Oxytocin As a Treatment for Addiction: The Effects of Exogenous Oxytocin on Heroin Taking and Seeking in Male Rats

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ABSTRACT

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Janie Duchesneau

Drug addiction is characterized by recurring episodes of relapse and has a profound impact on individuals' well-being as well as our economic and healthcare systems. Although many efforts have been put into developing solutions to treat drug addiction, an effective long-term treatment has yet to be found. Previous animal studies indicate that exogenous oxytocin (OXT) holds potential to treat and reduce relapse, withdrawal and help drug cessation. More particularly, a decrease in psychostimulant drugs (e.g., cocaine and methamphetamine) consumption has been noted while earlier studies also suggested a similar role for OXT in opiate drugs (e.g., morphine and heroin) reward and withdrawal.

In light of those studies, our objective was to continue investigating the role of OXT in inhibiting heroin self-administration (SA) as well as attenuating relapse by using an animal model of stress-induced reinstatement. We examined the effects of acute and chronic administrations of OXT on heroin SA and the reinstatement of extinguished heroin seeking in male rats. Rats were tested under fixed-interval 20 s (FI-20 s) and progressive-ratio (PR) schedules of reinforcement in order to assess heroin intake. Results suggest that OXT did not attenuate heroin taking during the SA sessions under various schedules of reinforcement. Next, we tested the effect of OXT on stress-induced reinstatement of extinguished heroin seeking, by using yohimbine (YOH) as our pharmacological stressor. Contrary to our expectations, central administration of OXT did not attenuate yohimbine-induced reinstatement. In conclusion,

contrary to recent findings with psychostimulant drug-trained rats and earlier reports with heroin-trained rats, OXT did not attenuate either heroin SA or yohimbine-induced heroin seeking.

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EPM	Elevated plus maze
FI-20 s	Fixed interval 20 seconds
FR1	Fixed ratio 1 schedule of reinforcement
ICV	Intracerebroventricular
IP	Intraperitoneal
NAc	Nucleus accumbens
OXT	Oxytocin
PFC	Pre-frontal cortex
PR	Progressive ratio
PVN	Paraventricular nucleus (of the hypothalamus)
SA	Self-administration
SC	Sub-cutaneous
SON	Supraoptic nucleus (of the hypothalamus)
VTA	Ventral tegmental area
YOH	Yohimbine

General Introduction

Defined as a chronic, relapsing disorder, drug addiction is characterized by compulsive drug taking and drug seeking (O'Brien & McLellan, 1996). It is one of today's most prevalent neuropsychiatric disorders (Piazza & Deroche-Gamonet, 2013). Drug addiction is multifaceted and, even though it results in a wide range of dysfunctions and negative impacts on motivation, emotions, learning and behaviours, individuals suffering from this disorder will maintain their drug usage despite its negative effects (Piazza & Deroche-Gamonet, 2013). For example, individuals who display recurrent drug use will suffer consequences preventing them from fulfilling major role obligations or induce precarious situations when under the influence of a substance. Drug addiction might also create pharmacological tolerance and induce withdrawal symptoms and lead to persistent social problems generated by the effects of the abused substance(s) (APA, 2013).

At the macro level, drug addiction causes a substantial drain on Canada's economy (Rehm et al., 2006) by directly affecting both the healthcare and the criminal justice systems, and by indirectly affecting work-related productivity. A study published in 2006 by the Canadian Centre on Substance Abuse estimated the overall societal cost of illegal drugs to be 39.80 billion dollars per year (Rehm et al., 2006). Illicit drugs account for 20.70 % of those costs, representing a total of 8.20 billion dollars (Rehm et al., 2006). In particular, heroin use has been a considerable public health issue for Canada with a known population of 60 000 to 90 000 users, aged 15 to 64 (Fischer et al., 2002).

Heroin users have a greater likelihood of morbidity, mortality and disability in comparison to the general population (Fischer et al., 2002), and 40 to 60 % of drug abusers who successfully cease to take drugs will relapse into their drug taking habit during the following year (McLellan et al., 2000). Relapse can be triggered by cues directly associated with the abused drug

(e.g., syringes, white powder, etc.) or by environmental cues such as being exposed to a specific location where someone used to take drugs, or re-exposure to the drug itself. Stress such as divorce, death of a relative, or financial difficulty is also a factor known to trigger relapse (Hser et al., 2000).

Its principal characteristics and the plethora of undesirable consequences suggest a grim reality: Drug addiction, and more specifically heroin usage, is still a pressing social issue, and an efficient and lasting treatment has yet to be found. Various behavioural strategies and pharmacological treatments have been suggested (e.g., Jain, Majumder, & Gupta, 2013; NIDA, 2011; Volkow et al., 2007), but with limited long-term success. To date, the most effective treatment programs have included various elements, each focusing on a specific aspect of the disorder and its related consequences. For example, treatments must not only address and treat the disorder itself, but also the more indirect negative consequences such as interpersonal conflicts and job loss. In fact, drug addiction treatment should empower addicts to maintain a drug-free regimen and help them achieve an adequate level of productivity in their family, at work, and in society (NIDA, 2011). However, due to its chronic and relapsing nature, drug addiction has been shown to be incredibly difficult to treat in the long term, and achieving and maintaining a drug-free life may thus require many attempts.

Various pharmacological treatments are available as treatment for addiction to various drugs and drug classes. Some treatments target the psychoactive drugs or their receptors in an effort to block their effects. For example, active immunization with vaccines showed treatment potential against nicotine (Pentel & LeSage, 2014), cocaine as well as methamphetamine (Shen, Orson, & Kosten, 2012), and pharmacological modulators (i.e., receptor antagonists or partial agonists) such as naltrexone that block euphoric effects associated with heroin, nicotine, and alcohol intake (NIDA, 2011). A different approach is substitution/drug replacement therapies,

including transdermal patches, chewing gum, inhalers, nasal spray, and lozenge used for nicotine addiction (Jain, Majumder, & Gupta, 2013). In heroin addicts, methadone and buprenorphine are commonly used as substitution for opiates (Mattick, Breen, Kimber, & Davoli, 2014). At present, however, the reported effects seem to be only mildly effective in the long term. Consequently, many former drug addicts, even after years of abstinence, will relapse and continue this deleterious cycle of drug taking and drug seeking (McLellan et al., 2000; Siegel, 2002). In the present thesis, we sought to investigate a new method to treat heroin addiction as it is has been shown to have the greatest rate of mortality among drug users and that neither methadone nor buprenorphine seem to cure heroin users from their addiction.

Oxytocin

Oxytocin, a neuropeptide primarily known for its role in parturition & lactation (Lee et al., 2009), has been reported to also affect social behaviours, learning and memory. Importantly, earlier research suggested that OXT had the potential to inhibit the development of tolerance (Kovács & Van Ree, 1985; Kovács & Telegdy, 1987), and to reduce both withdrawal symptoms and self-administration of opiates in rodents. Recent studies have also reported a decrease in the motivation to seek and self-administer stimulant drugs such as methamphetamine (Carson et al., 2010; Westenbroek et al., 2013) and cocaine (Leon et al., 2016; Zhou et al., 2014).

Oxytocin (OXT) has been widely studied since Dr. Vincent du Vigneaud isolated the molecule and synthesized it in its active form for the first time more than four decades ago (du Vigneaud et al., 1953). Oxytocin's major expression site is situated in the hypothalamus, more specifically in the magnocellular neurons of the hypothalamic paraventricular (PVN) and supraoptic (SON) nuclei (Gimpl & Fahrenholz, 2001; Marazzti et al., 2008; Rubin & Pfaff, 2009). In response to a plethora of stimuli such as mating, pregnancy, parturition, suckling

(Rubin & Pfaff, 2009), social bonding, and some types of stress, oxytocin is released from the posterior pituitary into the blood stream (Gimpl & Fahrenholz, 2001; Rubin & Pfaff, 2009). In addition to the hypothalamic projections of oxytocin, oxytocinergic neurons display widespread projections throughout the central nervous system and both originate and affect regions such as the basal ganglia, the limbic system, the thalamus, the brainstem, the spinal cord and other cortical regions (Gimpl & Fahrenholz, 2001; Rubin & Pfaff, 2009). Oxytocin is also synthesized in peripheral tissues such as the uterus (Gimpl & Fahrenholz; Rubin & Pfaff, 2009), the ovary (Rubin & Pfaff, 2009), the mammary gland (Rubin & Pfaff, 2009), the testis (Gimpl & Fahrenholz, 2001; Rubin & Pfaff, 2009), the liver (Rubin & Pfaff, 2009), and the heart (Gimpl & Fahrenholz, 2001; Rubin & Pfaff, 2009). This neurohormone has, to date, one known receptor: OXTR. The OXTR is a G protein-coupled receptor that is primarily coupled via Gq proteins to phospholipase C (Rubin & Pfaff, 2009) and is widely distributed throughout the brain, including in reward-related areas such as the VTA and nucleus accumbens (Love, 2013).

Oxytocin as a potential treatment for drug addiction

Oxytocin, a neuropeptide primarily known for its role in ensuring homeostasis, reproductive success and social behaviours, oxytocin has been shown to be involved in both learning and addiction processes (Sarnyai & Kovács, 2013). Importantly, earlier research suggested that OXT had the potential to inhibit the development of tolerance (Kovács & Van Ree, 1985; Kovács & Telegdy, 1987), and to reduce both withdrawal symptoms and self-administration of opiates in rodents. Recent studies have also reported a decrease in the motivation to self-administer and to seek stimulant drugs such as methamphetamine (Carson et al., 2010; Westenbroek et al., 2013) and cocaine (Leong et al., 2016; Zhou et al., 2014). These reports also suggested that neuropeptides, more specifically OXT, could modulate forms of

neuroadaptation such as learning and memory processes (e.g., Davis et al., 2014) and addiction (e.g., Carson et al., 2010; Carson et al., 2013; Sarnyai et al., 1990; Sarnyai & Kovács, 1994; Sarnyai, 2011; Thompson et al., 2007). Previous studies on the role of OXT in social behaviours (e.g., Sobota et al., 2015) and learning processes (e.g., Campbell-Smith, Holmes, Lingawi, Panayi, & Westbrock, 2015) have lead scientists to believe that OXT could also mediate pro-social effects of drugs of abuse such as MDMA (i.e., ecstasy) (Sarnyai & Kovács, 2013). Thompson et al. (2007) suggested that the effects of OXT on addiction, despite being limited, are perhaps related to the heightened reward obtained from social interactions instead of the reward induced following drug usage. They have also suggested that positive and pro-social interactions, when taking place outside a drug context, could serve as a protective factor against drug use. However, positive or negative social interactions taking place within a drug-related context might interact with other factors and either precipitate or inhibit drug consumption. Other research suggests that OXT mostly inhibits memory consolidation, retrieval and other learning and memory processes (Sarnyai & Kovács, 2013). As drug addiction is often referred to as a type of pathological learning, it seems reasonable to assume that OXT might inhibit or reduce drug-related responses.

Dopamine (DA), a monoamine neurotransmitter, is thought to have a central role in the reinforcing effects of drugs of abuse (Volkow et al., 2007). Specifically, the mesolimbic dopaminergic system is strongly involved in goal-oriented behaviours (Iversen et al., 2009), attention (Iversen et al., 2009), reward (Iversen et al., 2009; Berridge, 2007), and addiction (Volkow et al., 2007). The dopaminergic neurons in the mesolimbic pathway originate from the ventral tegmental area (VTA) and project to the nucleus accumbens (NAc) and to the prefrontal cortex (PFC) (Wise, 2002). Stimulants such as amphetamine and cocaine act on the dopamine transporter and directly increase dopaminergic transmission in the nucleus accumbens (Wise,

2002; Koob, 2008) while opiate drugs inhibit GABA-ergic neurons that, in turn, disinhibit dopaminergic neurons in the VTA (Koob, 2008).

This central neuromodulator has a vast number of effects on central nervous system functions, such as mood (Baskerville & Douglas, 2010), behaviour (Baskerville & Douglas, 2010), and pair-bonding consolidation (Liu & Wang, 2003). However, recent studies have raised an interesting observation: Individuals suffering from dopamine-related disorders like schizophrenia (Rosenfield, Lieberman, & Jarskog, 2010), anxiety disorder (Abi-Dargham, 2012; Amico et al., 2004) and addiction (Sarnyai & Kovács, 2013) also had irregular levels of peripheral and central oxytocin (Baskerville & Douglas, 2010). Consequently, oxytocin has been suggested to be a pivotal neural substrate that interacts with dopamine systems (Baskerville & Douglas, 2010; Liu & Wang, 2003). Such interactions have been revealed to modulate behaviours such as social affiliation, mating and drug-altered locomotion (Kovács et al., 1990). Moreover, it has been suggested that oxytocin mediates the mesolimbic dopamine system during drug addiction processes (Baskerville & Douglas, 2010; Kovács et al., 1990; Sarnyai et al., 1990; Sarnyai et al., 1992; Sarnyai & Kovács, 1994; Sarnyai & Kovács, 2013). Morphological and functional studies have shown that oxytocin acts as a neuromodulator in (meso-)limbic structures and spinal regions (McGregor, Callghan, & Hunt, 2008). Such structures, like the nucleus accumbens, the ventral tegmental area, and the hippocampus, are innervated with oxytocinergic neurons (Gimpl & Fahrenholz, 2001; Love, 2013). These structures have previously been shown to be effective in mediating drug reward and addiction (Wise, 2002).

The importance of animal models of drug addiction

A major challenge of addiction research has been to develop animal models in order to study the multifaceted aspects of drug addiction. The complexity of this neuropsychiatric disorder

makes it highly complicated to study its etiology and underlying neurocircuitry in humans, which stresses the importance of preclinical animal models in elucidating the neuropathophysiology of addiction (Piazza & Deroche-Gamonet, 2013). Drug addiction affects multiple brain circuits that are strongly involved in reward, motivation, learning, and memory (Iversen et al., 2009; NIDA, 2011). Clearly, understanding the neural mechanisms associated with drug-related behaviours such as drug taking, drug seeking, and relapse necessitates a cellular and subcellular examination of neuronal systems (Yap & Miczek, 2008). Animal models of drug addiction thus provide pivotal insight into the conditions and mechanisms affecting the multifaceted aspects of this disorder.

Numerous empirical studies have used animal models of drug addiction. Among the advantages to using animal models is the opportunity to investigate the different phases of the drug addiction cycle (Deroche-Gamonet & Piazza, 2014), to assess the reinforcing properties of novel drugs (O'Connor et al., 2010), to assess the abuse potential of a drug (Haney & Spealman, 2008), and to achieve a better understanding of the neural systems that are involved in the development and maintenance of the disorder (O'Connor et al., 2010). Based on these findings, we used a distinctive feature of the drug self-administration model that maximizes the internal validity and reproducibility of the design while aiming at a holistic approach of significant relevance to human situations as proposed in Sanchis-Segura & Spanagel (2006). The self-administration model consists of training an animal – in our case, a rat – to perform an operant behaviour leading to the consumption of a drug by different routes of administration (i.e., intravenous infusion of heroin). In addition to the "choice" made by the animal to self-administer a given drug – in our case, heroin –, the route by which the drug is administered is pivotal to its effects on both organism and behaviour. For example, there is a shorter latency between the time an animal self-administers a drug intravenously (i.e., performs an operant behaviour and then

receives a drug through an intravenous catheter) and the experienced physiological and psychoactive effects of the drug compared to the experimenter injecting it in the periphery, either by intraperitoneal (IP) or subcutaneous (SC) injections. The association made between operant behaviour (i.e., drug seeking and consumption) and the perceived effects following it (i.e., euphoria, relaxed state, etc.) resembles human drug usage. Furthermore, the animal has complete control over the amount of drugs consumed. In other words, the total drug dose administered within a session is dependent on the behaviour of the rat (Sanchis-Segura & Spanagel, 2006; O'Connor et al., 2010). Consequently, the self-administration procedure plays a fundamental role in advancing our understanding of the abuse liability of a drug and the reinforcing effects it has on the organism.

A vast number of self-administration studies have used simple fixed-ratio (FR) schedules of reinforcement (Arnold & Robert, 1997). A fixed-ratio schedule is a schedule where a response (e.g., a lever press) is reinforced once a specific number of responses has been performed. For example, if a rat were under a fixed-ratio 1 (FR1) schedule of reinforcement, it would have to respond once (i.e., pressing a lever) to receive reinforcement (i.e., drug delivery). Similarly, the fixed-interval ratio means that a reward will be made available when a response is performed after a certain amount of time has elapsed since the last reinforcement delivery (Cosgrave, 2016). Those fixed schedules are useful for exploring behavioural patterns of drug intake and for teaching new behaviours. However, those fixed schedules of reinforcement seem inappropriate when assessing changes in the reinforcing effects of drugs, leading to several issues associated with the interpretation of the resulting fixed-ratio data (Arnold & Roberts, 1997). Indeed, studies using a fixed-ratio schedule of reinforcement base their interpretation on the changes in the rate of self-administration. However, those changes in rate of self-administration under a fixed ratio might originate from an alteration in relation to a greater increase in drug tolerance (due to the

high number of drug infusions per session), which in turn would render the shifts in rats' drug self-administration more tenuous to interpret (Roberts & Bennett, 1993).

For these reasons, a progressive-ratio (PR) schedule is better suited to studying and understanding the reinforcing properties of stimulant and opiate drugs (Arnold & Roberts, 1997; Chiodo, Läck, & Roberts, 2008). Similar to a fixed ratio, a progressive-ratio schedule is described as a schedule of reinforcement used in operant conditioning settings when an animal (i.e., a rat) is trained to produce an operant response (e.g., pressing a lever) in order to obtain a reinforcer (e.g., a drug infusion or natural reward delivery). However, following each drug delivery, the number of lever presses required to receive the next drug infusion is increased until the rat ceases to engage in self-administration or lever pressing for a prolonged period of time. This state is called breakpoint. An important factor regarding breakpoint is the fact that it is dose-dependent, hence allowing for changes in the dose-response curve to be studied. During the early part of a self-administration session under progressive ratio, the response ratios are quite small, meaning that lever presses are regularly reinforced by drug infusions. As the name suggests, the lever pressing requirements progressively increases until it exceed the reinforcing efficacy of the drug, and lever responding ceases (Arnold & Roberts, 1997). This designates the breakpoint within a single self-administration session. The breakpoint under the progressive-ratio schedule is thus used as an assessment of the rat's motivation to self-administer a drug (Arnold & Roberts, 1997; Chiodo et al., 2003).

Oxytocin treatment in animal model of drug addiction

Studies performed since the early 1980s have tested oxytocin as a potential treatment for opiate addiction using animal models (e.g., Ibragimov et al., 1987; Kovács & Van Ree, 1985; Sarnyai et al., 1990). Those findings suggested that oxytocin administration could influence

opiate (heroin and morphine) self-administration as well as the development of tolerance to various effects of opiates, cocaine and alcohol (Sarnyai et al., 1992; Kovács et al., 1998). This includes, for example, the attenuation of behavioural adaptations (tolerance of stereotyped sniffing) following a repeated cocaine administration (Sarnyai et al., 1992). In addition, similar results have been reported in relation to stimulant drugs. In fact, treatment with exogenous oxytocin was reported to inhibit cocaine-induced locomotor hyperactivity, exploratory activity, and stereotyped behaviours (Sarnyai et al., 1992; Sarnyai & Kovács, 1994). Although oxytocin also seemed to facilitate cocaine sensitization (Sarnyai et al., 1992), it was also noted that the neuropeptide inhibited cocaine tolerance and cocaine self-administration (Sarnyai & Kovács, 1994).

More recently, Carson et al. (2010) studied self-administration, locomotor hyperactivity and relapse in methamphetamine-trained rats. Rats were first trained to self-administer methamphetamine on a FR1 schedule of reinforcement and then tested on a progressive-ratio schedule. Both medium (0.30 mg/kg; IP) and high (1.00 mg/kg; IP) doses of oxytocin resulted in reduced motivation to self-administer methamphetamine whereas only the low (0.10 mg/kg; IP), medium and high doses of exogenous oxytocin reduced locomotor hyperactivity within the self-administration period (Carson et al., 2010). Although the extent to which oxytocin crosses the blood-brain barrier is still unclear (Gimpl & Fahrenholz, 2001), the effect of oxytocin reported above was obtained following peripheral injections of the polypeptide, extending previous research that had shown similar drug-related behavioural effects following a central (i.e., ICV) administration of oxytocin (Kovács et al., 1998). While most of the studies described above tested the effects of oxytocin in male rats, Westenbroek et al. (2013) showed preliminary data suggesting that female rats that were chronically treated with oxytocin (0.30 mg/kg; IP) for a period of 14 days displayed reduced motivation to self-administer methamphetamine. Similarly,

other studies have reported that acute peripheral OXT reduced cocaine SA and active lever responses as well as reduced cocaine taking and seeking, and cocaine-induced locomotor activity in females (Leong et al., 2016), and a decrease in cocaine taking in males (Zhou et al., 2014), thus further supporting OXT as a potential drug therapies for addiction.

As mentioned above, even with the plethora of studies on drug addiction and the urgent need for a treatment, no long-term solution has been established yet. However, the few studies that have specifically investigated the role of oxytocin in drug addiction have shown potential in treating it. While those promising results have mostly been shown in stimulant drugs such as cocaine (Sarnyai et al., 1992; Sarnyai et al., 1991; Zhou et al., 2014; Leong et al., 2016) and methamphetamine (Carson et al., 2010; Carson et al., 2013; Westenbroek et al., 2013), previous research suggests similar effects of oxytocin treatment in heroin addiction (Kovács et al., 1985).

The current study

No studies to date have investigated the behavioural effects of exogenous oxytocin administration on heroin self-administration under both a fixed-interval (20 s) and a progressive-ratio schedule of reinforcement. In addition, the effects of treatment with oxytocin on the reinstatement of extinguished heroin seeking, a model for drug relapse, have not been previously investigated. Given the gap in our knowledge about the consequences of treatment with exogenous oxytocin on heroin taking and seeking, we explored the effects of acute (chapter 1) and chronic (chapter 2) oxytocin administration on heroin self-administration under FI-20 s and PR schedules of reinforcement. In addition we have investigated the effects of an acute (chapter 1) and chronic (chapter 2) treatment with oxytocin on yohimbine-induced reinstatement of heroin seeking, an animal model of stress-induced relapse to drugs (Shaham, Shalev, Lu, de Wit, & Stewart, 2003).

General Methods

Subjects

Male Long Evans rats weighing 300-350g (Charles River, St-Constant, QC) were used in all the experiments. Following their arrival at the animal care facility (ACF), rats were housed in pairs and handled for one week under reverse light/dark conditions (lights off at 9:30 a.m.). Rats then underwent intravenous (IV) catheterization and the intracerebroventricular (ICV) cannulation surgeries. Rats were returned to the ACF where they were single-housed in a shoebox cage and given two days of recovery. Following this recovery period, each rat was transferred into an operant-conditioning chamber designed for drug self-administration (SA). After a day of habituation to their new environment, the rats started heroin SA training. During training and extinction phases (see below), rats had unlimited access to food and water. Rats were treated according to the Canadian Council on Animal Care guidelines, and the Concordia University Animal Research Ethics Committee granted approval for all experiments.

Surgical procedures

To allow for heroin SA, all rats were implanted with IV silastic catheters (Dow Corning, Midland, MI, USA) under ketamine and xylazine anaesthesia (90.0 + 13.0 mg/kg; intraperitoneal [IP]). The catheter was inserted into the right jugular vein of the rat and then sutured to the vein. After securing the catheter to the vein, the silastic tubing was subcutaneously passed to the top of the head where it was attached to a modified 22-gauge cannula (Plastics One Inc., Roanoke, VA, USA). In addition, a 22-gauge unilateral cannula (Plastics One Inc., Roanoke, VA, USA) targeting the lateral ventricles was implanted in either right or left hemisphere (counterbalanced) to enable the ICV OXT injections (AP: -0.5; ML: ±1.3 DV: -3.0 mm, in relation to Bregma). The cannula was fixed on the rat's skull with dental cement and five screws (Boulonnerie de Montréal

Inc., Laval, QC). Before surgery, rats were injected with saline (0.90 %; CDMV, Saint-Hyacinthe, QC) and penicillin (450,000 IU/rat; CDMV, Saint-Hyacinthe, QC) in order to hydrate and prevent infection. After surgery, rats were subcutaneously (SC) administered saline and ketoprofen (5.0 mg/kg; CDMV, Saint-Hyacinthe, QC) to hydrate and reduce pain. Catheters were flushed daily with a heparin and gentamicin mix (7.50 IU + 12.00 mg/rat) to prevent blockage and infection.

