Shalev et al., 2001; Airvaara et al., 2010). For example, Shalev et al. (2001) trained rats to self-administer heroin for 10 days on a CR schedule. Following self-administration, rats were assigned to different withdrawal periods and subsequently responding was observed in extinction conditions. They found an increase in responding that followed an inverted U curve, after 6, 12, and
25 days from the last exposure to heroin. As a result, after a 4-day drug-free period a planned comparison was conducted on the first day of extinction (see Appendix).

Although unexpected, it was found that the Heroin Injection group emitted more responses on the active lever on the initial extinction session (Figure 10). These results cannot be attributed to an increase in responding on the active lever during acquisition because differences in responding between the groups disappear on day 10 of acquisition and on PR Test I. The current study may be the first to demonstrate that animals with greater heroin exposure during self-administration show an enhanced "incubation" effect. These results also provide further evidence that the manipulation had different effects in the two groups.

**Decrease in BP from PR Test I to PR Test II**

Another interesting finding was the significant drop in BP from PR Test I to PR Test II (see statistics in Appendix). This pattern of results is consistent with a study by Zhang et al. (2007). In their study, animals were trained to nose poke for sucrose under a PR schedule and were subsequently administered low or high doses of morphine. After a drug-free period, they found a significant decrease in BP for sucrose. In fact, this significant decrease in BP has also been seen with other classes of drugs like d-amphetamine after a drug-free period (Barr & Phillips, 1999).

Since rats obtained high BPs during PR Test I, but not PR Test II, it is possible that the high BPs were achieved because of behavioural momentum that was built up over the course of acquisition. However, the results of Experiment #2 eliminate "behavioural momentum" as an explanation. After abstinence and extinction animals re-introduced to heroin on a CR schedule, only once, showed responding for heroin similar
to levels obtained on the last day of acquisition. Subsequently, a PR test the next day (PR Test II), showed a reacquisition of heroin self-administration by rats. In fact, BPs during PR Test II were similar to those achieved during PR Test I (Figure 7).

Alternatively, it could be reasoned that after a 13-day drug-free period the potency of heroin increased because of diminished tolerance, resulting in a rate-limiting effect on responding that heroin induced because of its pharmacological effects. However, again the results of Experiment #2 suggest this is untrue. Rats re-introduced to heroin on a CR schedule of reinforcement (1 session) after abstinence and extinction show responding on the active lever and drug-intake similar to levels obtained on the last day of acquisition. It is possible, as previously mentioned, that the drop in BP is a result of extinction training interfering with the retrieval of original learning.

**Conditioned tolerance**

It could be possible that differences observed in acquisition were a result of conditioned tolerance. For example, Siegal & Patenall (1999) suggest that early drug onset cues may become signals for the later larger drug effects. Therefore, it could be possible that because the Heroin Injection group received subcutaneous heroin injections it induced a slow and gradual onset of drug effects, which allowed for the conditioning of the early drug onset cues to later larger drug effects. In this case, a heroin infusion may be more reinforcing for the Heroin Injection group. However, this is unlikely, as we should still expect to see differences on PR schedule during dependence and relapse, however, none were observed.
Methodological considerations

This study is not without its limitations. For example, Roberts & Bennett (1993) demonstrated that on PR schedules, once animals receive the first infusion of heroin, motivation for further infusions is diminished. Hence, it may be difficult to pick up differences in heroin reinforcement. However, Roberts and Bennett (1993) designed a modified PR schedule that better characterizes the differences in heroin reinforcement, which may have been a better alternative to the PR schedule employed in the current study. Additionally, active and passive drug exposure has different biological effects (see review Jacobs et al., 2003). For example, brain activity is altered preceding active, but not passive morphine self-administration. There are short and long-term neuroadaptive effects in the brain that depend on whether passive or active self-administration is used. Another potential limitation of the study is the difference in the number of rats excluded from the analysis. More than three times as many rats in the Heroin Injection group (n = 18) were excluded from the analysis than the Vehicle Injection group (n = 5). An analysis of operant responding (not shown) showed no evidence of discrimination between the active and inactive levers by both the Vehicle and Heroin Injection groups over the course of self-administration, suggesting that the heroin injections did not contribute to a decrease in operant responding (Figure 13). Perhaps the differences in the number of excluded rats was accidental. Lastly, the conclusions from this study would benefit from testing of additional heroin and yohimbine doses to better characterize the pharmacological aspects of the primary findings.
Future directions

*Behavioural studies using PR responding over acquisition*

Self-administration on a PR schedule, instead of a CR schedule (in Experiment #1) during acquisition may better characterize the motivational differences in heroin reinforcement. For example, Roberts and Bennett (1993) showed that a between session assessment of BP for heroin on a modified PR schedule was better suited for characterizing motivation towards heroin. On this modified schedule, the response requirement for the first infusion is two PR steps below the final ratio obtained on the previous day (see Roberts and Bennett, 1993). This between session approach has shown to be sensitive to changes in heroin reinforcement over the course of acquisition by Cummins et al. (2008).

*Behavioural studies using active drug exposure*

It may be possible that long access self-administration models (Ahmed et al., 2000; Chen et al., 2006) are better suited to study the effects of dependence. Previous studies have demonstrated drug induced alterations in active but not passive drug exposure, in the following areas: operant behaviour, concentrations of dopamine, acetylcholine, 5-HT, GABA etc, and expression of protein (Jacobs et al., 2003). Therefore, instead of using experimenter-administered injections, long access models used to create different levels of dependence may better characterize drug addiction.