Apparatus

Rats were housed in operant-conditioning chambers (Coulbourn Instruments, Whitehall, PA, USA; 29.00 cm x 29.00 cm x 25.50 cm) for the complete duration of the experiments. Sound attenuating chambers enclosed all operant chambers to reduce external sounds. Operant-conditioning chambers had a metal grid floor, a reusable waste pan, a Plexiglas door and a metal ceiling that allowed access to the drug harness system. Two retractable levers (Coulbourn Instruments, Whitehall, PA, USA) were located on the same sidewall, 11 cm above the grid floor. Presses on the *active* lever led to the heroin delivery (0.05 or 0.10 mg/kg/infusion) and the activation of the cue light/tone complex for 20 s. Presses on the *inactive* lever had no programmed consequences. The location of the active and inactive levers was counterbalanced between the chambers. The white cue-light (Coulbourn Instruments, Whitehall, PA, USA) was positioned just above the active lever, and the tone module (Sonalert, 2.90 KHz, Coulbourn Instruments) was installed above the active lever, close to the chamber ceiling. The infusion pump (Razel Single Syringe Pump, Razel Scientific Instruments, Fairfax, VT) was situated within the enclosure of the wooden compartment. Each infusion pump had a 20 ml syringe connected to a swivel that was then connected to the catheter via Tygon tubing (Norton Performance Plastics, Akron, OH, USA; OD 0.06, ID 0.02) and a liquid swivel (Instech, Boulder,

CO, USA). The Tygon tubing inside the operant-conditioning chamber was protected by a metal spring. A computer system (Graphic State 3.02, Coulbourn Instruments) controlled the chambers and recorded all infusions and lever responses.

Drug

Heroin (diacetylmorphine hydrochloride; National Institute for Drug Abuse, Research Triangle Park, NC, USA) was dissolved in sterile saline (5.00 mg/ml) to be diluted again with saline, for each rat (0.05 and 0.10 mg/kg/infusion). Oxytocin (OXT) lyophilized powder (50 IU; Sigma-Aldrich, St-Louis, MO) was dissolved in sterile saline (0.50, 1.00, 2.50 µg/rat [ICV]; 0.30, 1.00 mg/kg [IP]).

Procedure

Training. First, rats were introduced to their individual operant-conditioning chamber for a 24-h period (habituation). Following habituation, rats were trained to self-administer heroin for 10 days under a fixed-interval 20 s schedule of reinforcement (FI-20 s). Training sessions were 3 hours each, three times a day, with an interval of three hours in between each self-administration period. Following a 10-day SA training under a FI-20s, rats were switched to a PR schedule of reinforcement (one 5-h PR session per day). All self-administration training sessions were set to start immediately after the light went off (9:30 a.m.). For training under FI-20 s schedule of reinforcement, both retractable levers were extended at the beginning of the first daily session, the house light was on, and the cue light and tone complex were activated for 30 s. The inactive lever was not retracted for the next 23 h, to allow better dissociation between the levers. For training under PR schedule of reinforcement, both levers were retracted at the end of the 5-h self-administration session. The infusion pump was activated (5 s, 0.13 ml/infusion) following one

press on the active lever (FI-20 s) or following 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95… presses on the active lever (PR). The progressive ratio equation was calculated as follows: $response\ ratio = (5 \times e^{(0.2 \times infusion\ number)}) - 5$ (Roberts & Bennett, 1993). All numbers were rounded to the nearest integer function. A 20 s timeout period always followed the activation of the infusion pump, during which all responses were recorded, but not reinforced, and the light/tone complex cue remained on.

Test days. On test days, rats were removed from the operant-conditioning chambers and injected with OXT approximately 20 minutes prior to their SA session before the beginning of the session. Intracerebroventricular injections were performed using a micro infusion pump (Harvard Apparatus Model 11 Syringe Pump) over a 2-minute period at 1.0 µl/min for final doses of 0.30 µg/rat (chapter 2), 0.50 µg/rat (chapter 1), 1.00 (chapter 2) or 2.50 µg/rat (chapter 1) and an additional minute to allow proper absorption.

CHAPTER 1

THE EFFECTS OF ACUTE OXYTOCIN INJECTIONS ON HEROIN TAKING AND SEEKING IN MALE RATS

Janie Duchesneau, Loïc Welch, Cristina Casola, & Uri Shalev

Introduction

The regulation and functions of he neuropeptide OXT have been under scrutiny more than a century (Love, 2013). There are now clear indications that OXT impacts dopamine in the mesocorticolimbic system leading us to investigate its impact of reward and motivation (Love 2013). Earlier studies have explored the effect of exogenous OXT on drug intake as neurohypophyseal peptides have been tested to alter the reinforcing properties of opiates (Kovács et al., 1985a). Such studies have first investigated the role of acute exogenous OXT on naïve- and heroin-trained rats using an animal model of addiction using heroin self-administration (Kovács et al., 1985a; Ibragimov et al., 1987). Findings suggested that exogenous OXT decreased heroin SA on rats that were made tolerant to the drug, but not in experimentally naïve rats (Kovács et al, 1985). Other studies showed that oxytocin could also diminish heroin tolerance, and simultaneously reduce heroin intake (Kovács & et al. 1985b). More recent studies have shown that carbetocin, an OXT analogue, was effective in reducing priming-induced reinstatement of extinguished morphine conditioned place preference (Georgiou et al., 2015). Other recent studies have shown OXT to be effective in reducing both methamphetamine SA (Carson et al., 2010) and methamphetamine-induced reinstatement of methamphetamine seeking in male (Carson et al., 2010) and female rats (Cox et al., 2013). Other research has found similar positive results on the effects of OXT and cocaine SA (Zhou et al., 2014; Leong et al., 2016) as well as cocaine-primed (Zhou et al., 2014) and cue-induced reinstatement of extinguished cocaine seeking in both males (Zhou et al., 2014) and females (Leong et al., 2016).

Interestingly, OXT has been shown to be a robust anxiolytic and anti-stress factor of the brain (Slattery & Neumann, 2010). Considering that most of the influential theories of drug addiction claim that stress plays a significant role in increasing drug intake and relapse (Sinha,

2001) and the high ratio of heroin users relapsing over a prolonged withdrawal period (O'Brien, 1997), research investigating the effect of OXT on stress-induced relapse is more relevant than ever. The drug reinstatement procedure, one of the most widely used models for relapse in animals, includes many reinstatement methods, such as drug priming-induced reinstatement, cue-induced reinstatement, and stress-induced reinstatement (Shaham, Shalev, Lu, de Wit, & Stewart, 2003). In order to test for drug reinstatement, rats must first undergo a consistent drug SA regimen. They will then have to experience an extinction phase until they reach a specific criterion (< 15 lever responses). During the extinction phase, the heroin is not unavailable which will ultimately decrease responses on the lever previously associated with the drug. Finally, the rats will be faced with a stressor in order to induce reinstatement of the extinguished drug seeing.

While most recent studies investigating the impact of OXT on drug taking and drug reinstatement choose acute, systemic (i.e., IP) OXT injections, here, we present a novel investigation of the role of acute exogenous OXT on heroin self-administration under different schedules of reinforcement (FI-20 s and PR), using different doses of heroin (0.05 or 0.10 mg/kg/infusion) and OXT (0.50 and 2.50 μg/rat [ICV]; 0.30 and 1.00 mg/kg [IP]). Then, considering the anxiolytic properties of OXT, we aimed to investigate its behavioural impact of acute OXT doses on yohimbine-induced reinstatement of extinguished heroin seeking. We chose yohimbine, a pharmacological stressor, due its robust effect at promoting relapse in previously extinguished heroin seeking (Banna, Back, Do, & See, 2010).

We predicted a decrease in the maintenance of heroin-reinforced behaviour that would be translated by lesser active (and inactive) lever presses following an acute injection of OXT. Finally, we predicted a decrease in behavioural responses (lever presses) would be found in the yohimbine-induced reinstatement tests in rats being treated with the Low (*0.50* μg/rat [ICV]; 0.30 mg/kg [IP]) and high (2.50 μg/rat [ICV]; 1.00 mg/kg [IP]) doses of OXT compared to the rats

treated with saline for the specific session tested.

Experiment 1: Effects of acute exogenous oxytocin on heroin-taking behaviour in male rats

Material & Methods

Subjects

The same group of Long Evans rats (n = 9; 300-350g) was used throughout the experiment. The rats were housed and treated as described in the general methods section.

Surgical procedures

Rats were implanted with intravenous (IV) silastic catheters to enable heroin self-administration and intracerebroventricular (ICV) cannulae to allow OXT injections, as detailed above.

Apparatus

Operant-conditioning chambers: The operant-conditioning chambers used were identical to those described in the general methods section.

Micro infusion pumps: Micro infusion pumps (Harvard Apparatus Model 11 Syringe Pump) were used to inject a chosen dose of OXT (or saline) prior to SA sessions or test days as described in the general methods section.

Drug

Heroin (diacetylmorphine hydrochloride) and OXT lyophilized powder were dissolved as described in the general methods section.

Procedure

Before being placed in the operant-conditioning chambers, rats' guide cannula placements were verified using an angiotensin II-induced (100 nmol; ICV) short latency (< 60 s) drinking response. All ICV cannulae were successfully verified. Once the PR self-administration training stabilized (> 5 infusions/session), rats were tested under four different conditions. Rats received two sham injections two days before each experiment started.

Experiment 1A: Rats were kept on a heroin SA regimen of 0.10 mg/kg/infusion on a PR schedule of reinforcement as described in the general methods section. Rats were injected (ICV) with three doses of OXT over 3 test days (0.00, 0.50 and 2.50 μg/rat; counterbalanced order), 20 min prior to their daily SA session. Tests were separated by one training day.

Experiment 1B: A lower heroin SA dose (0.05 mg/kg/infusion) was used, and rats were tested with the same procedures and OXT doses as in Experiment 1A (ICV; 0.00, 0.50 and 2.50 μg/rat; counterbalanced order).

Experiment 1C: Rats were kept on a heroin SA regimen of 0.05 mg/kg/infusion on a PR schedule of reinforcement as described for Experiment 1B. However, OXT was administered IP 20 min before the daily SA session (0.00, 0.30 and 1.00 mg/kg; counterbalanced order).

Experiment 1D: Rats were re-trained on an FI-20 s schedule of reinforcement (one 5-h session/day) with a 0.05 mg/kg/infusion dose of heroin for 5 days. Next, rats were tested with

acute OXT injections 20 min before the first daily self-administration session (ICV; 0.00, 0.50 and 2.50 μg/rat; counterbalanced order).

Statistical Analyses

All analyses were conducted using SPSS software (IBM, SPSS Statistics, version 22). Self-administration training: Data for all rats were analyzed using a repeated measures analysis of variance (ANOVA), with *FI-20 s training day* (1-10) or *PR training day* (11-17) as the within-subject factor, and *active* and *inactive lever responses* as well as number of *infusions* as the dependent variables. The number of infusions earned in a session, which also represents the number of PR steps completed, was defined as the "breakpoint".

Test days: Number of *infusions* as well as *active* and *inactive lever responses* during the SA session following the acute OXT injection were analyzed using repeated measures ANOVAs, with *OXT dose* (Experiment 1A, 1B, 1D: Baseline, 0.00 [vehicle], 0.50 [low] and 2.50 [high] μg/rat; Experiment 1C: Baseline, 0.00 [vehicle], 0.30 [low] and 1.00 [high] mg/kg) as the within-subject factor. Baseline was calculated as the average infusions and lever responses over the last five days of training. The critical cut-off point for statistically significant results was $p \leq .05$.

Time course: Number of *infusions* as well as *active* and *inactive lever responses* during the first 60 minutes of the SA session following an acute OXT injection were analyzed using two-way repeated measures ANOVAs, with *OXT dose* (Experiment 1D: 0.00 [vehicle], 0.50 [low] and 2.50 [high] μg/rat) and *time* (6 x 10-min bins) as the within-subject factors. The critical cut-off point for statistically significant results was $p \leq .05$.

Results

Final analysis for self-administration training included 9 rats. Experiments 1A and 1B included 9 rats while Experiments 1C and 1D included 8 rats. One rat was excluded due to the loss of its head cap following the training phase and was never tested, and a second rat died following an acute 2.50 μg/rat (ICV) OXT injection during Experiment 1B.

The Greenhouse-Geisser correction was used when Mauchly's test of sphericity assumptions was violated.

Training

Heroin infusion: Under FI-20 s, there was a statistically significant increase in heroin infusions over time, $F(3.18, 28.59) = 7.46$, $p < .001$ (Figure 1A). However, there was no statistically significant change in infusions number over training days under PR, $F(6, 54) = 2.04$, $p = .076$ (Figure 2A).

Active lever responses: Similarly to heroin infusions, active lever responses increased throughout FI-20 s training ($F(2.25, 20.25) = 3.41$, $p = .048$; Figure 1B), while there was no change under PR ($F(2.75, 24.74) = 1.85$, $p = .168$; Figure 2B).

Inactive lever responses: No statistically significant change was observed for inactive lever responses throughout both self-administration training regimes under FI-20 s ($F(1.76, 15.88) = 2.66$, $p = .106$) or PR ($F(1.05, 9.44) = 1.36$, $p = .275$).

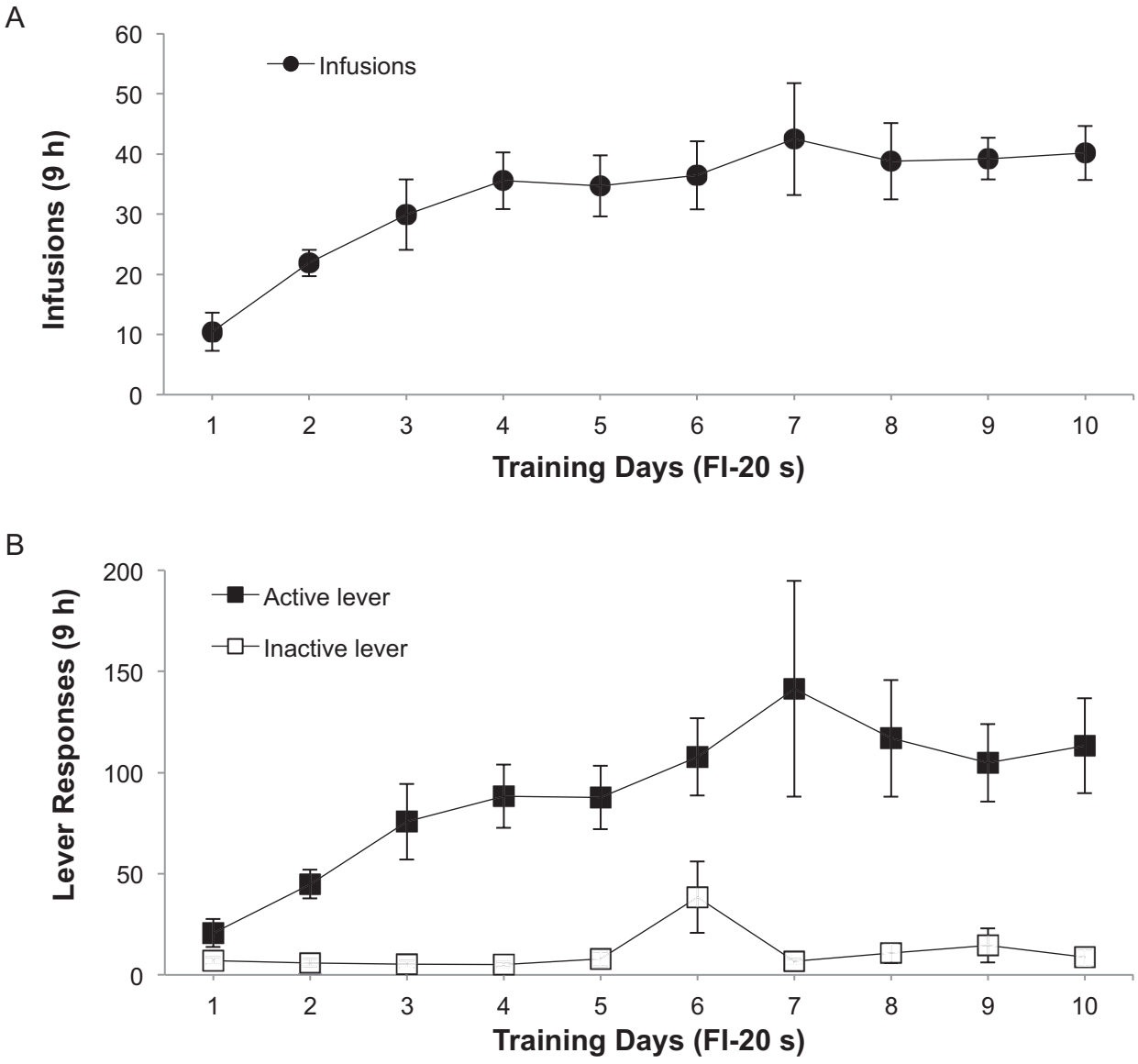


Figure 1. Mean (± *SEM*) number of infusions (A) as well as active and inactive lever responses (B) made during heroin self-administration training by rats in Experiment 1 ($n = 9$). Rats self-administered heroin (0.10 mg/kg/infusion) for three 3-h sessions under a fixed interval 20 s (FI-20 s) schedule of reinforcement, over a 10-day period. Both infusion and active lever responses increased at a statistically significant level ($p < .05$) during training. There was no statistically significant change for inactive lever responses.

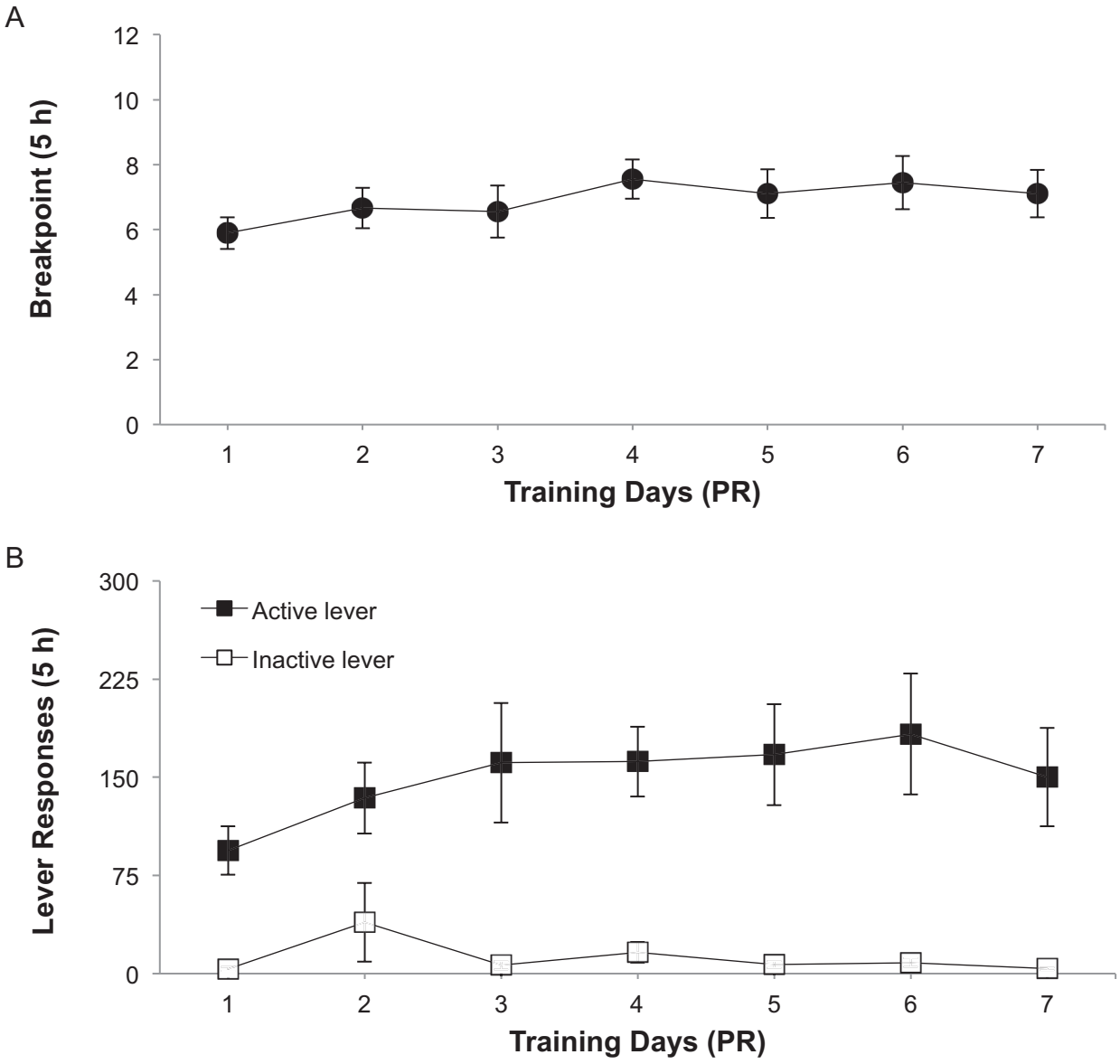


Figure 2. Mean (± *SEM*) number of infusions (breakpoint) (A) as well as active and inactive lever responses (B) made during heroin self-administration training by rats in Experiment 1 (n = 9). Rats self-administered heroin (0.10 mg/kg/infusion) for one 5-h session under a progressive-ratio (PR) schedule of reinforcement, over a 7-day period. There were no statistically different changes over time for the number of infusions, active and inactive lever responses.

Test days

Experiment 1A: Acute treatment with OXT reduced breakpoint under PR schedule of reinforcement with 0.10 mg/kg/infusion of heroin, but only for the Low dose (Figure 3). There was a statistically significant effect for *OXT dose* on breakpoint ($F(1.91, 15.28) = 4.09$, $p = .039$, $\eta_p^2 = 0.34$). *Post-hoc t*-tests with a Bonferroni-adjusted $\alpha = .008$ revealed a statistically significant decrease in breakpoint in rats treated with a Low (0.50 μg/rat) OXT infusion versus the Baseline condition ($t(9) = 2.31$, $p = 0.047$) and the high (2.50 μg/rat) *OXT dose*, $t(8) = -2.50$, $p = .037$ (Figure 3). A similar pattern was observed for active lever responses; however, no statistically significant changes were found for active ($F(1.06, 8.46) = 2.31$, $p = .165$, $\eta_p^2 = 0.22$; Figure 4A) or inactive ($F(3, 24) = 2.42$, $p = .095$, $\eta_p^2 = 0.26$; Figure 4B) lever presses.

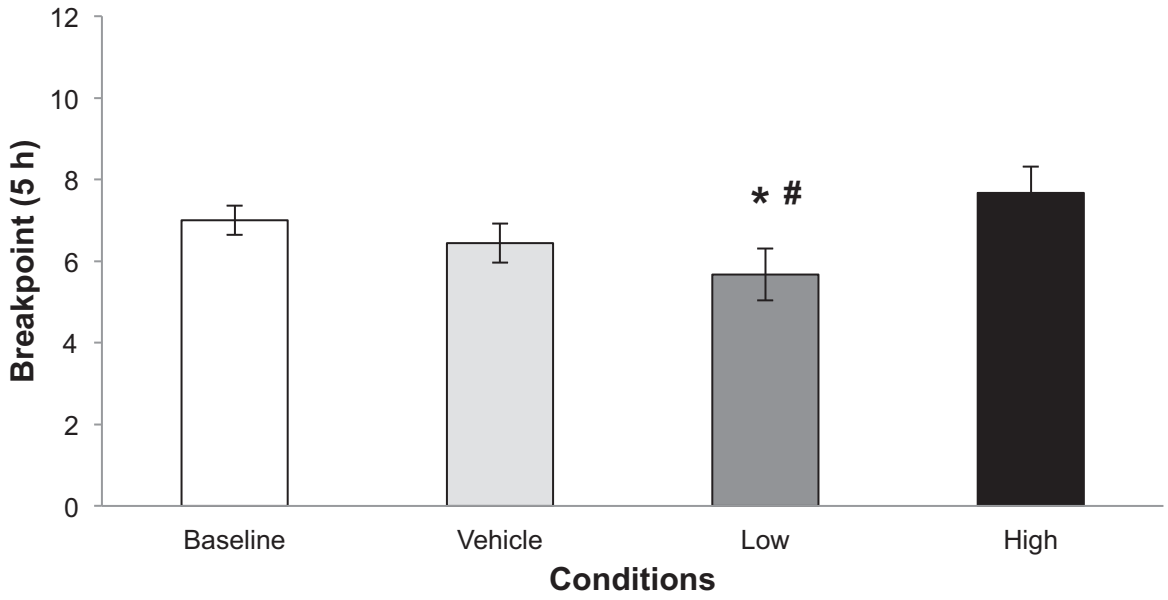


Figure 3. Mean (± *SEM*) number of infusions earned (PR breakpoint) following an acute OXT injection (0.00 [vehicle], 0.50 [low] and 2.50 [high] μg/rat, ICV) in Experiment 1A ($n = 9$). Rats self-administered heroin (0.10 mg/kg/infusion) during a 5-h session under a progressive-ratio (PR) schedule of reinforcement. Baseline was calculated as the average number of infusions over the last five days of SA training under PR schedule. *$p = .047$ compared to Baseline condition. $^{\#}p = .037$ compared to high condition.

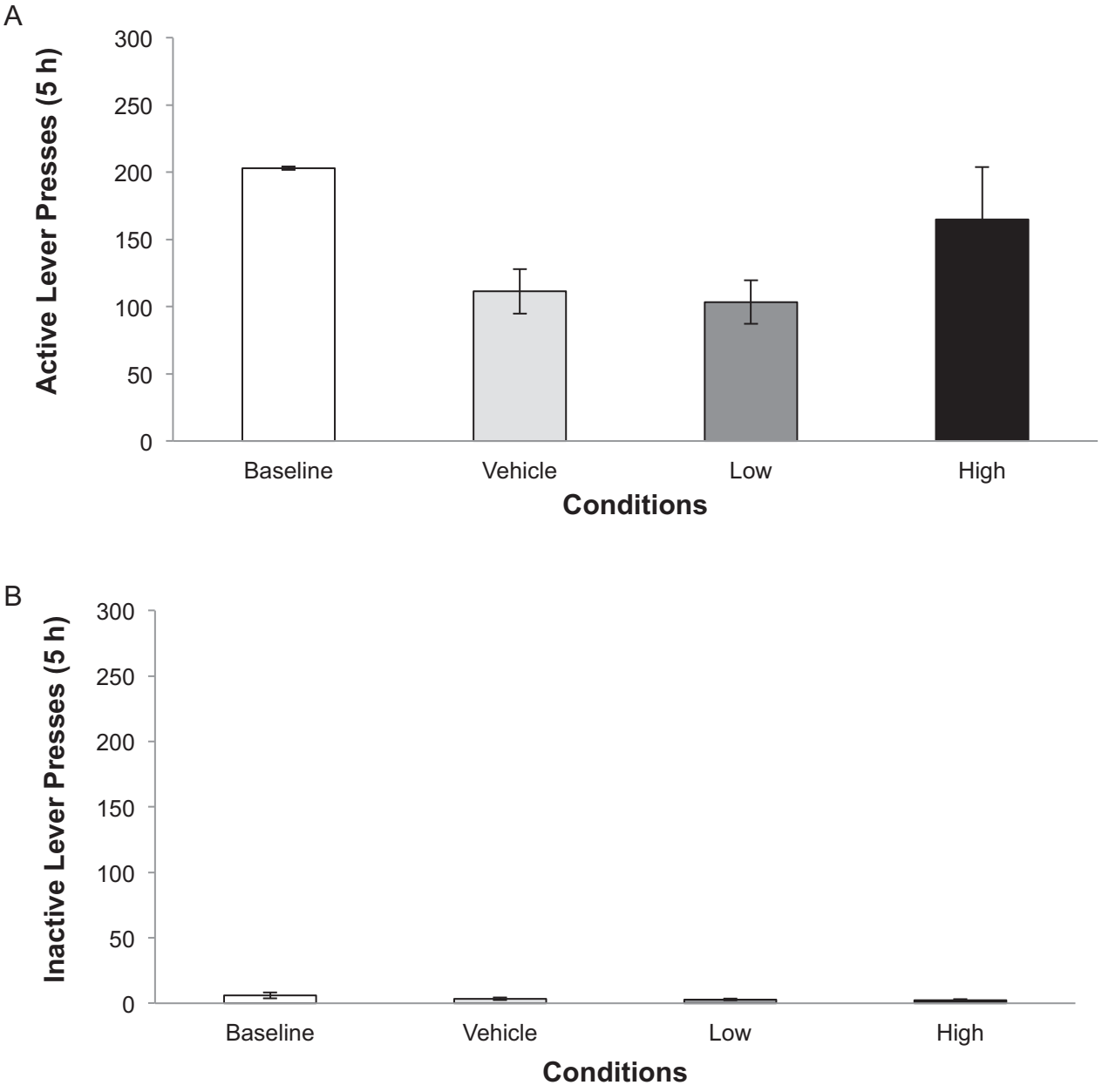


Figure 4. Mean (± *SEM*) number of active (A) and inactive (B) lever responses following an acute OXT injection (0.00 [vehicle], 0.50 [low] and 2.50 [high] μg/rat, ICV) in Experiment 1A ($n = 9$). Rats self-administered heroin (0.10 mg/kg/infusion) during a 5-h session under a PR schedule of reinforcement. Baseline was calculated as the average number of active lever responses over the last five days of SA training under PR schedule.

Experiment 1B: No statistically significant changes in breakpoint under PR schedule of reinforcement with 0.05 mg/kg/infusion of heroin were found following OXT treatment (*OXT dose*: $F(1.06, 0.52) = 0.59$, $p = .474$, $\eta_p^2 = 0.07$; Figure 5). Oxytocin infusions (ICV) seemed to reduce the number of active lever responses performed during the test session. Repeated measures ANOVA for active lever responses approached statistical significance ($F(3, 24) = 2.89$, $p = .057$, $\eta_p^2 = 0.27$; Figure 6A). There were no statistically significant differences in inactive lever responses between the different treatment conditions ($F(1.45, 11.59) = 1.72$, $p = .221$, $\eta_p^2 = 0.17$; Figure 6B).

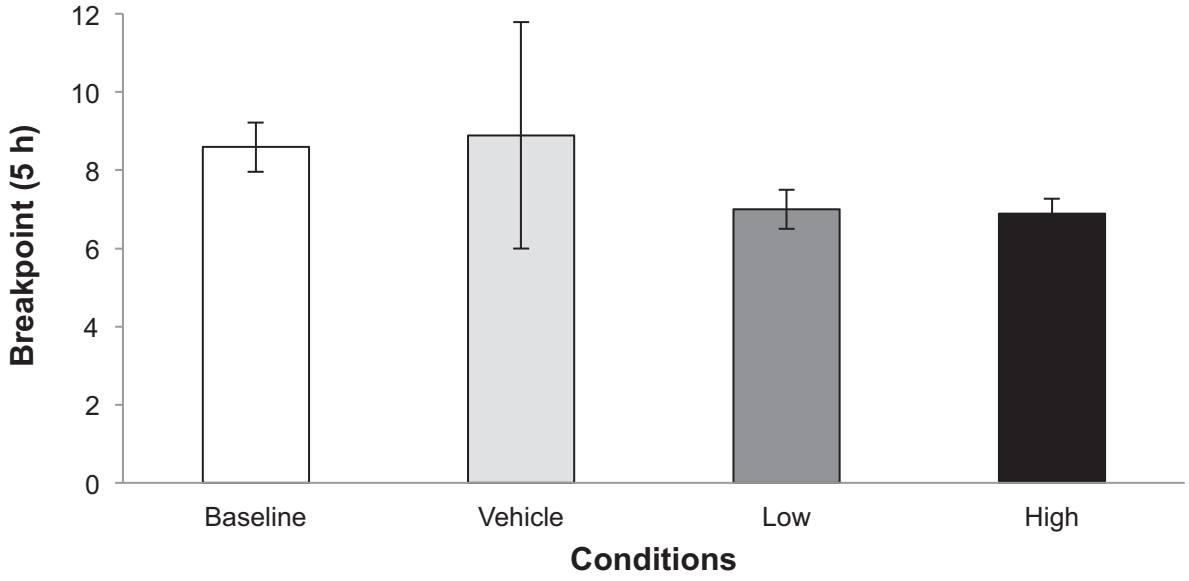


Figure 5. Mean (± *SEM*) number of infusions (PR breakpoint) following an acute OXT injection (0.00 [vehicle], 0.50 [low] and 2.50 [high] μg/rat; ICV) in Experiment 1B (n = 9). Rats self-administered heroin (0.05 mg/kg/infusion) during a 5-h session under a PR schedule of reinforcement. Baseline was calculated as the average number of infusions over the last five days of SA training under PR schedule.

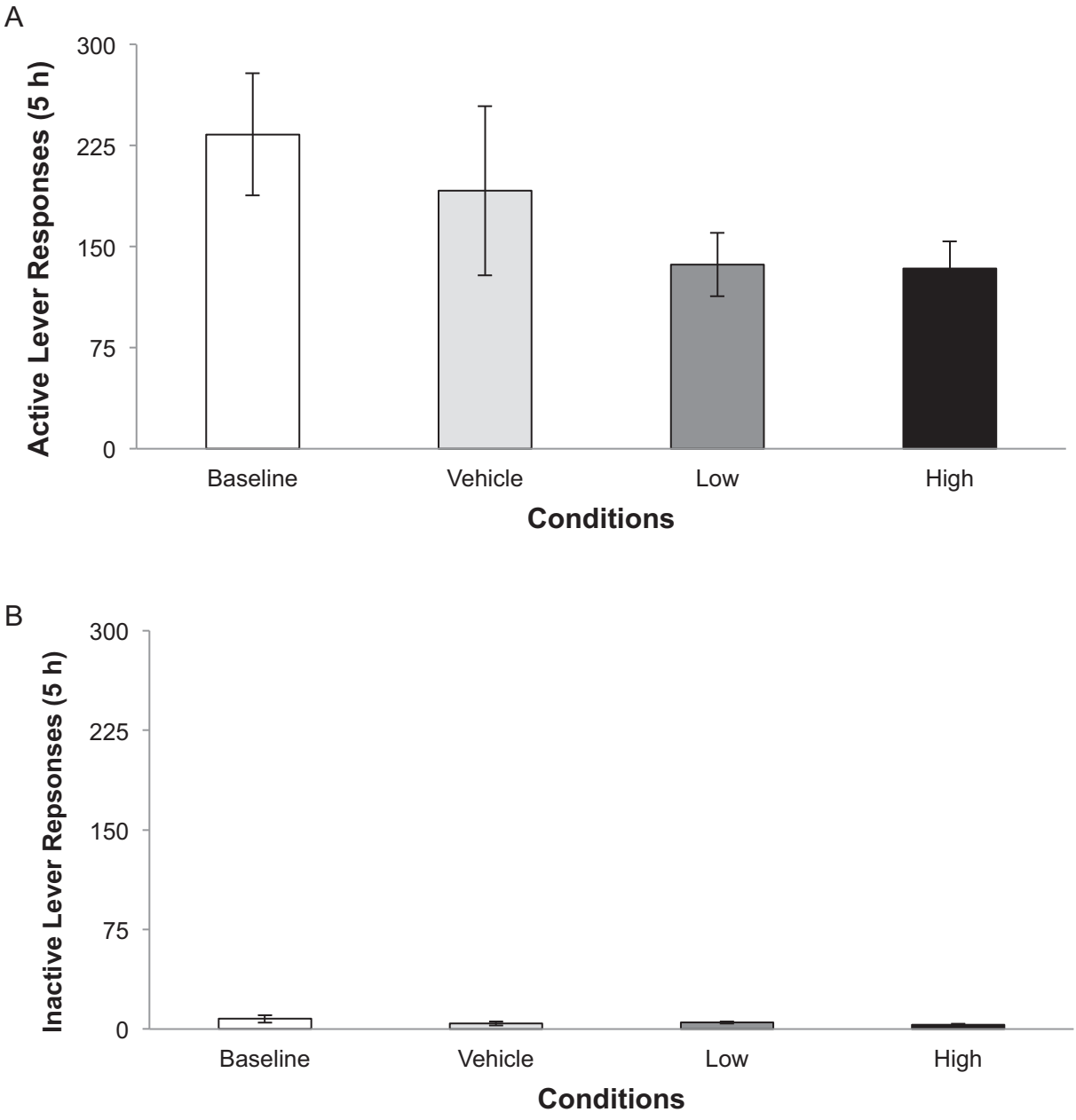


Figure 6. Mean (± *SEM*) number of active (A) and inactive (B) lever responses following an acute OXT injection (0.00, 0.50 and 2.50 µg/rat; ICV) in Experiment 1B (n = 9). Rats self-administered heroin (0.05 mg/kg/infusion) during a 5-h session under a PR schedule of reinforcement. Baseline was calculated as the average number of active lever responses over the last five days of SA training under PR schedule.

Experiment 1C: No statistically significant differences were found for any of the analyzed variables following an acute IP administration of OXT to rats under a PR schedule of reinforcement with 0.05 mg/kg/infusion of heroin (*OXT dose:* $F_{\text{infusions}}(3, 21) = 2.91$, $p = .059$, $\eta_p^2 = 0.29$; $F_{\text{active}}(3, 21) = 2.34$, $p = .102$, $\eta_p^2 = 0.25$; and $F_{\text{inactive}}(3, 21) = 1.83$, $p = .172$, $\eta_p^2 = 0.21$; Figures 7, 8A, 8B.

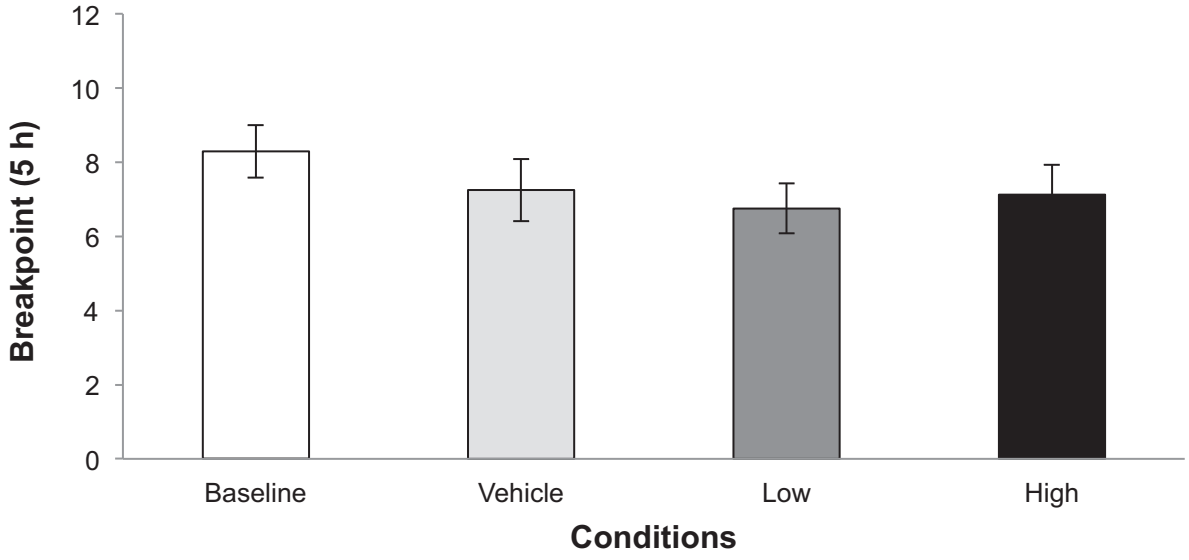


Figure 7. Mean (± *SEM*) number of infusions (PR breakpoint) following an acute OXT injection (0.00 [vehicle], 0.30 [low] and 1.00 [high] mg/kg; IP) in Experiment 1C (n = 8). Rats self-administered heroin (0.05 mg/kg/infusion) during a 5-h session under a PR schedule of reinforcement. Baseline was calculated as the average number of infusions over the last five days of SA training under PR schedule.

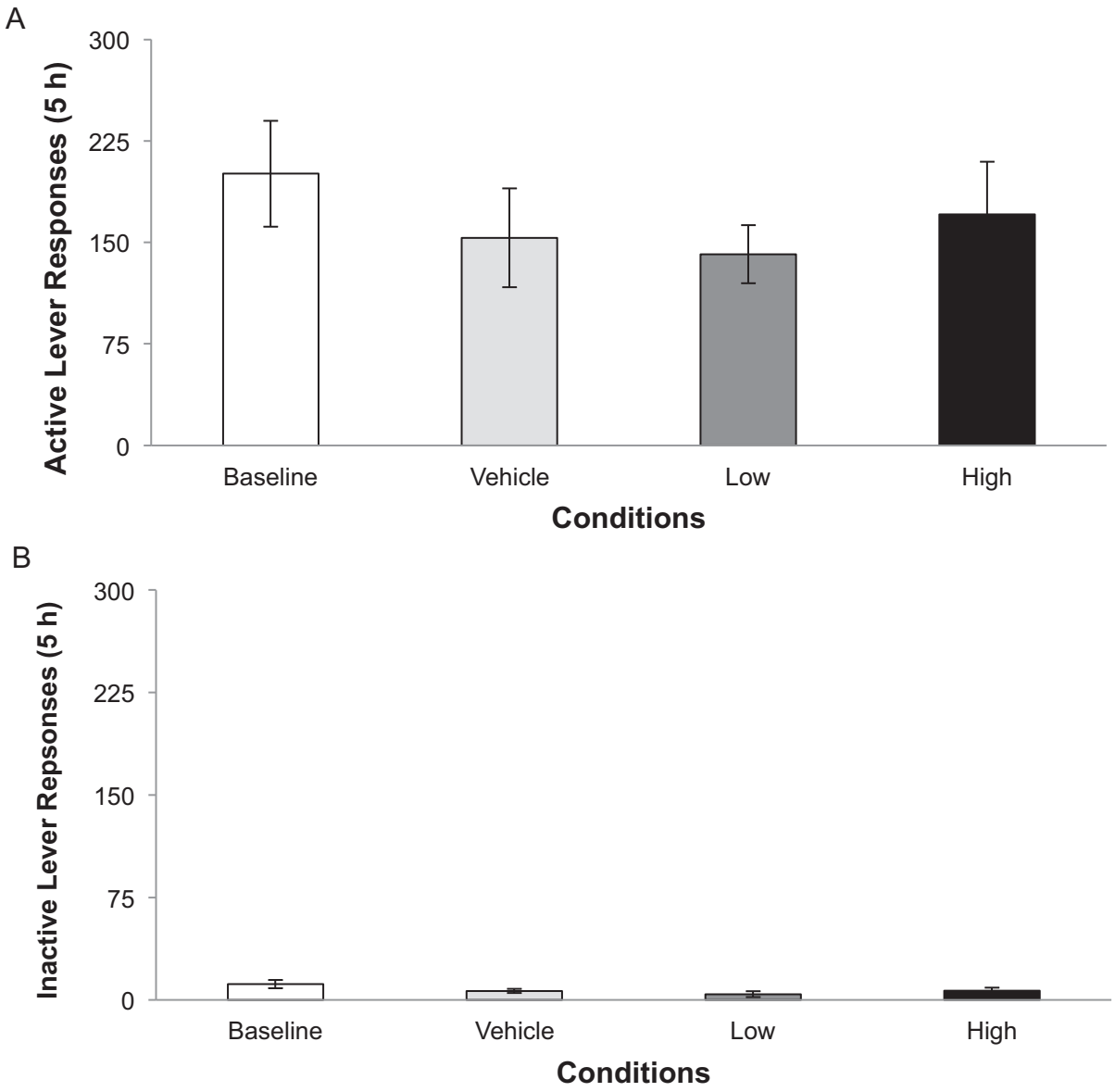


Figure 8. Mean (± *SEM*) number of active (A) and inactive (B) lever responses following an acute OXT injection (0.00 [vehicle], 0.30 [low] and 1.00 [high] mg/kg; IP) in Experiment 1C (n = 8). Rats self-administered heroin (0.05 mg/kg/infusion) during a 5-h session under a PR schedule of reinforcement. Baseline was calculated as the average number of active lever responses over the last five days of SA training under PR schedule.

Experiment 1D: No statistically significant effects were found for the OXT treatment on the number of heroin infusions under FI-20 s schedule of reinforcement ($F(1.35, 9.42) = 1.15$, $p = .331$, $\eta_p^2 = 0.14$; Figure 9), the active lever responses ($F(3, 21) = 1.23$, $p = .322$, $\eta_p^2 = 0.15$; Figure 10A), or the inactive lever responses ($F(1.09, 7.61) = 0.88$, $p = .385$, $\eta_p^2 = 0.11$; Figure 10B).

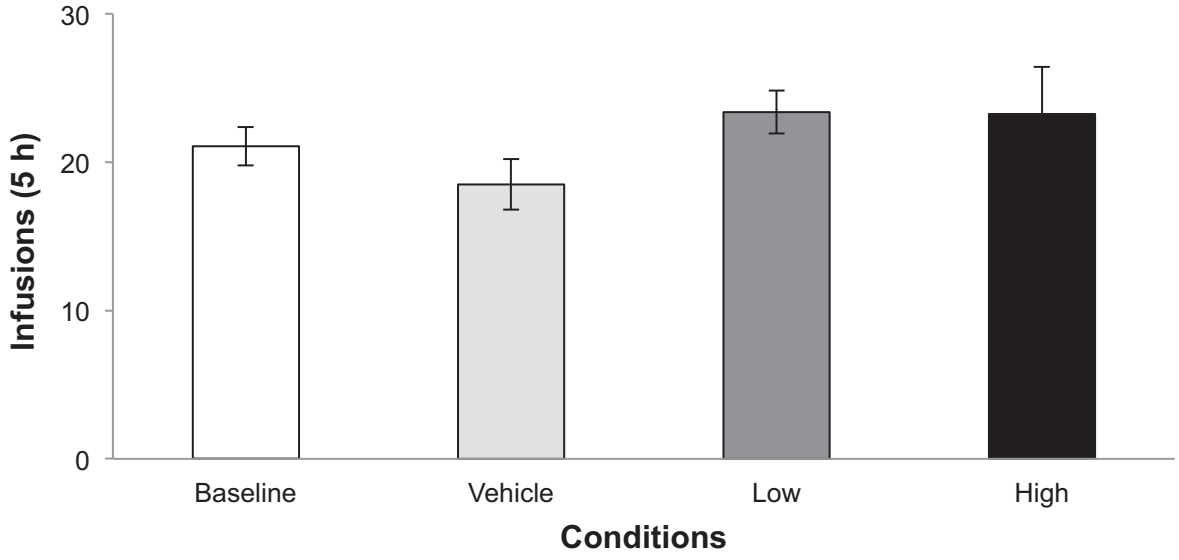


Figure 9. Mean (± *SEM*) number of infusions following an acute OXT injection (0.00, 0.50 and 2.50 μg/rat; ICV) in Experiment 1D (n = 8). Rats self-administered heroin (0.05 mg/kg/infusion) for one 5-h session under a FI-20 s schedule of reinforcement. No statistically significant difference was found for any of the conditions. Baseline was calculated as the average number of infusions over the last five days of SA re-training under FI-20 s schedule.

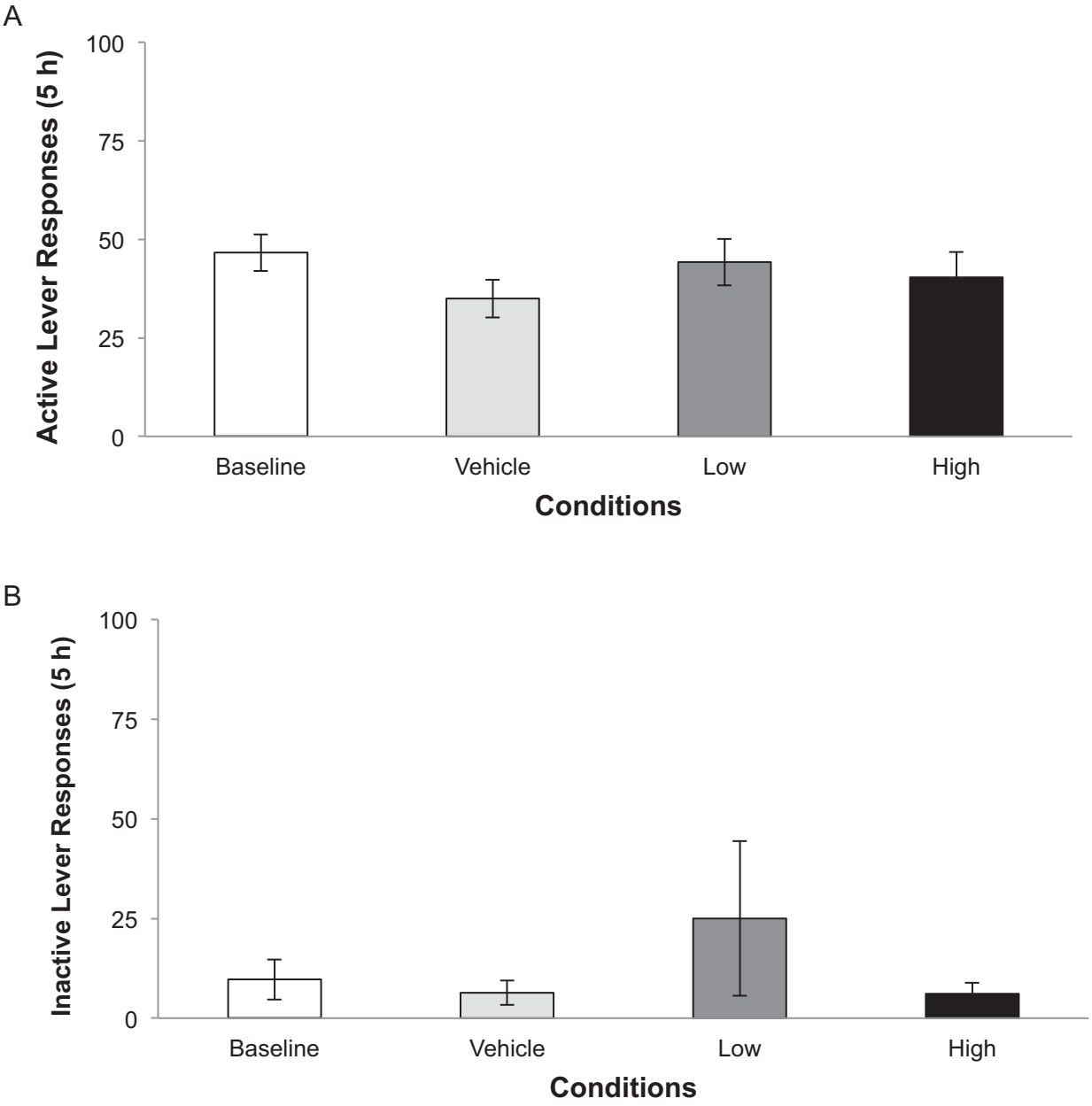


Figure 10. Mean (± *SEM*) number of active (A) and inactive (B) lever responses following an acute OXT injection (0.00, 0.50 and 2.50 µg/rat; ICV) in Experiment 1D (n = 8). Rats self-administered heroin (0.05 mg/kg/infusion) for one 5-h session under a FI-20 s schedule of reinforcement. Baseline was calculated as the average number of active lever responses over the last five days of SA re-training under FI-20 s schedule.