*Behavioural studies exploring resumption on CR/PR combination schedules*

Based on the findings from the current experiments, it is a possibility that the lack of difference in resumption (Experiment #1) was mediated by interference in the retrieval of previous learning during conditioning. Therefore, the question of whether
heroin dependence affects resumption of heroin self-administration may not have been answered. Perhaps, resumption on a CR schedule prior to tests on a PR schedule is a better method of characterizing the effects on resumption of heroin self-administration.

Additionally, it would be interesting to explore how YOH pre-treatment during relapse on a CR schedule would affect PR responding for heroin the following day. It would also be interesting to look at whether this new method produces BPs that are sensitive to unit doses of heroin.

**Heroin dependence and incubation of craving**

Another extension of the current experiments would be the exploration of the effects of prior heroin dependence on incubation of drug craving. It may help to further elucidate the role of prior dependence on goal directed behaviour. Additionally, an exploration of the molecular mechanisms involved may help to inform treatments.

**Neurobiological studies of YOH-induced reinstatement and YOH induced resumption**

A logical extension of the current experiments would be to explore why YOH affected instrumental responding, but not consummatory. Perhaps, heroin gains control over responding. Hence, it would be interesting to use c-fos immunohistochemistry to explore whether NA and dopamine systems in the central nucleus of the amygdala, BNST, NAcc, prefrontal cortex, all which have been implicated in stress induced reinstatement (review Stewart, 2003), are affected when heroin self-administration is resumed on a PR schedule.

**Enhancement of interoceptive conditioning**

It could also be possible that since the maximum effect of a drug is more immediate during an intravenous infusion, there was little or no opportunity for the early
drug onset cues to be associated with the reduction of withdrawal. As a result, there would be no differences in the amount of interoceptive conditioning. Future studies would benefit from the addition of methods that could enhance the opportunity for interoceptive conditioning. For example, a protracted infusion may allow for the gradual onset of drug effects therefore promoting the reestablishment of interoceptive conditioning (Kim et al., 1999).

**Conclusions**

In conclusion, although results did not support the interoceptive conditioning hypothesis, the results of experiment #1 and #2 suggest that the schedule of reinforcement employed has a profound effect on responding after abstinence and extinction. Although the ability of the drug to maintain responding was lost after extinction (Experiment #1) the introduction of one CR session, elevated PR responding to levels observed during dependence (Experiment #2). Therefore, Experiment #2 provides a technique for studying the resumption of opioid self-administration in rats on a PR schedule. Perhaps we have not answered the question of whether interoceptive conditioning plays a role in relapse. This is an important area of research as avoiding relapse is an important component of recovery from drug addiction; and identifying factors that underlie relapse will lead to a greater understanding of what drives individuals to relapse to drug use.
References


Appendix

Results

Extinction

An ANOVA comparing responses on the active and inactive lever in the Vehicle and Heroin Injections groups identified a significant Session by Lever interaction \[F(8, 672) = 42.62, p < 0.001\], as well as main effects of Session \[F(8, 672) = 87.46, p < 0.001\] and of Lever \[F(1, 85) = 96.11, p < 0.001\]. Multiple comparisons indicated that responding on the active lever and inactive lever decreased significantly in both groups from session 1 to session 9. On the last session of extinction, both groups displayed similar responding on the active and inactive levers. A planned comparison on day 1 of extinction revealed a significant difference between Vehicle and Heroin Injection groups. The Heroin Injection group showed greater responding on the active lever than the Vehicle Injection group (Figure 10).

Weight loss over abstinence

An ANOVA comparing weight loss over the 4-day Abstinence period in the Vehicle and Heroin Injection groups identified a significant Day by Injection interaction \[F(3, 252) = 19.53, p < 0.001\], as well as main effects of Day \[F(3, 252) = 15.52, p < 0.001\] and Injection \[F(1, 84) = 17.14, p < 0.001\]. Multiple comparisons indicated that there was significant difference in weight gain between Vehicle and Heroin Injection groups. In fact the Heroin Injection group lost weight, while the Vehicle injection group gained weight on the first day of Abstinence.
Assessment of locomotion through tolerance

An ANOVA comparing total distance moved after an 8 mg/kg on day 8 & 10 of main effects of day \[F(1, 11) = 8.12, p < 0.05\] and time \[F(11, 121) = 2.38, p < 0.05\]. Multiple comparison of 10 minute bins revealed differences between day 8 & 10 at 10, 70, 80, 90, 100, & 110 minutes.

Decrease in BP from PR Test I to PR Test II

There were no differences in BP in the resumption conditions after pre-treatment with Vehicle or 0.5 mg/kg YOH. Consequently, the data was collapsed across pre-treatment and a two-factor mixed design ANOVA was conducted using Injection Group (Vehicle or Heroin) and PR Test (PR test I and PR test II) as factors. According to the significant main effect of test \[F(1, 48) = 11.52, p = 0.01\], there was a significant decrease in BP from PR Test I to PR Test II in both Vehicle and Heroin Injection groups for 0.05 mg/kg/inf.
Figure 9. Mean (SEM) breakpoints on a progressive ratio schedule of reinforcement for 0.05 mg/kg/inf heroin on PR Test I and PR Test II in Vehicle and Heroin Injection animals.
Figure 10. Mean (SEM) active and inactive lever responses on a continuous reinforcement schedule during extinction. The * indicates significant differences within session between Vehicle and Heroin Injection groups.
Figure 11. Mean (SEM) weight change (g) from the previous day after termination of heroin. The * indicates a significant difference between Vehicle and Heroin Injection groups.
**Figure 12:** Mean (SEM) distance moved (cm) during 2 hour test of motor behaviour after 8 mg/kg heroin injection Vehicle (n = 12) and Heroin (n = 12) Injection groups. The * indicates a significant difference between day 8 and day 10.