Experiment 1D: Time course analysis for test days

The number of infusions decreased quickly over the first 20 min of the daily 5-h session. There was a statistically significant effect of *time* ($F(3, 35) = 10.20$, $p < .01$, $\eta_p^2 = 0.59$) and *OXT dose* ($F(2, 14) = 5.29$, $p = .019$, $\eta_p^2 = 0.43$), but no *OXT dose* x *time* interaction effect ($F(4.27, 29.88) = 0.87$, $p = .498$, $\eta_p^2 = 0.11$; Figure 11). *Post-hoc t*-tests with a Bonferroni-adjusted $\alpha = .003$ revealed that there was no statistically significant difference from the first to the last 10-min bins for the low and high OXT doses while there were statistically significant differences between the 10 to 30-min ($t(7) = 7.00$, $p < .01$) bins and the 10 to 40-minute ($t(7) = 5.16$, $p < .01$) bins for the vehicle dose.

The number of active lever responses decreased quickly over the first 20 min of the session. There was a statistically significant effect of *time* ($F(1.64, 11.45) = 18.53$, $p < .01$) and *OXT dose* ($F(2, 14) = 18.94$, $p < .01$), but no *OXT dose* x *time* interaction effect ($F(2.49, 17,45) = 2.26$, $p = 0.125$; Figure 12A). *Post-hoc t*-tests with a Bonferroni-adjusted $\alpha = .003$ revealed that there was no statistically significant difference from the first to the last 10-min bins for the high OXT dose, while there was a statistically significant decrease in the 10 to 30, 40, and 50-min bin in the vehicle and in the 10 to 20, 30, 40, and 50-min bin in the low OXT dose.

No statistically significant changes in inactive lever responses were found for *time* ($F(1.06, 7.41) = 0.74$, $p = .426$), *OXT dose* ($F(1.02, 7.15) = 1.07$, $p = .336$) or the interaction between *time* x *OXT dose* ($F(1.05, 7.34) = 0.94$, $p = .367$); see Figure 12B.

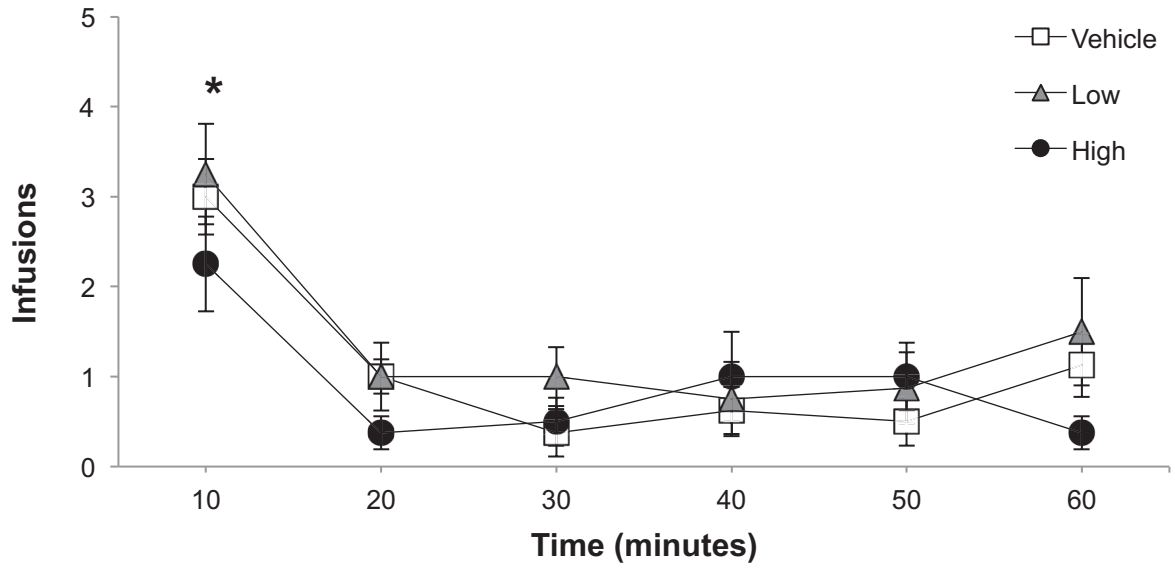


Figure 11. Mean (± *SEM*) number of infusions following an acute OXT injection (0.00 [vehicle], 0.50 [low] and 2.50 [high] µg/rat, ICV) in Experiment 1D (n = 8). Rats self-administered heroin (0.05 mg/kg/infusion) during the first 60 minutes of their one 5-h session under a FI -20 s schedule of reinforcement. * Vehicle: $p < .01$ compared to the 10 to 30-min and 40-min bins.

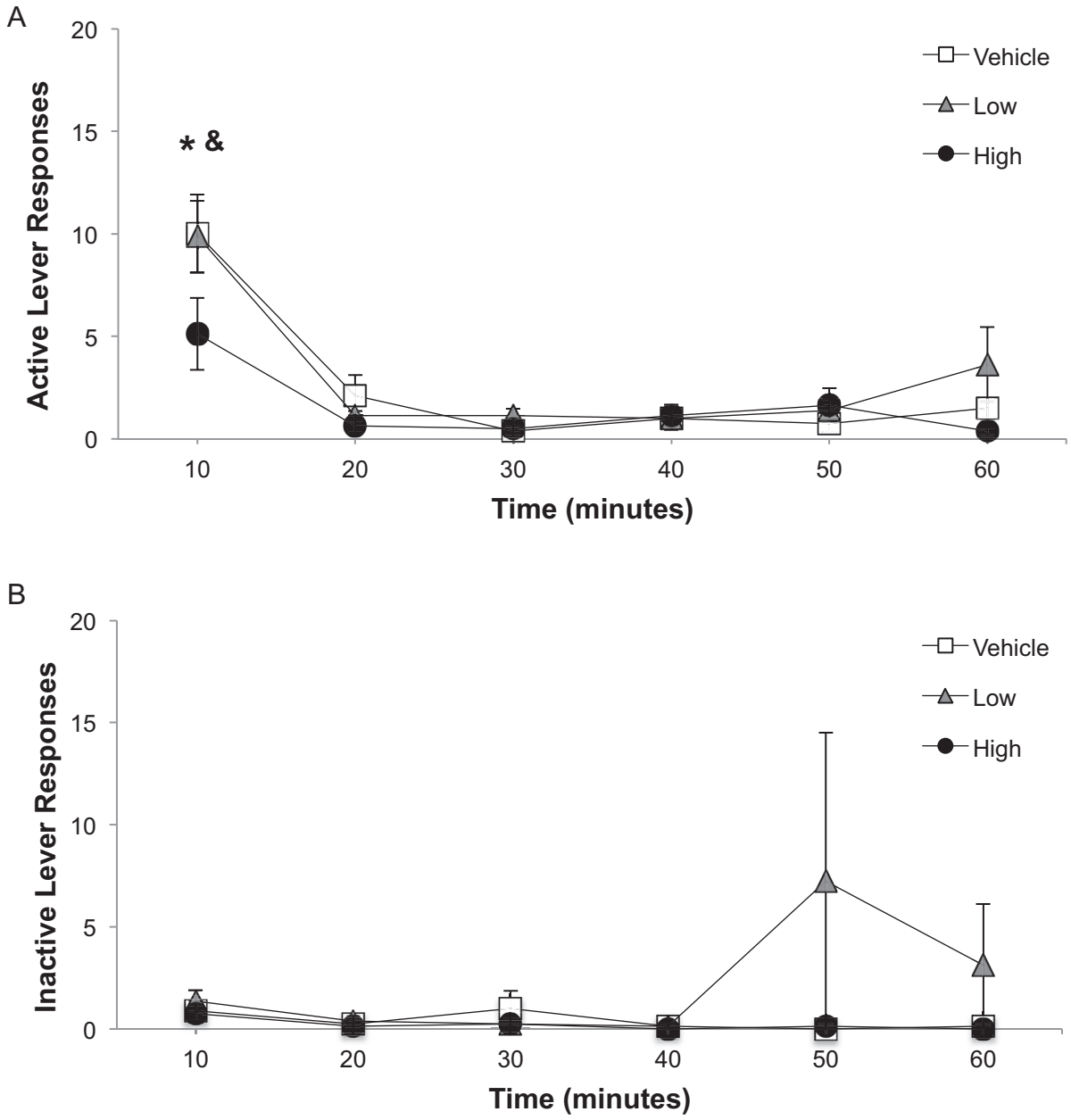


Figure 12. Mean (± *SEM*) number of active (A) and inactive (B) lever responses following an acute OXT injection (0.00 [vehicle], 0.50 [low] and 2.50 [high] μg/rat, ICV) in Experiment 1D ($n = 8$). Rats self-administered heroin (0.05 mg/kg/infusion) during the first 60 minutes of their one 5-h session under a FI-20 s schedule of reinforcement. * Vehicle: $p < .01$ compared to the 10 to 30, 40, and 50-min bins. # Low: $p < .01$ compared the 10 to the 20, 30, 40, and 50-min bins.

Experiment 2: Effects of acute exogenous oxytocin on yohimbine-induced reinstatement of extinguished heroin seeking

Material & Methods

Subjects

The same rats used in Experiment 1 were used in Experiment 2A (n = 7). In Experiment 2B, only six rats completed both reinstatement tests. Rats were separated into two groups (OXT or saline). First, we calculated the mean of infusions as well as active and inactive lever presses obtained during the last 5 days of training (FI-20 s). Then, we counterbalanced the two groups and randomly chose the one in which the rats were assigned.

Apparatus

Operant-conditioning chambers: The operant-conditioning chambers used were identical to those described in the general methods section.

Micro infusion pump: The micro infusion pump (Harvard Apparatus Model 11 Syringe Pump) was used to inject a chosen dose of OXT (or saline) prior to the reinstatement test as described in the general methods section.

Drug

Yohimbine hydrochloride powder (Sigma-Aldrich, St-Louis, MO) was dissolved in sterile saline (2.00 mg/kg) and injected IP. Oxytocin lyophilized powder was dissolved and injected as described in the general methods section.

Procedure

Extinction: Following the completion of Experiment 1, rats were exposed to the same conditions and cues as described in the general methods section; however, no heroin was made available. The first day of extinction consisted of three 3-h extinction training sessions. Subsequently, extinction training included one 3-h daily session. Extinction training continued for at least 4 days and until the rats reached the extinction criterion of ≤ 15 active lever responses during the 3-h session.

Yohimbine-induced reinstatement: Once the rats met the extinction criterion, they were tested for yohimbine-induced reinstatement on the next day. Rats were tested twice for reinstatement following an injection of saline and yohimbine (2.00 mg/kg; IP) in counterbalanced order, administered approximately 30 min before the test session. Exogenous OXT (Experiment 2A: 2.50 μg/rat; Experiment 2B: 1.00 μg/rat; ICV) or saline were injected immediately before the session. Reinstatement tests were separated by at least one day of extinction training. Active and inactive lever responses were recorded during all sessions, and Baseline of active and inactive lever responses was calculated by averaging the lever responses made during the extinction session that preceded each test.

Statistical Analyses

All analyses were conducted using SPSS software (IBM, SPSS Statistics, version 22). Separate repeated measures ANOVA were conducted for the active and inactive lever responses. The Greenhouse-Geisser correction was used when Mauchly's test of sphericity assumptions was violated. Analyses included a between-subject factor of *treatment* (vehicle, OXT) and a within-subject factor of *condition* (baseline, saline, yohimbine).

The critical cut-off point for statistically significant results was $p \leq .05$.

Results

One rat was excluded due to poor health (Experiment 2A). A second rat died following an acute 2.50 µg/rat OXT injection (Experiment 2B).

Experiment 2A

Although the number of active lever presses increased following the yohimbine injection, the effect was not statistically significant (*condition*: $F(1.11, 5.55) = 4.25$, $p = .087$, $\eta_p^2 = 0.46$; Figure 13). Moreover, no significant effect was found for the *treatment* ($F(1, 5) = 0.02$, $p = .897$, $\eta_p^2 = 0.004$) or the *treatment* x *condition* interaction ($F(1.11, 5.55) = .064$, $p = .833$, $\eta_p^2 = 0.01$; Figure 13A).

There was no statistically significant difference in inactive lever responses for either the *condition* ($F(1.05, 5.25) = 3.73$, $p = .108$, $\eta_p^2 = 0.43$), *treatment* ($F(1, 5) = 1.42$, $p = .287$, $\eta_p^2 = 0.22$) or the *treatment* x *condition* interaction ($F(1.05, 5.25) = .182$, $p = .699$, $\eta_p^2 = 0.04$; Figure 13B).

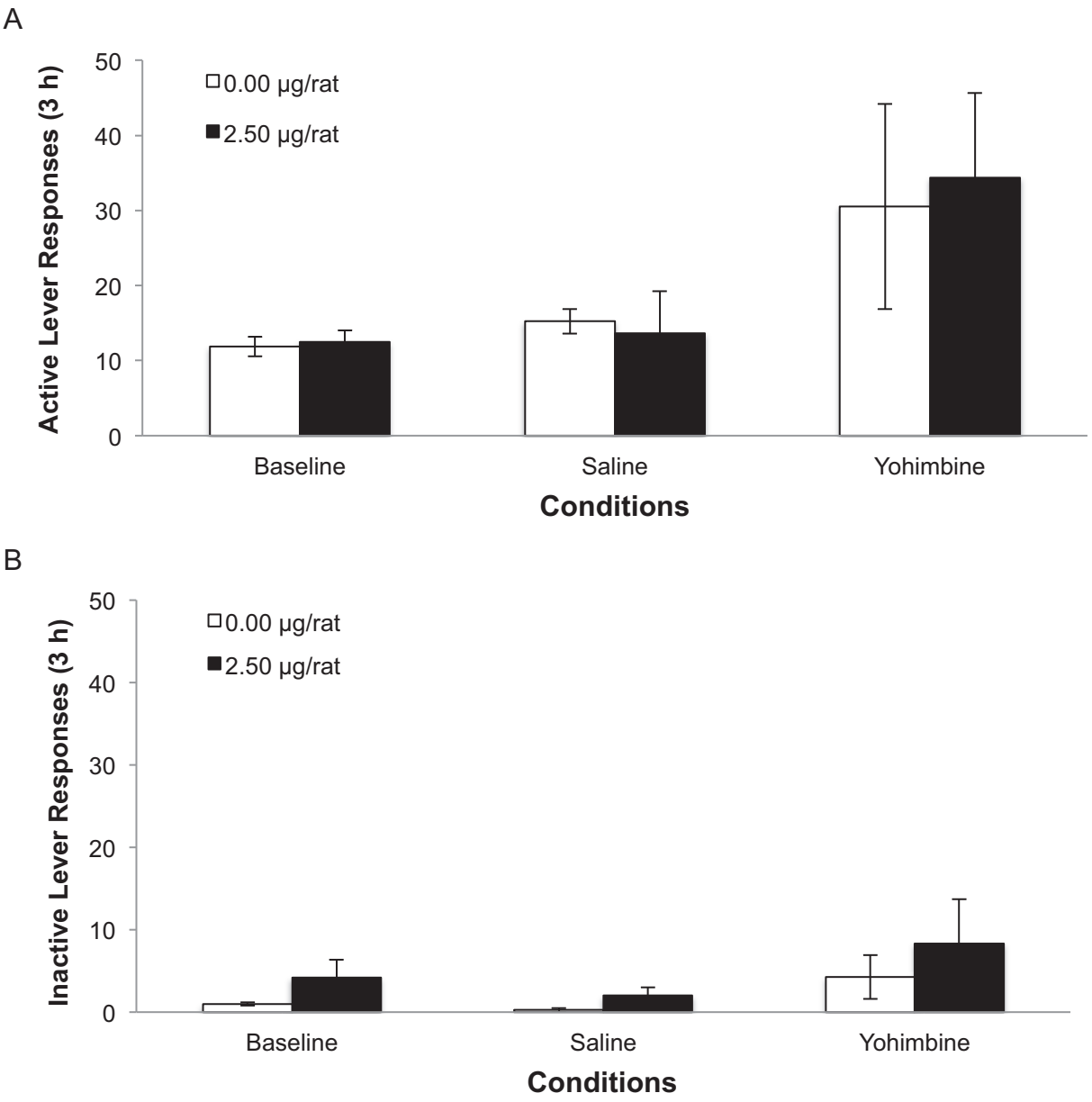


Figure 13. Mean (± *SEM*) number of active (A) and inactive (B) lever responses following an acute OXT (2.50 µg/rat) or saline injection (0.00 µg/rat; ICV; $n_{saline} = 4$, $n_{OXT} = 3$). All rats were tested following a yohimbine (2.00 mg/kg) and saline injection (counterbalanced; IP). The reinstatement tests took place under extinction conditions for a 3-h session under a FI-20 s schedule of reinforcement. Baseline was calculated by averaging the lever responses made during the extinction session that preceded each test. No statistically significant difference was found for any of the conditions.

Experiment 2B

Yohimbine injection resulted in an increase in the number of active lever responses performed during the test session (*condition:* ($F(2, 8) = 12.17$, $p < .01$, $\eta_p^2 = 0.75$; Figure 15). *Post-hoc t*-tests with Bonferroni-adjusted $\alpha = .017$ revealed a statistically significant difference between the Baseline active lever and the yohimbine condition ($t(6) = -3.74$, $p = .01$). There was no statistically significant effect for the *treatment* x *condition* interaction ($F(2, 8) = 2.20$, $p = .174$, $\eta_p^2 = 0.35$); however, the statistically significant yohimbine-induced increase in active lever responses seemed to be driven mostly by the OXT treated group. No significant effect was found for the *treatment (*$F(1, 4) = 5.45$, $p = .08$, $\eta_p^2 = 0.36$)

Moreover, there was no statistically significant difference in inactive lever responses for either the *condition* ($F(2, 8) = 3.70$, $p = .073$, $\eta_p^2 = 0.48$) or the *treatment* x *condition* interaction ($F(2, 8) = 0.48$, $p = .635$, $\eta_p^2 = 0.11$; see Figure 16). No significant effect was found for the *treatment (*$F(1, 4) = 0.02$, $p = .906$, $\eta_p^2 = 0.004$), as well.

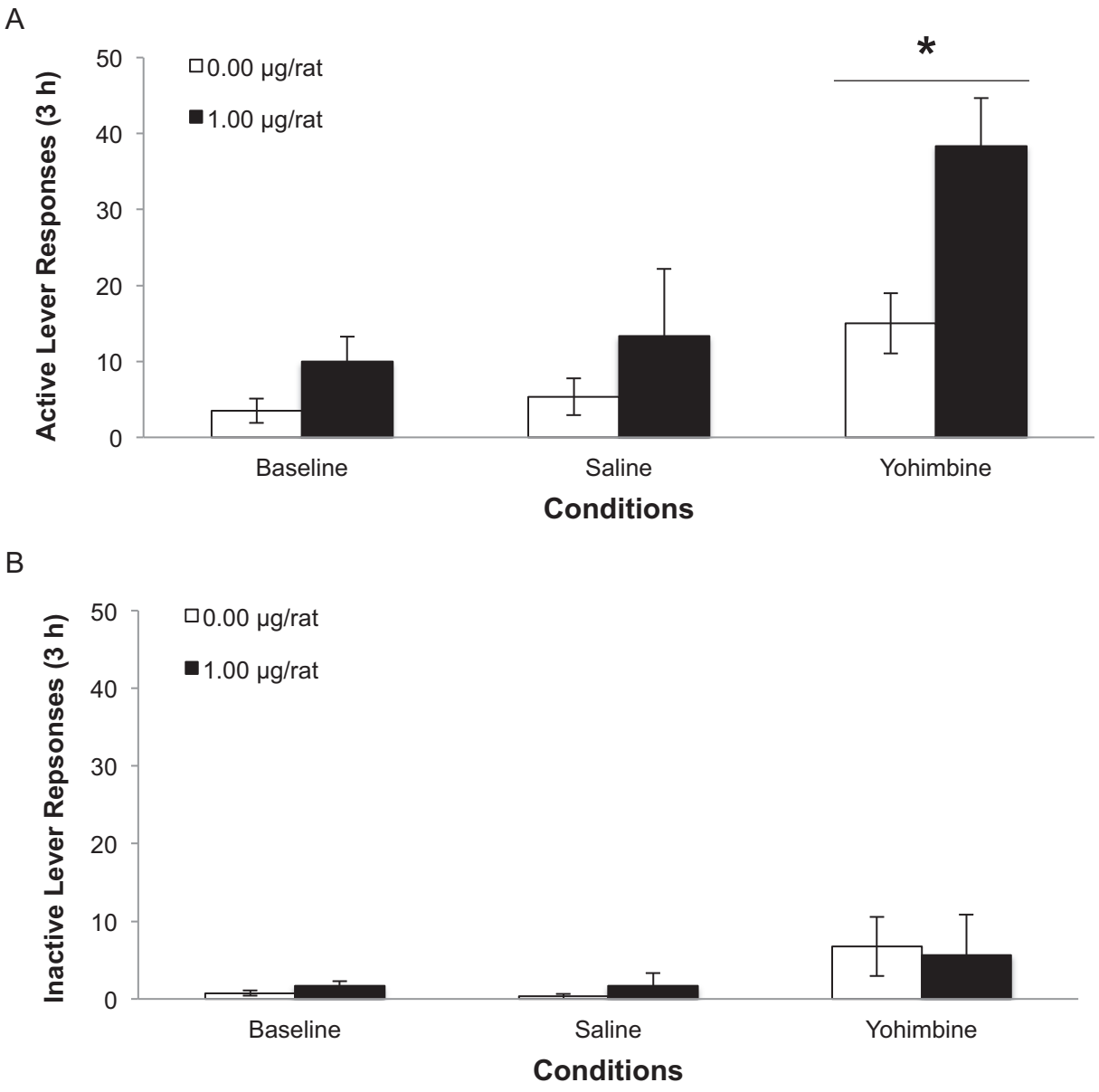


Figure 14. Mean (± *SEM*) number of active (A) and inactive (B) lever responses following an acute OXT (1.00 µg/rat) or saline injection (0.00 µg/rat; ICV; $n_{saline} = 3$, $n_{OXT} = 3$). All rats were tested following a yohimbine (2.00 mg/kg) and saline injection (counterbalanced; IP). The reinstatement tests took place under extinction conditions for a 3-h session under a FI-20 s schedule of reinforcement. Baseline was calculated by averaging the lever responses made during the extinction session that preceded each test. *p < .01, Yohimbine condition > Baseline.

Experiment 3: Effects of acute exogenous oxytocin on anxiety-related behaviours in relation to locomotor activity

Material & Methods

Subjects

The same rats (n = 7) used in Experiments 1 & 2A were used for the following experiments.

Apparatus

Elevated plus maze: A wooden elevated plus maze (EPM) was used in Experiment 3A. The EPM consisted of a wooden plus sign-shaped maze, which was elevated 50 cm from the ground and had two closed arms (10 cm x 50 cm) and two open arms (10 cm x 50 cm), both of which separated by a neutral centre zone (10 cm x 10 cm). The open arms were located opposite of each other, resembling a platform, as they did not have arms. The closed arms, also located opposite each other, had a wall of 50 cm height each.

Open field: An open field, made of metal and painted white (14 733.66 cm^2), was used to conduct Experiment 3B. The open field consisted of a wide circular metallic arena (diameter of 137 cm and walls 46 cm in height) and was placed in a brightly lit room with one food pellet weighing 5 g in the center.

Micro infusion pump: The micro infusion pump (Harvard Apparatus Model 11 Syringe Pump) was used to inject 1.00 μg/rat of OXT (or saline) prior to the anxiety-related behavioural tests.

Drug

Oxytocin lyophilized powder was dissolved and injected as described in the general methods section.

Procedure

Following Experiments 1 and 2, rats were tested for anxiety related-behaviours in procedures such as the elevated plus maze (EPM) and the open field.

Elevated plus maze: Rats were individually transported inside a covered bucket to the testing room where they received an OXT injection (1.00 μg/rat; ICV) or saline, in counterbalanced order. Twenty minutes after the injection, rats were placed in the central zone with their head facing one of the closed or open arms (counterbalanced between rats and treatment conditions). A video camera was suspended above the EPM, and the rats' behaviour was recorded over 5 minutes for off-line scoring. The test was performed in a brightly lit room with no outside interaction. An entry to an arm was defined as the condition in which the front paws and half the body of the rat were in one of the EPM arms (open or closed). The entire EPM was cleaned with 70 % ethanol solution between rats. Finally, four variables were scored: time spent in each arm (open, closed and central area) and number of entries in each arm. Forty-eight hours were given between tests in order for the drugs to wash out.

Open field: Before testing the rats in the open field, the animals were food restricted to 90% of their baseline body weight for 7 days in order to increase motivation to seek a food pellet that was placed in the middle of the open field. On test days, rats were brought into a dimly lit room where they were injected with OXT (1.00 μg/rat; ICV) or saline, in counterbalanced order.

Twenty minutes after the injection, rats were introduced into the open field. Rats were placed facing either the north, south, west or east positions of the arena and were then allowed to explore the open field for 10 minutes. A video camera was suspended above the open field apparatus in order to record movement for off-line scoring. Three variables were scored: latency to consumption of the food pellet, number of approaches to the food pellet, and number of moves per quadrant in the arena. Forty-eight hours were given between tests in order for the drugs to wash out.

Statistical Analyses

All analyses were conducted using SPSS software (IBM, SPSS Statistics, version 22). Behaviours scored were analyzed using a paired-sample *t*-test with *OXT condition* (saline or OXT [1.00 μg/rat]) as the independent variable, and *arm entries* (EPM), *time* spent *in each arm* (EPM), *latency to food consumption* (open field), *number of food approaches* (open field), and *number of moves per quadrant* (open field) as the dependent variables. The critical cut-off point for statistically significant results was $p \leq .05$.

Results

Elevated plus maze

Open arm entries: Paired-sample *t*-tests revealed a statistically significant higher number of entries into the open arms under the 0.00 μg/rat compared to the 1.00 μg/rat *OXT condition*, $t(6) = -3.03$, $p < .05$, $d = 0.28$ (Figure 15A).

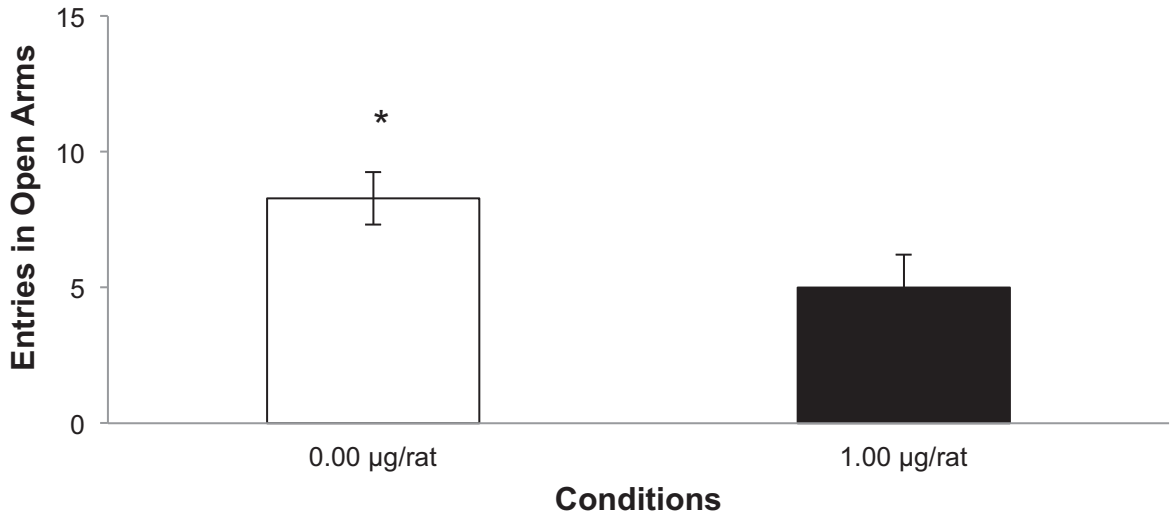
Time spent in open arms: Paired-sample *t*-tests revealed no statistically significant difference between the 0.00 μg/rat and the 1.00 μg/rat *OXT condition*, $t(6) = -0.46$, $p = .665$, $d = 0.02$ (Figure 15B).

Closed arm entries: Paired-sample *t*-tests revealed no statistically significant difference between the 0.00 μg/rat and the 1.00 μg/rat *OXT condition*, $t(6) = -0.082$, $p = .448$, $d = 3.38$ (Figure 16A).

Time spent in closed arms: Paired-sample t-tests revealed no statistically significant difference between the 0.00 μg/rat and the 1.00 μg/rat *OXT condition*, $t(6) = 1.04$, $p = .339$, $d = 0.23$ (Figure 16B).

Time spent in centre area: Paired-sample t-tests revealed no statistically significant difference between the 0.00 μg/rat and the 1.00 μg/rat *OXT condition*, $t(6) = -1.60$, $p = .161$, $d = 0.09$ (Figure 17).

A



B

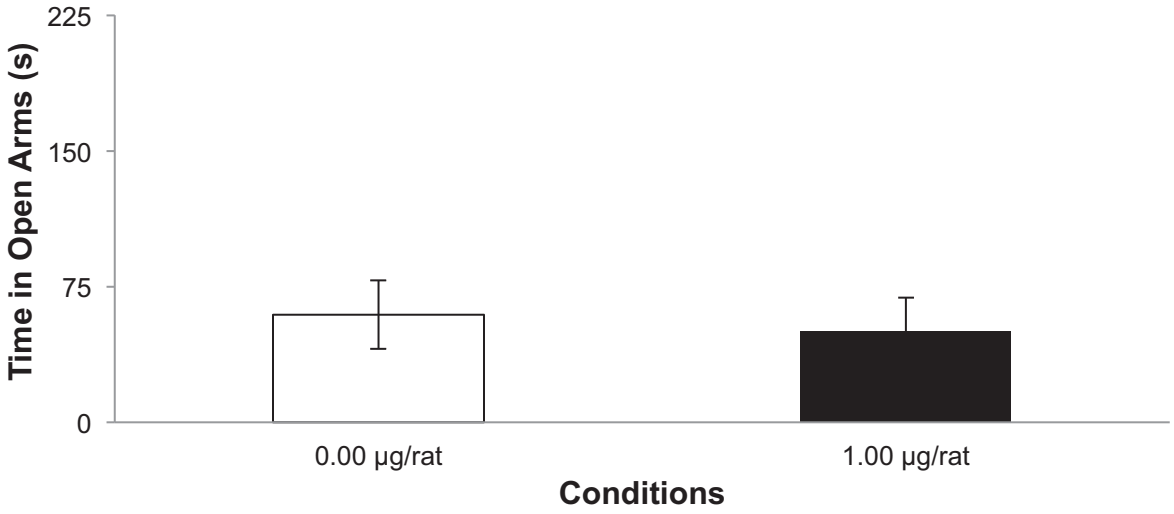
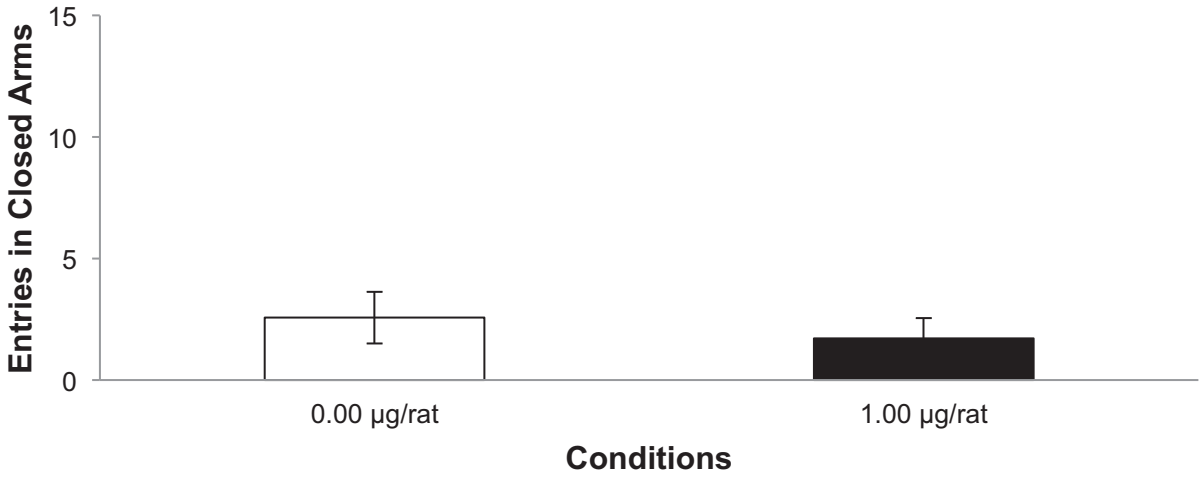


Figure 15. Mean (± *SEM*) of the number of entries (A) and the time spent (in s) (B) during a 5-min observation in the open arms of the EPM following either a saline (0.00 µg/rat) or an acute OXT (1.00 µg/rat) injection (ICV; $n = 7$). * $p < .05$.

A



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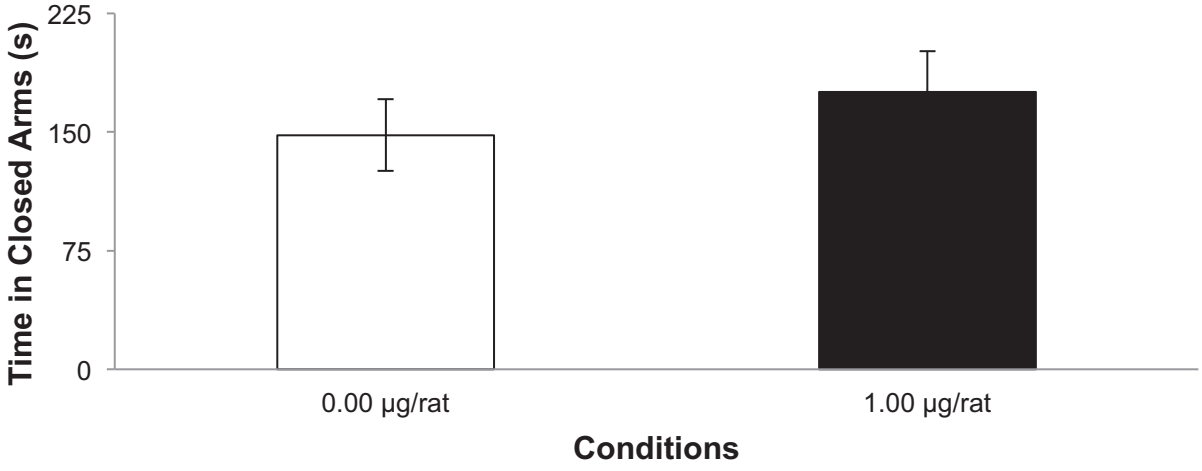

Figure 16. Mean (± *SEM*) of (A) the number of entries and (B) the time spent (in s) during a 5-min observation in the closed arms of the EPM following either a saline (0.00 µg/rat) or an acute OXT (1.00 µg/rat) injection (ICV; $n = 7$). No statistically significant differences were found.

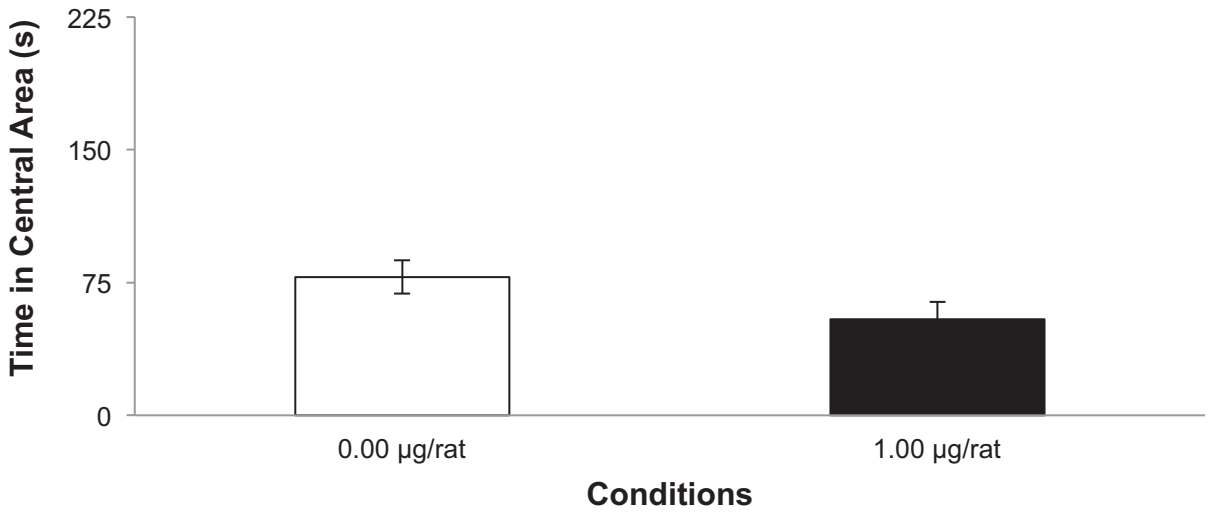

Figure 17. Mean (± *SEM*) of the number of entries during a 5-min observation in the central area of the EPM following either a saline (0.00 µg/rat) or an acute OXT (1.00 µg/rat) injection (ICV; *n* = 7). No statistically significant differences were found.

Open field

Latency to food pellet consumption: Paired-sample *t*-tests revealed no statistically significant difference between the saline and the acute OXT (1.00 μg/rat) in the latency to the consumption of the food pellet placed at the centre of the open field, $t(6) = 1.58$, $p = .769$, $d = 0.02$ (Figure 18).

Number of approaches to central area of the field: Paired-sample *t*-tests revealed no statistically significant difference between the saline and the acute OXT (1.00 μg/rat) in the number of approaches attempted towards the centre of the open field, $t(6) = 1.75$, $p = .131$, $d = 0.11$ (Figure 19).

Number of moves per quadrant: Paired-sample *t*-tests revealed no statistically significant difference between the saline and the acute OXT (1.00 μg/rat) in the number of moves per quadrant performed by rats in the open field, $t(6) = -0.31$, $p = .166$, $d = 0.07$ (Figure 20).

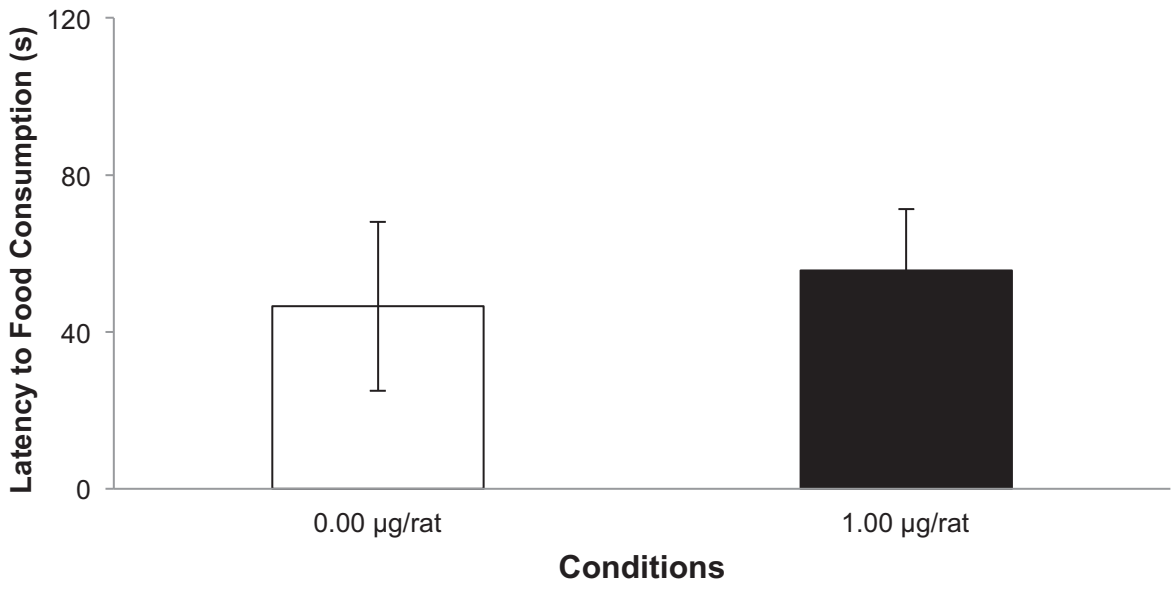

Figure 18. Mean (± *SEM*) of latency to food consumption in (in s) between the saline (0.00 µg/rat; $n = 7$) and OXT (1.00 µg/rat; $n = 7$) conditions. No statistically significant differences were found.

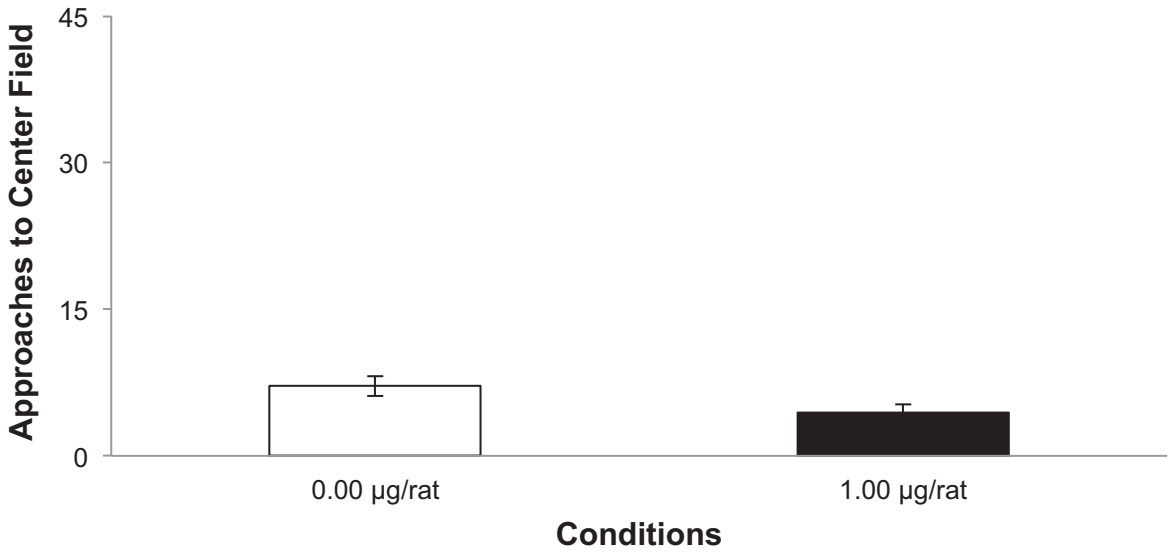

Figure 19. Mean (± *SEM*) number of approaches to the central area of the field during the 10-min observation period between the saline (0.00 µg/rat; $n = 7$) and OXT (1.00 µg/rat; $n = 7$) conditions. No statistically significant differences were found.

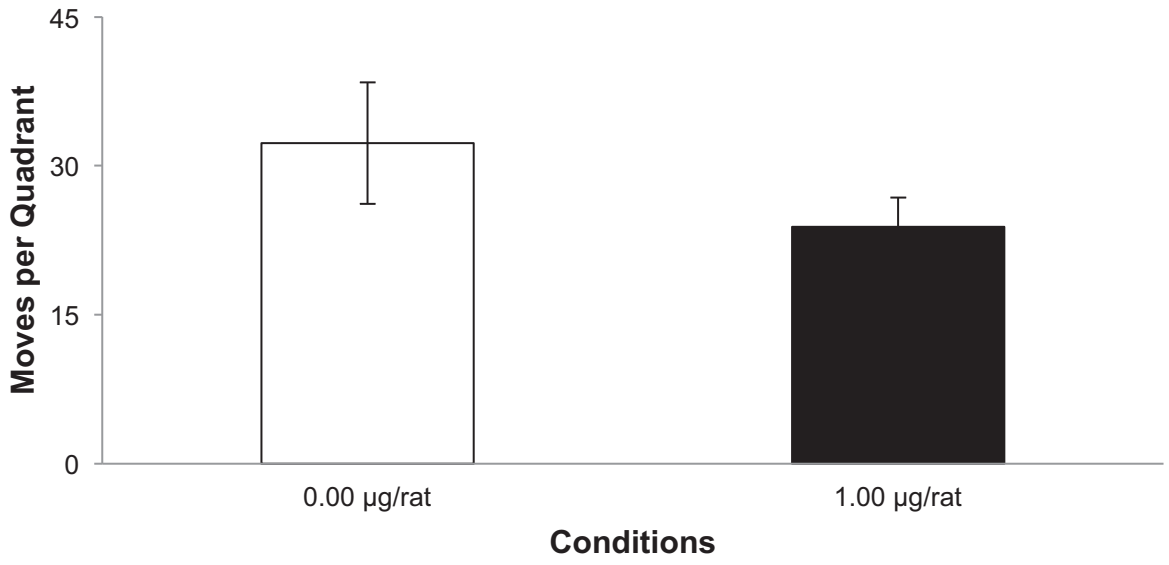

Figure 20. Mean (± *SEM*) number of moves per quadrant during the 10-min observation period between the saline (0.00 µg/rat; $n = 7$) and OXT (1.00 µg/rat; $n = 7$) conditions. No statistically significant differences were found.

Summary

Contrary to our expectations, exogenous OXT did not attenuate heroin taking or heroin seeking in male rats, hence failing to replicate previous findings with both psychostimulant- and opiate-trained rats. Analysis of time course throughout the first hour of the self-administration test session in Experiment 1 revealed reduced lever pressing, which might be due to a non-specific locomotor effect. In Experiment 2, oxytocin failed to reduce the anxiogenic effect induced by yohimbine, a pharmacological stressor, during reinstatement of extinguished heroin seeking. In addition, many cases of barrel rolling were observed following ICV injections of the highest dose of oxytocin used (i.e., 2.50 μg/rat). Experiment 3 aimed to investigate the effects of OXT on anxiety-related behaviours. Contrary to our hypothesis, OXT did not seem to have an anxiolytic effect on the rats in either tasks like the elevated plus maze or the open field.

CHAPTER 2

THE EFFECTS OF CHRONIC EXOGENOUS OXYTOCIN ON HEROIN TAKING AND HEROIN SEEKING IN MALE RATS

Janie Duchesneau, Leon Mayers, Stacy Pollack, & Uri Shalev

Introduction

Contrary to reported findings, both central and systemic acute OXT injections have failed to show any noticeable impact on either the acquisition or the maintenance of heroin self-administration (chapter 1). However, other studies have reported using a different procedure than the one introduced in chapter 1 by injecting OXT over a period of time (chronic injections) instead of acute injections.

Unfortunately, very few studies implicating the effect of OXT on drug addiction have been conducted and such chronic administrations have stated inconsistent findings (Peters, Slattery, Uschold-Schimdt, Reber, & Neumann, 2014). Prior research has shown that chronic injections of OXT inhibited stimulated release of dopamine in the basal forebrain (Kovács et al., 1986), key area for both OXT binding sites and drug-related behaviours (Sarnyai & Kovács, 1994); and OXT dose-dependently decreased cocaine-induced hyperlocomotion and stereotyped grooming behaviour (Kovács et al., 1998). While recent studies have found that acute systemic OXT injections seem to positively impact (e.g., reduce intake or drug seeking) cocaine SA and cocaine reinstatement (see chapter 1), very little is known on the effects of chronic OXT injections on the motivation to consume and seek drugs. However, Westenbroek et al. (2013) have shown that OXT, chronically injected over 14 days, reduced motivation to self-administer methamphetamine in rats (females).

In regards to our previous chapter, recent studies have shown how acute OXT could be of use in both drug intake and relapse prevention. As mentioned in chapter 1, OXT is well known to reduce anxiety-related behaviours. Peters et al.'s study (2014) aimed to investigate the impact of the neuropeptide in a clinically relevant animal paradigm modelling modern society's chronic stress. They assessed the effect of chronic OXT (injected over 15 days; ICV) on chronic

subordinate colony housing, a model for chronic psychosocial stress, and reported that low doses of OXT could be beneficial and protect against such stress.

In light of the very few studies that examined chronic OXT administration in relation to drug addiction, we investigated the role of chronically administered exogenous OXT on heroin self-administration on a progressive ratio schedule of reinforcement. Then, we aimed to investigate the behavioural impact of OXT on yohimbine-induced reinstatement of extinguish heroin seeking. As in chapter 1, rats were first trained to self-administer heroin for a period of 10 days on a FI-20 s and were then put on a progressive ratio schedule for 7 days. Following self-administration training, rats were assigned to three groups: vehicle (0.00 μg/rat; ICV), low (0.30 μg/rat; ICV) or high (1.00 μg/rat; ICV) OXT. Rats receive their assigned dose of OXT for 14 continuous days. Following this 14-day self-administration period on which the behavioural effect of OXT was measured, rats were kept in their operant-conditioning chambers for extinction training, as described in the general methods section. Finally, rats received either a yohimbine or a saline injection (2.00 mg/kg [IP]; counterbalanced) following their chronic OXT infusion on their reinstatement test day.

We predicted that, over the 14-day OXT treatment period, we would see a decrease in heroin intake. We also predicted that a decrease in behavioural responses (lever presses) would be found in the yohimbine-induced reinstatement tests in rats being treated with the low and high doses of OXT compared to the saline group.

Experiment 1: Effects of chronic exogenous oxytocin on heroin-taking behaviour in male rats

Material & Methods

Subjects

Long Evans rats (n = 17; 300-350g) were used. Rats were housed and treated as described in the general methods section.

Surgical procedures

Rats were implanted with intravenous (IV) silastic catheters to enable heroin self-administration and intracerebroventricular (ICV) cannulae to allow OXT injections, as detailed in the general methods section.

Apparatus

Operant-conditioning chambers: The operant-conditioning chambers used were identical to those described in the general methods section.

Micro infusion pumps: Micro infusion pumps (Harvard Apparatus Model 11 Syringe Pump) were used to inject a chosen dose of OXT (or saline) prior to SA sessions or test days as described in the general methods section.

Drug

Heroin (diacetylmorphine hydrochloride) and oxytocin lyophilized powder were dissolved as described in the general methods section.

Procedure

Before being placed in the operant-conditioning chambers, rats' guide cannula placements were verified using an angiotensin II-induced (100 nmol; ICV) short latency (< 60 s) drinking response. All ICV cannulae were successfully verified. Rats were first trained to self-administer heroin for 10 days under a fixed-interval 20 s schedule of reinforcement (FI-20 s; 3 x 3 h). Then, rats were switched to a PR schedule of reinforcement (one 5-h PR session per day) for 5 days until the PR self-administration training stabilized (> 5 infusions/session; see the general methods section). Following SA training, ICV exogenous oxytocin injections were administered over 14 days before the daily self-administration session. Rats first received two sham injections over two days before the daily OXT treatment started. Next, rats were matched based on their average breakpoints and active lever presses, then split into three equivalent groups and injected (ICV) with their respective dose of OXT (0.00, 0.30 and 1.00 μg/rat) immediately before their daily SA session.

Statistical Analyses

All analyses were conducted using SPSS software (IBM, SPSS Statistics, version 22).

Self-administration training: Data for all rats were analyzed using a repeated measures analysis of variance (ANOVA), with FI-20 s training day (1-10) and PR training day (11-15) as

the within-subject factor, and active and inactive lever responses or number of infusions as the dependent variables. The number of infusions earned in a session, which also represents the number of PR steps completed, was defined as the "breakpoint".

Test days**:** Number of infusions as well as active and inactive lever responses during the SA session following daily OXT injection were analyzed using repeated measures ANOVAs, with a between-subject factor of *OXT dose* (0.00 [vehicle], 0.30 [low] and 1.00 [high] μg/rat) and a within-subject factor of *day (1-14)*. The critical cut-off point for statistically significant results was $p \leq .05$.

Results

Two rats were excluded due to loss of their head cap and to poor health. Final analysis included 17 rats.

The Greenhouse-Geisser correction was used when Mauchly's test of sphericity assumptions was violated.

Training

Heroin infusions: There was a statistically significant increase in heroin infusions over time under the FI-20 s schedule, $F(3.44, 61.93) = 20.68$, $p < .01$ (Figure 21A), as well as for the PR schedule, $F(4, 68) = 2.94$, $p = .027$ (Figure 22A).

Active lever responses: Similar to heroin infusions, active lever responses increased throughout both FI-20 s training ($F(3.10, 55.79) = 7.02$, $p < .01$; Figure 21B) and PR training ($F(4, 68) = 3.01$, $p = .024$; Figure 22B).

Inactive lever responses: No statistically significant change was observed for inactive lever responses throughout both self-administration training regimes under FI-20 s ($F(3.06, 55.12) = 1.44$, $p = .240$) or PR ($F(2.41, 40.88) = 1.08$, $p = .358$).

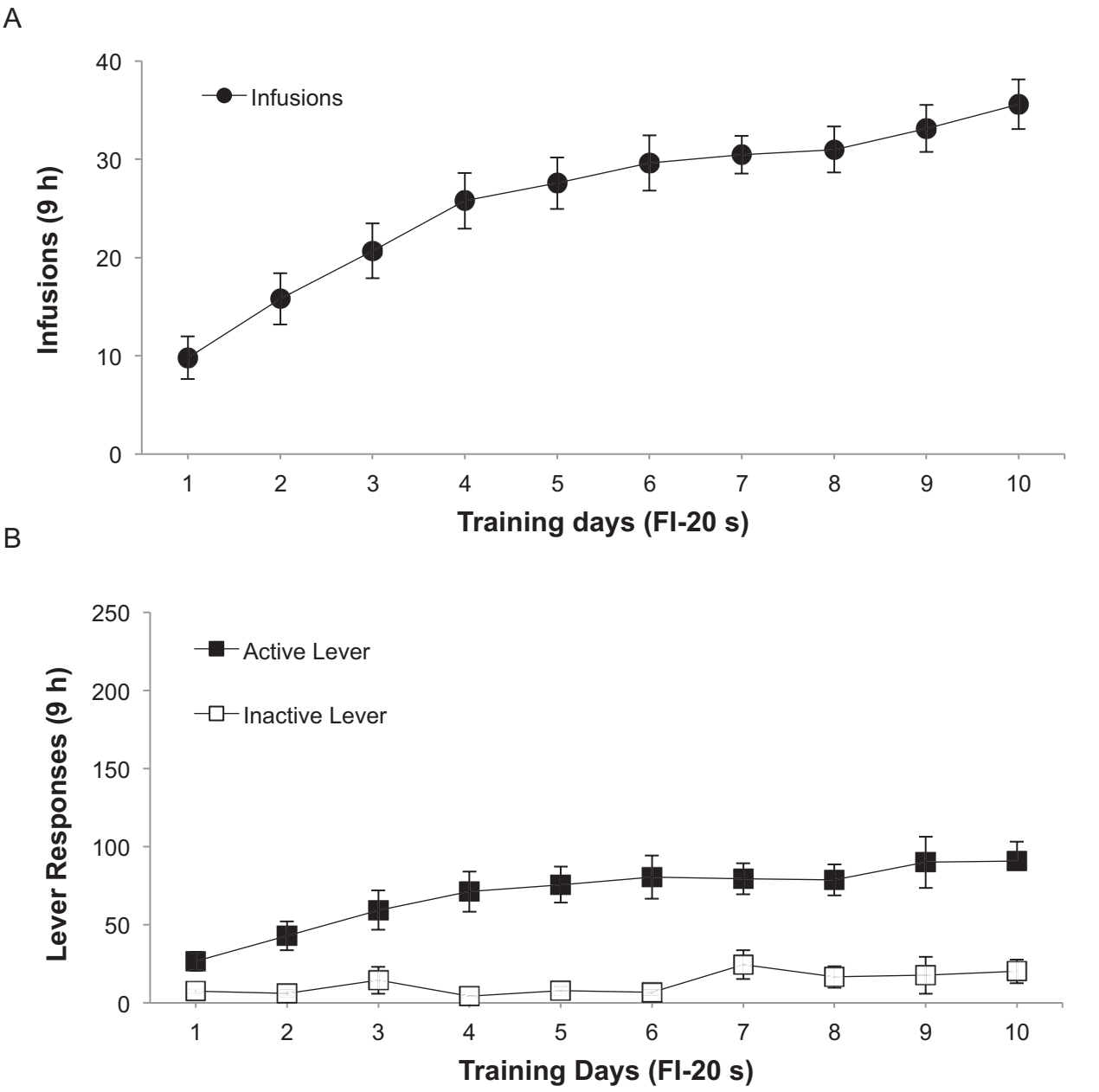

Figure 21. Mean (± *SEM*) number of infusions (A), active and inactive lever responses (B) during heroin self-administration training by rats in Experiment 1 ($n = 17$). Rats self-administered heroin (0.10 mg/kg/infusion) daily in three 3-h sessions under a fixed interval 20 s (FI-20) schedule of reinforcement, over a 10-day period. Both infusion and active lever responses increased at a statistically significant level ($p < .05$) during training. There was no statistically different change for inactive lever responses.

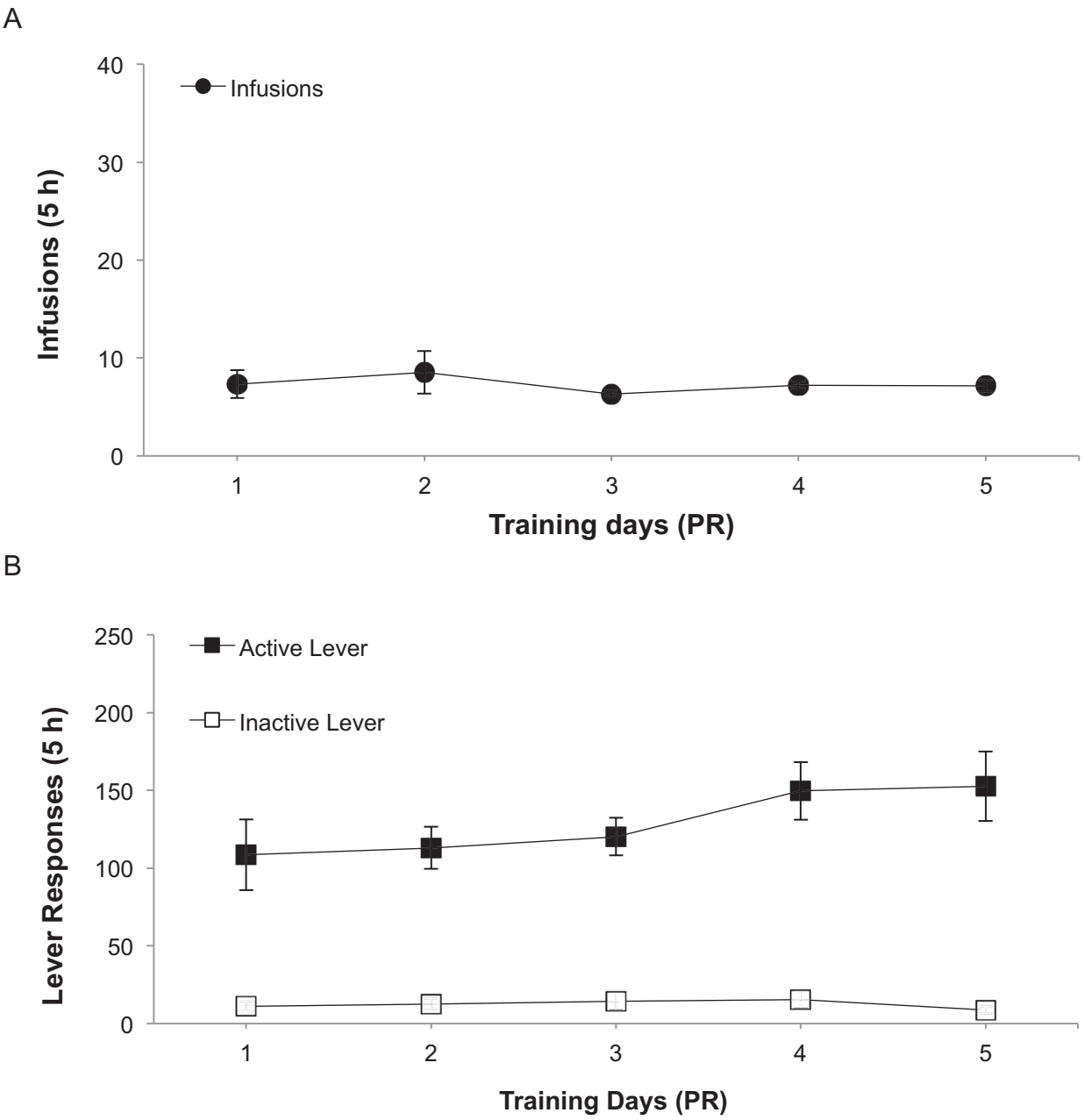

Figure 22. Mean (± *SEM*) number of infusions (breakpoint) (A), active and inactive lever responses (B) during heroin self-administration training by rats (n = 17). Rats self-administered heroin (0.10 mg/kg/infusion) daily in one 5-h session under a progressive-ratio (PR) schedule of reinforcement, over a 5-day period. Both infusion and active lever responses increased at a statistically significant level ($p < .05$) during training. There was no statistically different change for inactive lever responses.

Test days

Heroin infusions: There was no statistically significant change in heroin infusions over the 14-day period under PR schedule of reinforcement, $F_{day}(1.37, 19.17) = 0.94$, $p = .374$, $\eta_p^2 = 0.06$ (Figure 23). OXT administration had no statistically significant effect on the number of infusions obtained ($F_{OXT\ dose}(2, 14) = 0.59$, $p = .568$, $\eta_p^2 = 0.08$).There also was no statistically significant interaction effect for *day* (14 days) x *OXT dose* ($F(2.74, 19.17) = 1.13$, $p = .359$, $\eta_p^2 = 0.16$; Figure 23).

Active lever responses: There was no statistically significant change in active lever responses over the 14-day period under PR schedule of reinforcement, $F_{time}(4.92, 68.88) = 1.57$, $p = .180$, $\eta_p^2 = 0.10$ (Figure 24). OXT administration had no statistically significant effect on the number of active lever responses ($F_{OXT\ dose}(2, 14) = 0.09$, $p = .918$, $\eta_p^2 = 0.001$). Moreover, there was no statistically significant interaction effect for *day* x *OXT dose (*$F(9.84, 68.88) = 1.88$, $p = .065$, $\eta_p^2 = 0.21$; Figure 24).

Inactive lever responses: There was no statistically significant change in inactive lever responses over the 14-day period under PR schedule of reinforcement, $F_{time}(4.28, 59.90) = 1.74$, $p = .150$, $\eta_p^2 = 0.11$ (Figure 25). Furthermore, there was no statistically significant difference for *OXT dose (*$F_{OXT\ dose}(2, 14) = 1.90$, $p = .186$, $\eta_p^2 = 0.18$*)* or the interaction of *day* x *OXT dose*, $F(8.56, 59.90) = 1.26$, $p = .281$, $\eta_p^2 = 0.15$; Figure 25).

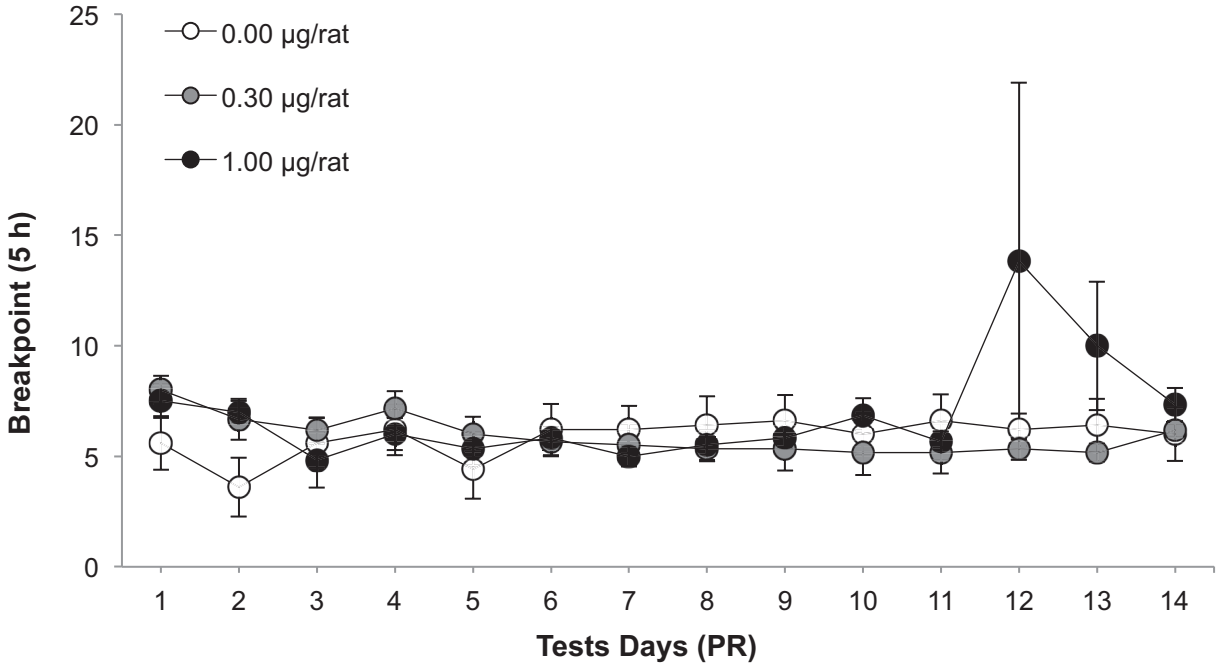

Figure 23. Mean (± *SEM*) number of heroin infusions (breakpoint) during the 14 days of chronic OXT (0.00 [vehicle], 0.30 [low] and 1.00 [high] µg/rat) administration. Rats self-administered heroin (0.10 mg/kg/infusion) daily in one 5-h session under a PR schedule of reinforcement. There were no statistically significant differences in heroin infusions for any of the groups ($n_{Vehicle}$ = 5; n_{Low} = 6; n_{High} = 6) over the 14 days of chronic OXT administration (ICV).

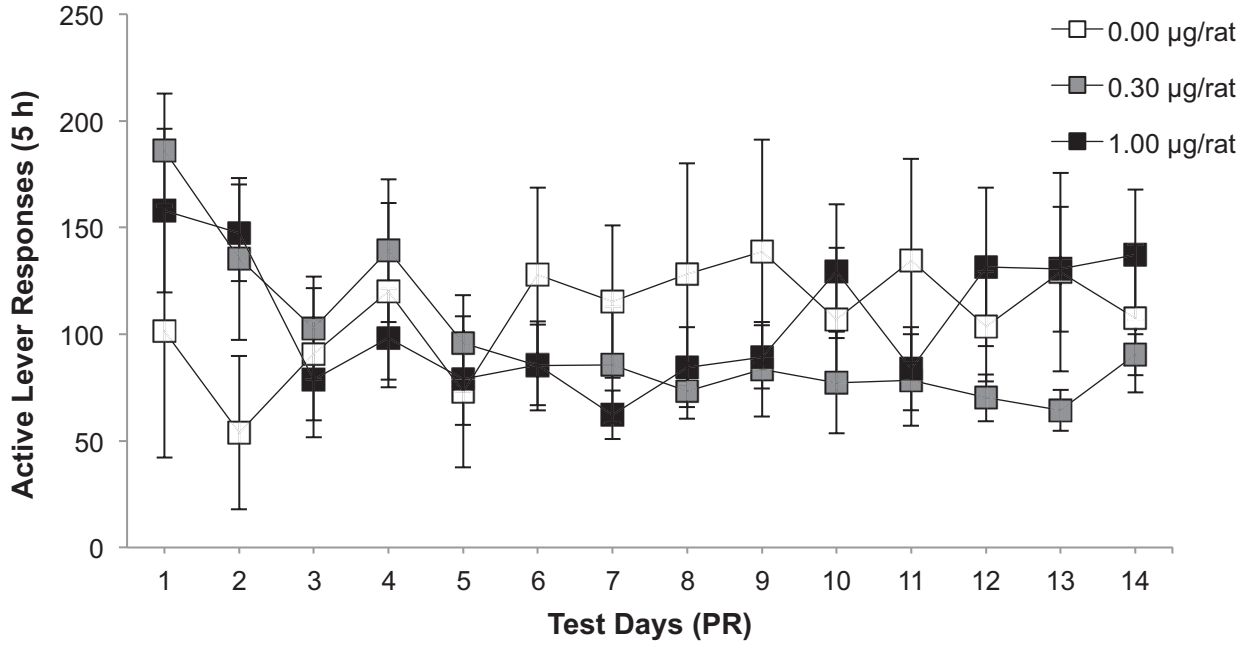

Figure 24. Mean (± *SEM*) number of active lever responses during the 14 days of chronic OXT (0.00 [vehicle], 0.30 [low], and 1.00 [high] µg/rat) administration. Rats self-administered heroin (0.10 mg/kg/infusion) daily in one 5-h session under a PR schedule of reinforcement. There were no statistically significant differences in active lever responses for any of the groups ($n_{Vehicle}$ = 5; n_{Low} = 6; n_{High} = 6) over the 14 days of chronic OXT administration (ICV).

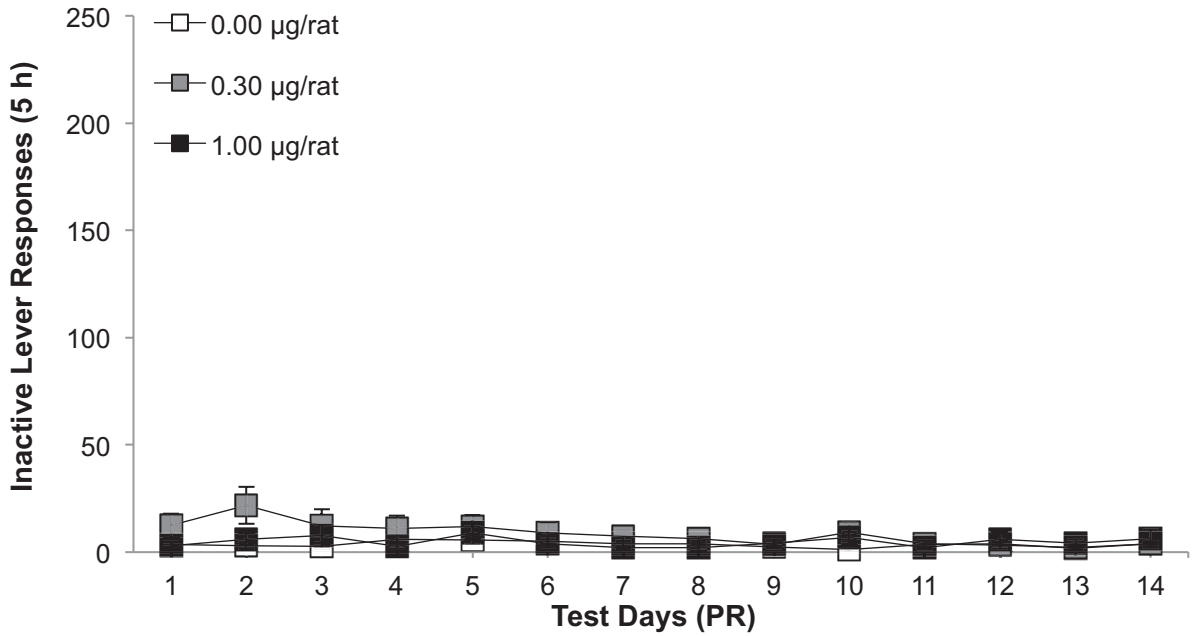

Figure 25. Mean (± *SEM*) number of inactive lever responses during the 14 days of chronic OXT (0.00 [vehicle], 0.30 [low], and 1.00 [high] µg/rat) administration. Rats self-administered heroin (0.10 mg/kg/infusion) daily in one 5-h session under a PR schedule of reinforcement. There were no statistically significant differences in inactive lever responses for any of the groups ($n_{Vehicle}$ = 5; n_{Low} = 6; n_{High} = 6) over the 14 days of chronic OXT administration (ICV).

Experiment 2: Effects of chronic exogenous oxytocin on yohimbine-induced reinstatement of extinguished heroin seeking

Material & Methods

Subjects

The same rats used in Experiment 1 were used in Experiments 2A (n = 9) and 2B (n = 7). However, one rat was excluded due to poor health.

Apparatus

Operant-conditioning chambers: The operant-conditioning chambers used were identical to those described in the general methods section.

Micro infusion pumps: Micro infusion pumps (Harvard Apparatus Model 11 Syringe Pump) were used to inject a chosen dose of OXT or saline, every day, prior to the rats' reinstatement test, as described in the general methods section.

Drug

Yohimbine hydrochloride powder (Sigma-Aldrich, St-Louis, MO) was dissolved in sterile saline (2.00 mg/kg) and injected IP. Oxytocin lyophilized powder was dissolved and injected as described in the general methods section.

Procedures

Extinction: Following the 14 days of testing under chronic OXT injections (Experiment 1), rats underwent extinction while continuing to receive daily OXT injections, until they reached the extinction criterion (< 15 active lever responses per 5 h session). The rats were exposed to the same conditions and cues as described in the general methods section; however, no heroin was made available. Extinction training consisted of one daily 5-h session under PR schedule of reinforcement (Experiment 2A) or one 3-h session under FI-20 s schedule of reinforcement (Experiment 2B). Extinction training continued for at least 4 days and until the rats reached the extinction criterion of ≤ 15 active lever responses during one 5-h session under PR (Experiment 2A) or during one 3-h session under FI-20 s (Experiment 2B).

Yohimbine-induced reinstatement: Once the rats met the extinction criterion they were tested for yohimbine-induced reinstatement on the next day. The effects of chronic OXT injections on reinstatement were first tested in Experiment 2A over one 5-h PR session. Then, the effects of chronic OXT injections were tested in Experiment 2B over one 3-h FI-20 s. For both experiments, rats in each group were tested twice for reinstatement following an injection of saline or yohimbine (2.00 mg/kg; IP) in counterbalanced order, administered approximately 30 minutes before the test session. OXT (0.00 [vehicle], 0.30 [low], and 1.00 [high] μg/rat; ICV) doses were injected immediately before the session. Reinstatement tests were separated by at least one day of extinction training. Active and inactive lever responses were recorded during all sessions, and Baseline of active and inactive lever responses was calculated by averaging the lever responses made during the extinction session that preceded each test.

Statistical Analyses

All analyses were conducted using SPSS software (IBM, SPSS Statistics, version 22). Separate repeated measures ANOVAs were conducted for the active and inactive lever responses. First, lever responses (active and inactive; dependent variables) during the first 4 days of extinction training were analyzed with separate repeated measures ANOVA with *day* (1-4) as the within-subject factor and *OXT dose* as a between-subject factor (vehicle, low, and high). Second, reinstatement test analyses included a between-subject factor of *OXT dose* (vehicle, low, and high) and a within-subject factor of *condition* (baseline, saline, yohimbine). The critical cut-off point for statistically significant results was $p \leq .05$.

Results

Final analysis for extinction training (> 4 days) included 9 rats (Experiment 2A). Two rats were then excluded due to poor health (experiment 2B).

The Greenhouse-Geisser correction was used when Mauchly's test of sphericity assumptions was violated.

Experiment 2A: Extinction training

Active lever responses: There was a statistically significant decrease in active lever responses over the 4 days of extinction training, $F_{day}(3, 18) = 3.90$, $p = .026$, $\eta_p^2 = 0.39$ (see Figure 26A). Also, OXT administration had no statistically significant effect on the number of active lever presses: $F_{OXT\ dose}(2, 6) = 0.91$, $p = .452$, $\eta_p^2 = 0.23$. Moreover, no significant effect was found for the *OXT dose* x *day* interaction ($F(6, 18) = 0.61$, $p = .717$, $\eta_p^2 = 0.17$).

Inactive lever responses: There was no statistically significant difference in inactive lever responses during extinction training for either the *day* ($F(3, 21) = 0.82$, $p = .495$, $\eta_p^2 = 0.04$) or the *OXT dose* x *day* interaction ($F(6, 21) = .61$, $p = .721$, $\eta_p^2 = 0.14$; see Figure 26B). Finally, OXT administration had no statistically significant effect on the number of inactive lever presses: $F_{OXT\ dose}(2, 7) = 0.89$, $p = .452$, $\eta_p^2 = 0.30$).

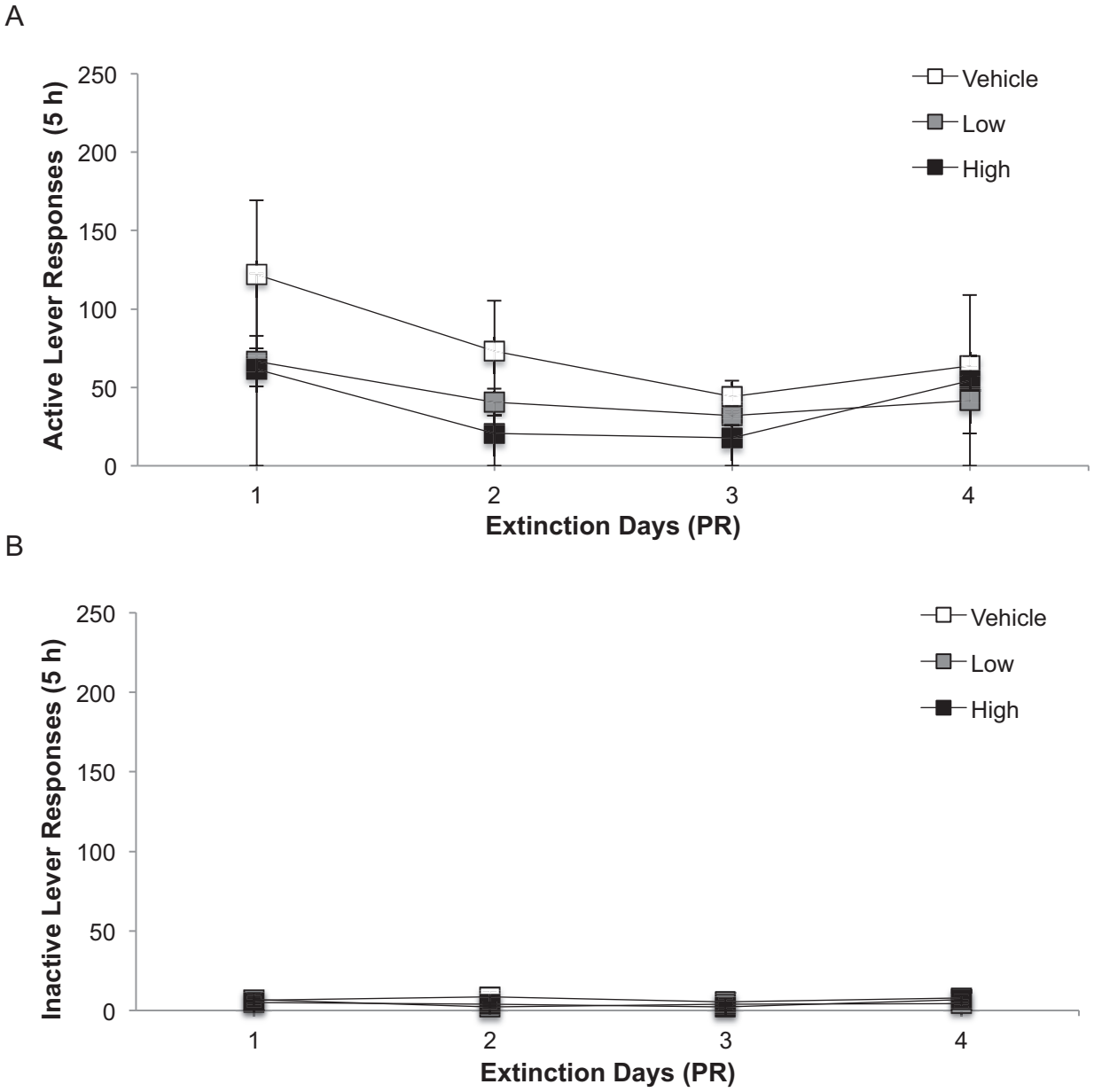

Figure 26. Mean (± *SEM*) number of active (A) and inactive (B) lever responses during the 4 days of extinction training with one daily 5-h session under a PR schedule of reinforcement. Rats continued to receive their daily OXT (0.00 [vehicle], 0.30 [low], and 1.00 [high] μg/rat) administration. There was no statistically significant decrease in active or inactive lever responses for any of the groups ($n_{Vehicle} = 4$; $n_{Low} = 3$; $n_{High} = 2$) over the 4 days of extinction training.

Experiment 2A: Yohimbine-induced reinstatement

Active lever responses: There was no statistically significant difference in active lever responses during yohimbine-induced reinstatement test for either the *Condition* ($F(2, 12) = 3.31$, $p = .072$, $\eta_p^2 = 0.39$) or for the *OXT dose* x *Condition* interaction ($F(4, 12) = 1.84$, $p = .186$, $\eta_p^2 = 0.17$; see Figure 27A). No significant effect was found for the *OXT dose* ($F(2, 6) = 0.41$, $p = .683$, $\eta_p^2 = 0.23$).

Inactive lever responses: There was no statistically significant difference in inactive lever responses following yohimbine-induced reinstatement tests for either the *Condition* ($F(2, 12) = 2.02$, $p = .175$, $\eta_p^{2 =} 0.25$) or the *OXT dose* x *Condition* interaction ($F(4, 12) = 0.52$, $p = .721$, $\eta_p^{2 =} 0.15$; see Figure 27B). Moreover, no significant effect was found for the *OXT dose* ($F(2, 6) = 1.91$, $p = .228$, $\eta_p^2 = 0.39$).

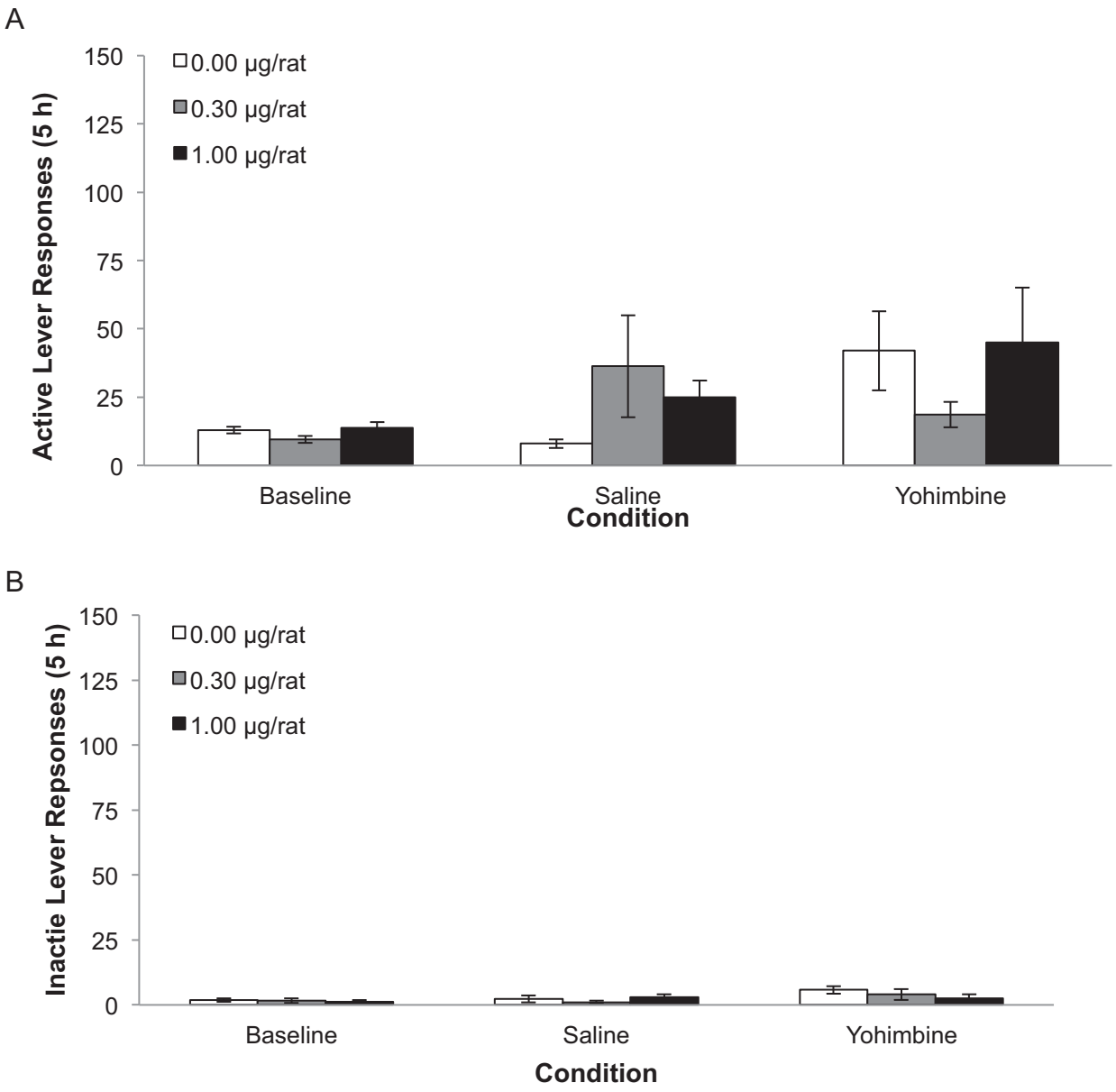

Figure 27. Mean (± *SEM*) number of active (A) and inactive (B) lever responses following chronic OXT injection (0.00, 0.30, 1.00 µg/rat; ICV; $n_{Vehicle} = 4$; $n_{Low} = 3$; $n_{High} = 2$). All rats were tested following yohimbine (2.00 mg/kg) and saline injections (counterbalanced order; IP). The reinstatement tests took place under extinction conditions during a 5-h session under a PR schedule of reinforcement. Baseline was calculated by averaging the lever responses made during the extinction session that preceded each test. No statistically significant difference was found for any of the conditions.

Experiment 2B: Extinction training

Active lever responses: There was no statistically significant difference in active lever responses during the extinction training, $F_{day}(1.03, 4.11) = 2.84$, $p = .166$, $\eta_p^{2 =} 0.42$ (see Figure 28A). No significant effect was found for the *OXT dose* x *day* interaction, either ($F(2.05, 4.11) = 0.36$, $p = .721$, $\eta_p^{2 =} 0.15$). Moreover, no significant effect was found for the *OXT dose* ($F(2, 4) = 0.17$, $p = .848$, $\eta_p^2 = 0.08$).

Inactive lever responses: There was no statistically significant difference in inactive lever responses during extinction training for either the *day* ($F(3, 12) = 0.52$, $p = .678$, $\eta_p^2 = 0.11$) or the *OXT dose* x *day* interaction ($F(6, 12) = .54$, $p = .770$, $\eta_p^2 = 0.21$; see Figure 28B). Furthermore, no significant effect was found for the *OXT dose* ($F(2, 4) = 0.59$, $p = .594$, $\eta_p^2 = 0.23$).

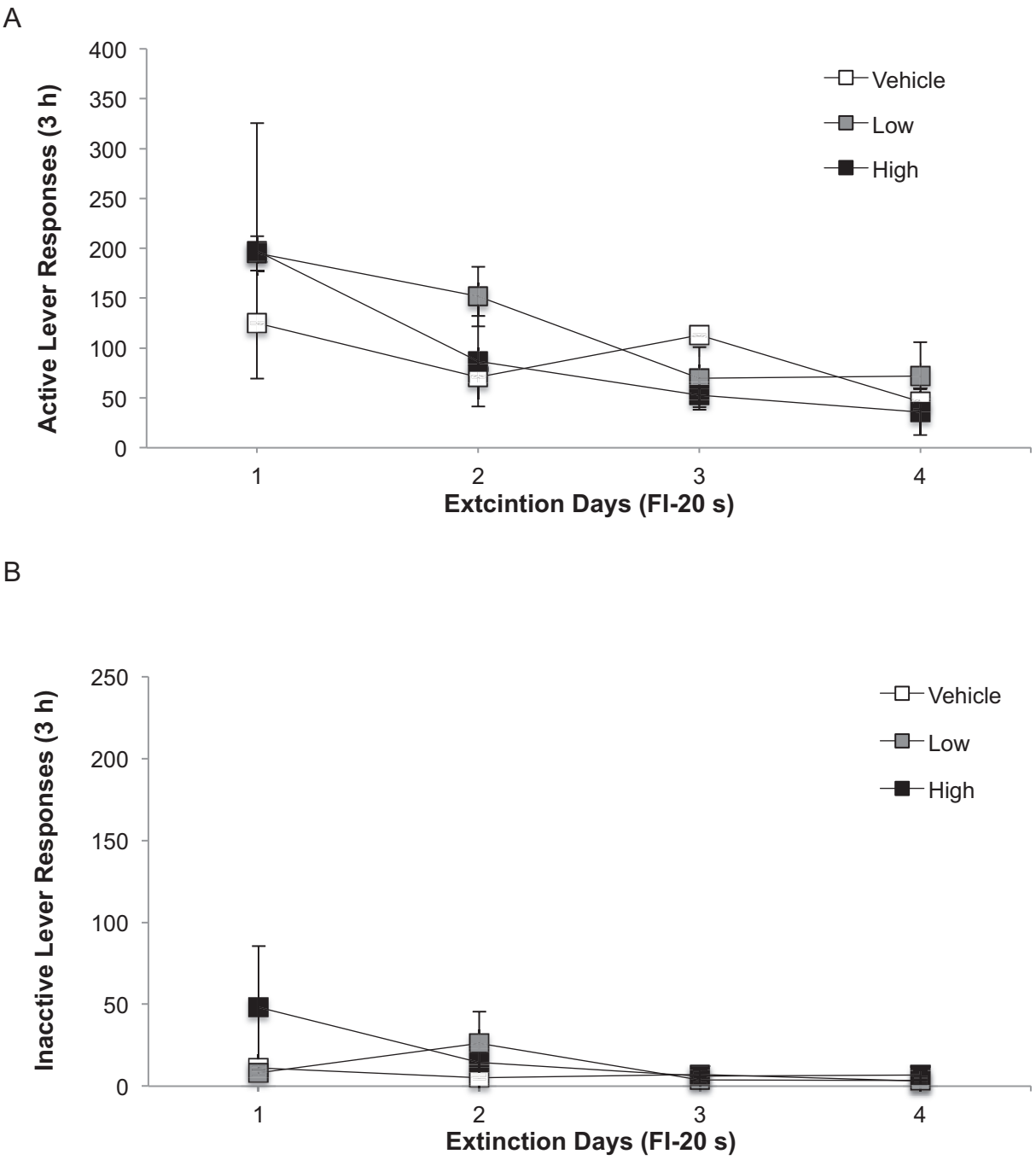

Figure 28. Mean (± *SEM*) number of active (A) and inactive (B) lever responses during the 4 days of extinction training for one 3-h session under a FI-20 s schedule of reinforcement. Rats continued to receive their daily OXT (0.00 [vehicle], 0.30 [low], and 1.00 [high] μg/rat) administration. There were no statistically significant differences in inactive lever responses for any of the groups ($n_{Vehicle}$ = 1; n_{Low} = 3; n_{High} = 3) over the 4 days of extinction training.

Experiment 2B: Yohimbine-induced reinstatement

Active lever responses: There was no statistically significant difference in active lever responses during yohimbine-induced reinstatement tests for either the *Condition* ($F(1.06, 4.23) = 2.57$, $p = .181$, $\eta_p^{2 =} 0.39$) or for the *OXT dose* x *Condition* interaction ($F(2.12, 4.23) = 0.52$, $p = .640$, $\eta_p^{2 =} 0.20$; see Figure 29A). No significant effect was found for the *OXT dose* ($F(2, 4) = 0.19$, $p = .838$, $\eta_p^2 = 0.08$).

Inactive lever responses: There was no statistically significant difference in inactive lever responses following yohimbine-induced reinstatement test for either the *Condition* ($F(2, 8) = 0.24$, $p = .792$, $\eta_p^{2 =} 0.06$) or the *OXT dose* x *Condition* interaction ($F(4, 80) = 0.32$, $p = .860$, $\eta_p^{2 =} 0.14$; see Figure 29B). Moreover, no significant effect was found for the *OXT dose* ($F(2, 4) = 0.36$, $p = .721$, $\eta_p^2 = 0.15$).

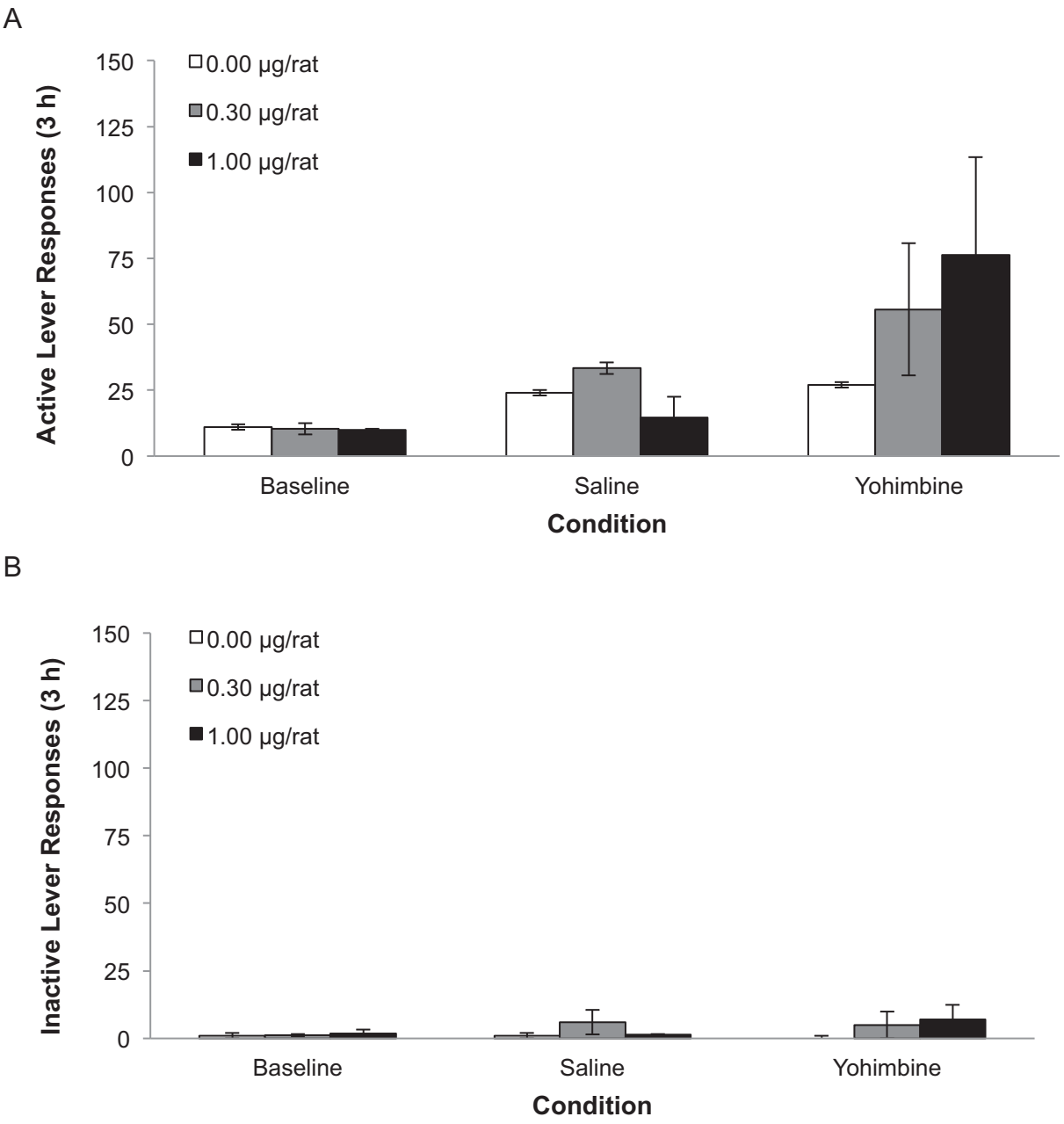

Figure 29. Mean (± *SEM*) number of active lever responses following chronic OXT (0.00, 0.30, 1.00 µg/rat; ICV; $n_{Vehicle}$ = 1; n_{Low} = 3; n_{High} = 3). All rats were tested following yohimbine (2.00 mg/kg) and saline injections (counterbalanced order; IP). The reinstatement tests took place under extinction conditions for a 3-h session under a FI-20 s schedule of reinforcement. Baseline was calculated by averaging the lever responses made during the extinction session that preceded each test. No statistically significant difference was found for any of the conditions.

Experiment 3: Effects of chronic exogenous oxytocin on stress-induced locomotor activity

Materials & Methods

Subjects

The same rats (n = 14) used in Experiment 1 through Experiments 2A and 2B were used in Experiment 3. Unfortunately, two rats were excluded due to minor barrel rolling following an OXT injection (1.00 μg/rat; ICV) and testicle swelling (one rat), and a blockage of the ICV cannula (one rat).

Apparatus

Locomotor activity chamber: Locomotor activity was measured on the 28th day of consecutive OXT injections (0.00, 0.30, 1.00 μg/rat; ICV) with the help of infrared activity-monitoring devices (TruScan Systems, Coulbourn Instruments, Whitehall, PA, USA) designed for rats. The apparatuses were made of clear polyethylene test chambers (39 cm x 42 cm x 50 cm) with 32 light-emitting diode photo detectors evenly spaced (2.50 cm) across the base of the apparatus. All locomotor boxes featured a slide-out floor for easier maintenance. The slide-out floor of each respective device was cleaned with ethanol (70 %) between each rat. Data were recorded using TruScan 2 (TS 2.0) software, and the following variables were selected for analysis: total distance travelled (in cm) and rest time. All rats were tested throughout the experiment in the same respective activity chamber at the same time of day they would self-administer in operant-conditioning chambers.

Forced swim stress: Water buckets (diameter: 30 cm and depth: 40 cm) were filled with lukewarm water.

Micro infusion pump: The micro infusion pump (Harvard Apparatus Model 11 Syringe Pump) was used to inject OXT (or saline) prior to SA sessions on test days as described in the general methods section.

Drug

Oxytocin lyophilized powder was dissolved and injected as described in the general methods section.

Procedures

Habituation: Rats were first introduced to their locomotor box for 30 min before receiving their daily OXT injection.

Forced swim stress: Rats were gently dropped into their respective bucket of water and stayed there for a total of 10 min. Rats were then collected, dried off and put back into their respective locomotor activity box.

Locomotor activity: Rats were put back in the same chamber they first habituated in and data were recorded for the following 60 min.

Statistical Analyses

All analyses were conducted using SPSS software (IBM, SPSS Statistics, version 22). Separate repeated measures ANOVAs were conducted for distance travelled and rest time using *time* as the within-subject factor (12 x 5-min bins) for the 60 minutes following the habituation and injection period. Analyses included a between-subject factor of *OXT dose* (vehicle, low, and high).

Total *distance travelled* (in cm), and total *rest time* (dependent variables) were analyzed with separate one-way ANOVAs with *OXT dose* (0.00, 0.30, 1.00 μg/rat) as the between-subject factor.

The critical cut-off point for statistically significant results was $p \leq .05$.

Results

Final analysis for stress-induced locomotor activity included 14 rats.

The Greenhouse-Geisser correction was used when Mauchly's test of sphericity assumptions was violated.

Experiment 3: Locomotor activity (distance travelled)

Distance travelled (by 5-min bins): Distance travelled over time decreased over the first five 5-min time bins, and then stabilized. There was a statistically significant effect of *time* ($F(3.35, 36.84) = 3.57$, $p = .020$, $\eta_p^2 = 0.26$). There were no statistically significant difference for

either the *OXT dose* x *time* interaction ($F(6.70, 36.84) = 1.99$, $p = .085$, $\eta_p^{2 =} 0.27$) or the effect of *OXT dose* ($F(2, 11) = 0.15$, $p = .862$, $\eta_p^{2 =} 0.03$).

Total distance travelled (cm): Homogeneity of variance (Levene's Test) was violated ($p = .002$), thus the Welch-Satterthwaite correction was applied. The one-way ANOVA revealed no statistically significant decrease in the total distance travelled during the 60 min spent in the locomotor activity box following an *OXT dose* administration ($F(2, 5.73) = 1.73$, $p = .258$; see Figure 31).

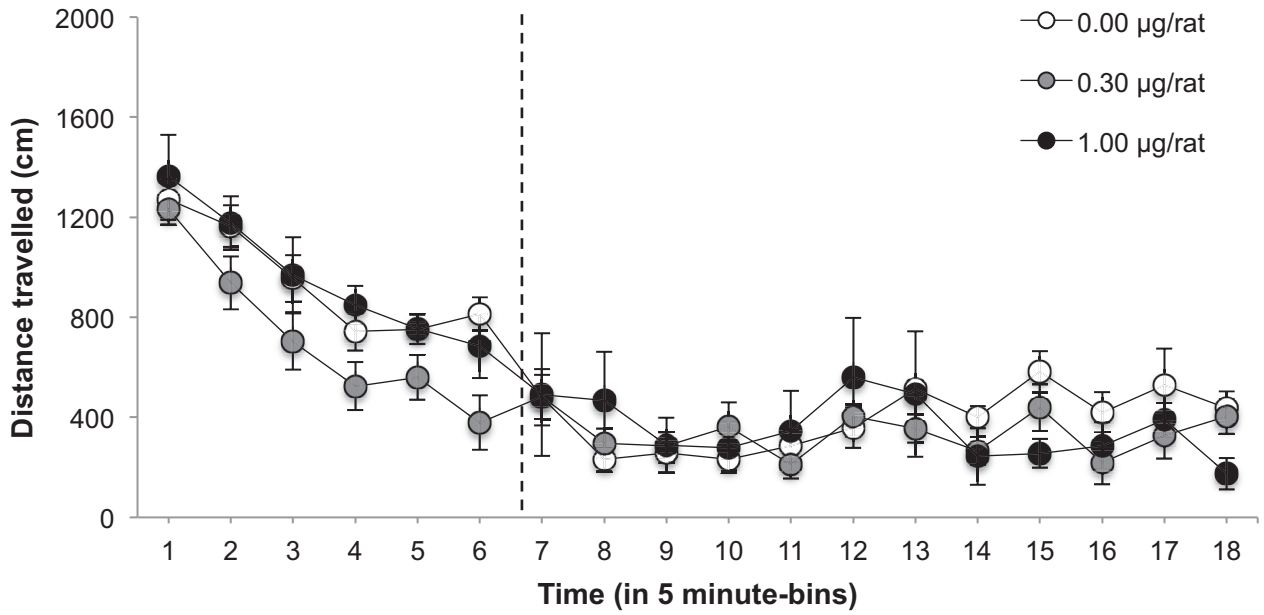

Figure 30. Mean (± *SEM*) of distance travelled (cm) following an OXT injection (ICV; $n_{0.00\ \mu g/rat} = 5$; $n_{0.30\ \mu g/rat} = 5$; $n_{1.00\ \mu g/rat} = 4$).

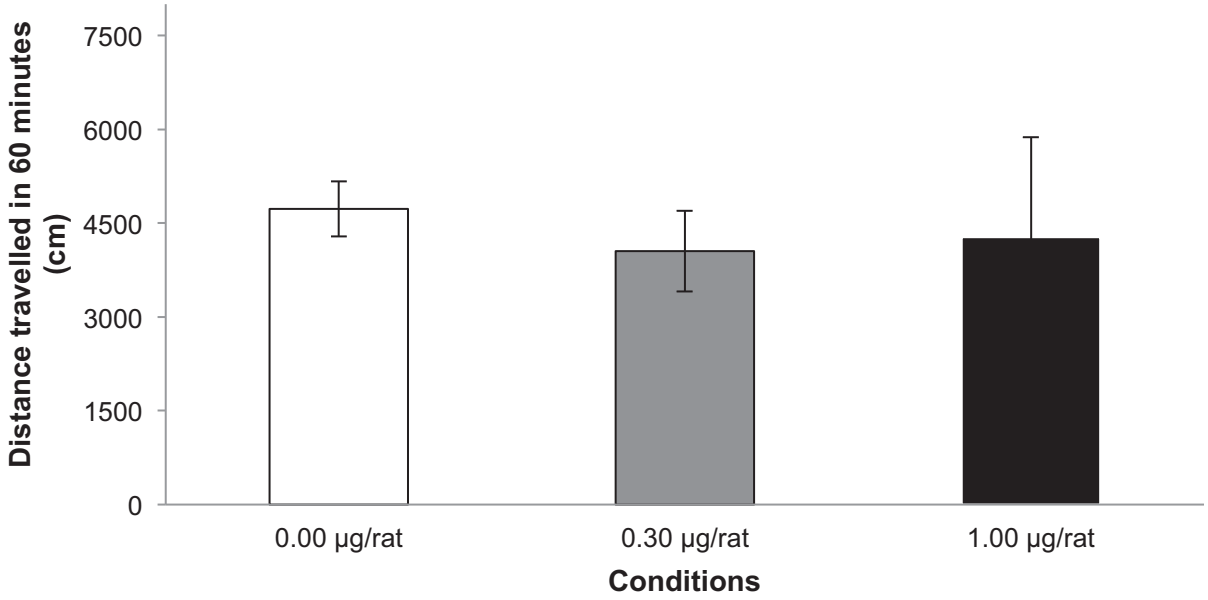

Figure 31. Mean (± *SEM*) of total distance travelled (in cm) during the 60 min following an OXT injection (ICV; $n_{0.00\ \mu g/rat} = 5$; $n_{0.30\ \mu g/rat} = 5$; $n_{1.00\ \mu g/rat} = 4$). No statistically significant differences were found when comparing the high dose to both the vehicle and low doses of OXT.

Experiment 3: Locomotor activity (resting time)

Rest time (by 5-min bins): There was no statistically significant difference in the time used by rats to rest in the locomotor box in relation to the *time* ($F(4.60, 50.55) = 2.15$, $p = .079$, $\eta_p^2 = 0.16$) or the *OXT dose* x *time* interaction ($F(9.19, 50.55) = 1.81$, $p = .088$, $\eta_p^2 = 0.25$). Also, there was no effect of *OXT dose* ($F(2, 11) = 0.44$, $p = .658$, $\eta_p^2 = 0.07$; see Figure 32).

Total rest time (in s): Homogeneity of variance (Levene's Test) was violated ($p < .001$), thus the Welch-Satterthwaite correction was applied. The one-way ANOVA revealed no statistically significant decrease in the total rest time during the 60 minutes spent in the locomotor activity box following an *OXT dose* administration ($F(2, 5.94) = 1.10$, $p = .392$; see Figure 33).

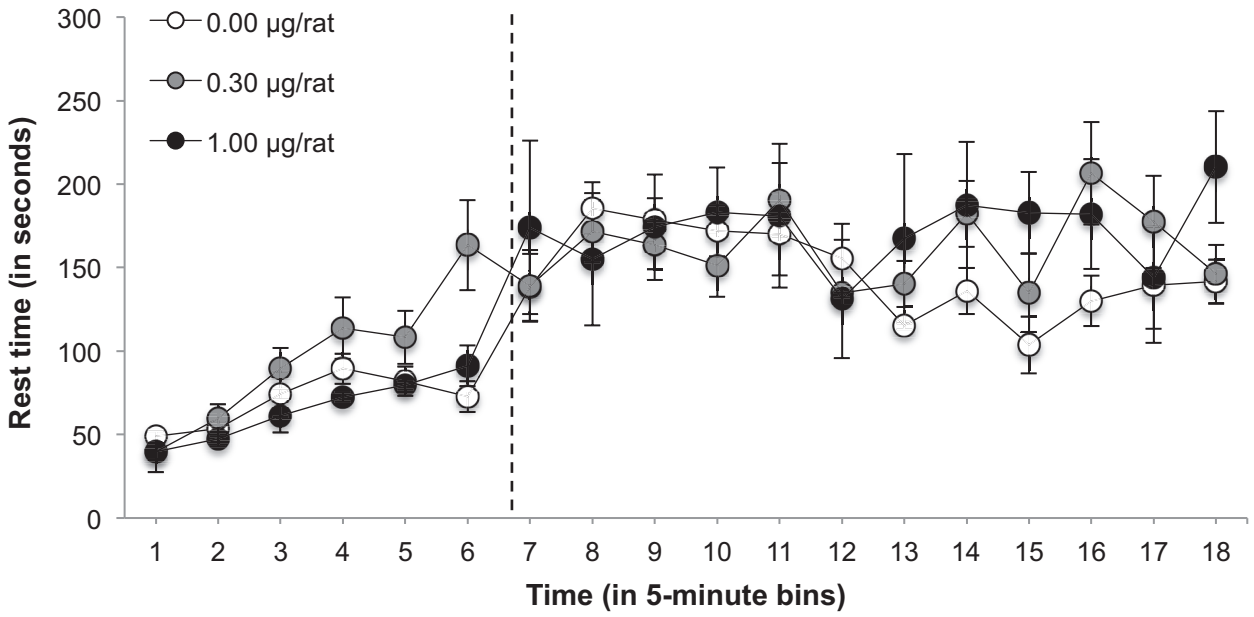

Figure 32. Mean (± *SEM*) of rest time following an OXT injection (ICV; $n_{0.00\ \mu g/rat} = 5$; $n_{0.30\ \mu g/rat} = 5$; $n_{1.00\ \mu g/rat} = 4$). The number of moves was compared starting at the 7th 5-min bin until the end of the locomotor activity task. No statistically significant difference was found for any of the conditions throughout the 60 min following an OXT injection.

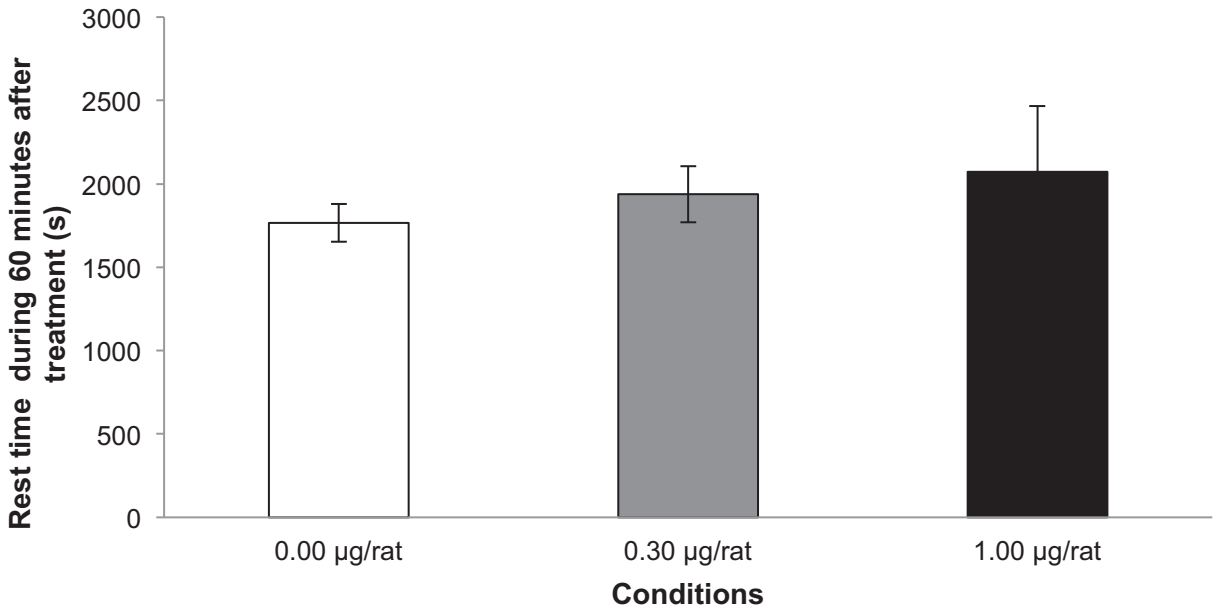

Figure 33. Mean (± *SEM*) of rest time during the 60 min following an OXT injection (ICV; $n_{0.00\ \mu g/rat} = 5$; $n_{0.30\ \mu g/rat} = 5$; $n_{1.00\ \mu g/rat} = 4$). No statistically significant difference was found for any of the conditions across the 60 min following an OXT injection.

Summary

In contrast to our expectations, no statistically significant changes were found during the 14-day heroin SA following chronic injections of exogenous OXT in Experiment 1. We noted high variability and inconsistency in active lever responses over the treatment period, and OXT did not decrease the motivation for heroin self-administration.

Experiment 2A checked whether OXT (low or high) would reduce yohimbine-induced reinstatement during a 5-h test under a PR schedule of reinforcement. Surprisingly, the low OXT dose (0.30 μg/rat) seemed to reduce this stress-induced reinstatement the most, but there was no statistically significant difference. Then, Experiment 2B tested yohimbine-induced reinstatement during a 3-h test under a FI-20 s schedule of reinforcement. In contrast to findings in Experiment 2A, OXT consistently increased the active lever responses for both the low and high doses of OXT. However, no statistical differences were found.

Finally, the effects of exogenous OXT were tested in locomotor activity. Contrary to our expectations, OXT did not seem to change the distance travelled, nor did it change the rest time displayed by the rats.

General Discussion

This thesis first examined the effects of exogenous OXT on heroin self-administration under fixed-interval (20 s) and progressive schedules of reinforcement. Then, we investigated the role of OXT on yohimbine-induced reinstatement of previously extinguished heroin seeking. Given the gap in our understanding of the behavioural consequences of treatment with exogenous oxytocin on heroin taking and seeking, we explored the effects of acute (chapter 1) and chronic (chapter 2) OXT administration on heroin self-administration in male rats.

Contrary to our expectations, both acute and chronic administration of exogenous oxytocin appeared to be ineffective in reducing heroin taking as well as in preventing or reducing yohimbine-induced reinstatement of extinguished heroin seeking. This is a surprising finding because previous studies had reported that OXT decreased heroin self-administration in heroin-tolerant rats (Kovács et al., 1985; Kovács & Van Ree, 1985; Ibragimov et al., 1987), methamphetamine self-administration (Carson et al., 2010), and cocaine self-administration (Leong et al., 2016; Zhou et al., 2014). Moreover, OXT has also been shown to reduce reinstatement of morphine (Georgiou et al., 2015), of cocaine (Morales-Rivera et al., 2014; Zhou et al., 2014; Leong et al., 2016), and of methamphetamine seeking in rats (Baracz et al., 2014). In contrast to these prior findings, the results of our experiments did not confirm the expected correlation, as detailed in the following summary and discussion.

In chapter 1, we hypothesized that OXT treatment would result in lower "breakpoints" under progressive-ratio schedule of reinforcement or, in other words, lower motivation for heroin intake. However, no statistically significant changes in heroin intake were found under any of the schedules of reinforcement. Interestingly, a pattern of decreased rate of responses on the active lever seemed to emerge under FI-20 s in oxytocin-treated rats (high dose) over the initial 10 min of a heroin self-administration session. That period could then correspond to the peek of the very

short half-life of oxytocin (Gonser, 1995). Thus, this finding may suggest that oxytocin reduces rats' locomotor activity and responses in their operant-conditioning chambers. However, this short-term decrease in rats' active lever responses contrasts with the substantial decrease in heroin self-administration reported by Kovács et al. (1985) that lasted at least 24 h.

In Experiment 2, contrary to our expectations, OXT did not reduce yohimbine-induced reinstatement and instead provoked, in a few instances, severe cases of barrel rolling. In fact, many cases of barrel rolling were observed, from short (3 to 20 s) and seemingly minor to long (over a minute) and lethal. Barrel rolls are characterized by unusual motor disturbances, ataxia, body swaying, spastic limbs abduction and so on (Diamant et al., 1993). It has been previously reported that some peptides, such as lysine vasopressin, arginine vasopressin, and OXT had induced similar responses when administered ICV (e.g., Kruse et al., 1977; Wurpel et al., 1985; Diamant et al., 1993) and were often fatal. Our results replicate the same deleterious effects as acute doses of arginine vasopressin, a similar neuropeptide to OXT also synthetized in the PVN and the SON, when centrally injected in the lateral ventricles of conscious rats (Kruse et al., 1977; Wurpel et al., 1985; Diamant et al., 1993). While the underlying causes of barrel rolling are mostly unknown, some pharmacological compounds such as phenytoin and diazepam (anti-epileptic drug) as well as muscle relaxant drugs (e.g., chlorpromazine) have been found to reduce such occurrences. Barrel rolling (or barrel rotation) has mostly been noted as an epileptic phenomenon considering the inhibiting effects induced by anti-epileptic medications (Diamant et al., 1993). Finally, following their results, Diamant et al. (1993) suggested that the V1 receptor, a receptor involved in cerebral water homeostasis regulation, was involved in barrel rolling. One plausible cause of those seizure-like incidents could be water intoxication in relation to high doses of OXT. While high doses of OXT were recommended for the treatment of missed abortions for women in the 1950s, water intoxication was quickly attributed to this type of

treatment since the first clinical case in 1962 (Ahmad et al., 1975). Interestingly, most water intoxication cases have been reported for acute doses of OXT in humans (Ahmad et al., 1975). As OXT and arginine vasopressin are also antidiuretic, it is possible that it creates a lower plasma sodium concentration, which in turns evokes hyponatremia, a severe water intoxication symptom (Ahmad et al., 1975).

Finally, we tested the effect of OXT on anxiety-related tasks such as the elevated plus maze and an open field. Even though OXT has been acknowledged to regulate anxiety response and diminish anxious behaviours during those tasks (e.g., time spent and distance travelled in closed arms, grooming, and rearing) (Klenerová et al., 2011; Mak et al., 2012; Morales-Rivera et al., 2014), we were unable to replicate such results.

In several studies (e.g., Klenerová et al., 2011, Mak et al., 2012), OXT acts as an anxiolytic agent to attenuate the fear reaction evoked in the EPM and open field tests. Although these studies have observed immediate behavioural modifications following an OXT injection, Klenerová et al. (2011) also reported a long-lasting attenuation of fear and anxiety when the animals were again exposed to stress 4 days later (treatment-free). Hence, it is possible that the rats in our experiments that received ICV and IP injections of OXT and vehicle (counterbalanced) had an attenuated response to stress on the second test, due to the long-lasting effect of OXT over time. Finally, when testing the effect of OXT on locomotor activity, our data did not show any statistically significant changes in the distance travelled and rest time in the locomotor box within a 90-min period, in contrast to findings from previous studies (Morales-Rivera et al., 2014; Zhou et al., 2015)

In chapter 2, rats (3 groups; vehicle, low and high) received chronic injections of OXT over a 14-day period. This treatment regimen was based on a recent study that reported that chronic OXT administration (0.30 mg/kg, IP) could reduce motivation to self-administer

methamphetamine in female rats (Westenbroek et al., 2013). However, we were unable to replicate such results for both heroin intake and heroin seeking. It has previously been mentioned that the effects of chronic OXT are inconsistent, with a high dose being anxiogenic, while a low dose protected against some of the effects of chronic stress (Peters et al., 2014). The discrepancies found between our results and Westenbroek's et al. (2013) study might be explained by investigating the effect of OXT on sex (males instead of female) and the type of drug (heroin instead of methamphetamine). Even though other studies (Leong et al., 2016; Zhou et al., 2015) suggest that OXT affects male as well as female rats, it seems that female rats are more responsive to OXT, at least in tests for locomotor activity, anxiety, and sucrose intake (Zhou et al 2015).

Oxytocin, drug self-administration, and schedules of reinforcement

Interestingly, our results failed to replicate previous findings in studies that suggested oxytocin has the potential to decrease opiate (Kovács et al., 1985; Kovács & Van Ree, 1985; Ibragimov et al., 1987) and psychostimulant (Carson et al., 2010; Morales-Rivera et al., 2014; Zhou et al., 2015; Leong et al., 2016) drug self-administration. One possible explanation for the lack of effect of oxytocin on heroin self-administration is that the progressive-ratio schedule of reinforcement, in the way it was implemented here, might not be the best way to assess motivation for heroin intake. In fact, instead of providing rate measures that generate a stream of data with which it is possible to determine the time course of a drug action, the progressive ratio only provides a single data point for a whole self-administration session (Arnold & Roberts, 1997). Recently, Zhou et al. (2014) reported that OXT statistically significantly reduced cocaine SA and active lever responses on FR1, FR5, and PR schedules of reinforcement. Arnold and Roberts (1997) suggest the possibility of fundamental and qualitative differences between

psychostimulant and opiate self-administration, and they hypothesized that while rats self-administering heroin are highly motivated to self-administer the first injection of the session, they might lose interest for each subsequent heroin infusion. To address this caveat, a different progressive-ratio schedule has been developed to better assess the motivation to self-administer opiates (Roberts & Bennett, 1993), and this schedule produces breakpoints sensitive to manipulations of the unit dose of heroin (Arnold & Roberts, 1997).

A recent pilot study conducted in humans aimed to assess how intranasal OXT would affect heroin craving in patients receiving opioid replacement therapy compared to control individuals (Woolley et al., 2016). This pilot study in humans has found that while patients under opioid replacement therapy tolerated the OXT treatment well, it did not affect their cue-induced heroin craving. Surprisingly and, as problematic as our study was, the results obtained in both chapters seem to be closer to the human condition. However, many results might be dependent on the methodological issues that arose in comparison to all aforementioned studies.

Methodological considerations

Given the necessary limitations in scope of this thesis, it would first be recommended to replicate these experiments with a much bigger sample size in order to see if a clearer pattern emerges in comparison to our preliminary findings. Moreover, various studies have investigated the effect of oxytocin in both male and females suggesting that even though they often show similar effects in psychostimulant drugs self-administration and reinstatement (Leong et al., 2016), some differences in locomotor activity and sucrose-seeking tests are still present (Zhou et al., 2015). Considering that very few recent studies have investigated the effect of OXT on opiate-related behaviours, it would be important to address the effects of OXT on opiate drugs intake and seeking in female rats.

Another crucial consideration would be the oxytocin doses used during acute OXT injections in Experiments 1, 2 and 3 (chapter 1). Indeed, the higher dose used (2.50 μg/rat) caused multiple cases of barrel rolling that led to the death of an animal and to the severe deterioration of health in many other laboratory subjects. Thus, the doses used in chapter 2 (Walter et al., 1978) seemed to have less drastic consequences on health while still seemingly affecting the rats during their daily SA sessions or tests. In fact, while we were not able to observe a quantifiable anxiolytic effect for OXT, which plants doubt regarding the efficacy of the treatment in all our experiments, we were able to observe a strong effect of OXT on non-specific locomotor activity at the beginning of every SA session.

Conclusion

In summary, the findings presented in this thesis suggest that there are no statistically significant changes in heroin SA or yohimbine-induced reinstatement for either acute or chronic treatment of oxytocin. While we could anecdotally report a reduction in locomotion following and OXT injection, it is very difficult to draw specific conclusions regarding the effects of OXT on heroin-related behaviours. Unless we conduct a broader follow-up study, we can only speculate about the potential implications of these findings.

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