EFFECTS OF SOCIAL-INTERACTION ON REWARD SENSITIVITY IN ADOLESCENT MICE

A Senior Scholars Thesis

by

SHANNON LEE COLE

Submitted to the Honors and Undergraduate Research
Texas A&M University
in partial fulfillment of the requirements for the designation as

UNDERGRADUATE RESEARCH SCHOLAR

May 2012

Major: Psychology

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Approved by:

Research Advisor: Shoshana Eitan
Associate Director, Honors and Undergraduate Research: Duncan MacKenzie

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ABSTRACT

Effects of Social-interaction on Reward Sensitivity in Adolescent Mice. (May 2012)

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This study examined alterations in the rewarding properties of opioids due to social interaction. The first experiment explored the role of peer interactions on drug reward. Adolescent male mice were administered morphine for 6 days while socially housed with cage-mates receiving different pharmacological treatments (i.e., exposure to different social interaction with peers). Then, drug reward was examined, using the conditioned place preference (CPP) paradigm. It was shown that exposure to drug-naïve peers had a protective effect on drug-administered mice, reducing the amount of morphine CPP, and that exposing drug-naive to drug-administered mice had a harmful effect. The second experiment explored the possible role the oxytocin (OT) system plays in mediating the effects of social interaction on drug reward. This was accomplished by examining the effect of an OT antagonist on the acquisition of CPP in mice that are housed in different social environments. It appears that suppressed or under-functioning OT system correlates with increases in morphine CPP, and thus, the potential for

morphine abuse. Ultimately, these studies sought to better elucidate the role that social interaction plays in the development of drug abuse, dependence, and addiction.

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CHAPTER I

INTRODUCTION

Adolescence is a critical time period in human development during which key habits are formed. The previous abuse of drugs is a large predictor of future abuse, especially during such a critical stage in development (Chen, Storr, & Anthony JC 2009; Grant & Dawson 1998; Hawkins *et al.* 1997; Odgers *et al.* 2008). Furthermore, early drug abuse has been shown to be a major risk factor for developing mood and anxiety disorders (Gfroerer, Wu, & Penne 2002; Caspi *et al.* 2005; Tucker *et al.* 2006; Mathers *et al.* 2006; Degenhardt *et al.* 2007). With current illicit drug abuse levels as high as 14% of US 8th graders, 27% of 10th graders, and 37% of 12th graders (Johnston *et al.* 2008), adolescent drug abuse represents a major concern.

Adolescence is a time of intense social interaction and susceptibility to social influence, and as such, the effects of peer illicit drug abuse may play a major role in the development and maintenance of drug abuse behaviors. In rodent models, adolescent rats have shown a preference for ethanol scents when exposed to intoxicated peers. However, this same effect was not seen after adolescent rats were exposed to the ethanol scent alone or after interaction with an intoxicated, anaesthetized peer (Fernández-Vidal & Molina 2004). Futhermore, adolescent rats increased ethanol intake after exposure to an intoxicated peer (Hunt, Holloway, & Scordalakes 2001).

This thesis follows the style of Addiction Biology.

Our lab has seen some interesting interactions between age, sex, and social effects using behavioral sensitization as a measure. Behavioral sensitization is an increase in response to a drug after previous exposure, and has been linked to an increase in rates of drug use in rodent, self-administration models (Deroche-Gamonet, Belin, & Piazza 2004; Horger, Giles, & Schenk 1992; Piazza *et al.* 1989; Robinson and Berridge1993) As such, sensitization is thought to be a large contributor to the development of drug abuse and dependence. Our previous studies used morphine-induced locomotion, where by morphine is administered to mice and an increase in locomotion believed to index an increase in morphine abuse potential. We have seen an enhancement in morphine-induced hyperlocomotion in drug-naïve, adolescent male mice housed with morphine-treated adolescent males. However, this enhancement was not seen in drug-naïve adults housed with morphine-treated adults (Hodgson *et al.* 2010). Interestingly, no significant increase in locomotor activity was observed between drug-naïve adolescent female housed with morphine-treated animals and drug-naïve females housed with drug-naïve females. Yet, a significant decrease in locomotor response was seen in morphine-treated adolescent females housed with drug-naïve females as compared to morphine-treated females housed with other morphine-treated animals, suggesting that social interactions with drug-naïve animals may have lowered the sensitizing effects of previous morphine exposure and provided a protective effect to drug-treated females (Hofford *et al.* 2010). Similar effects were also observed in adolescent rats, in which morphine-treated rats housed with saline-treated (drug-naïve) rats experienced a significantly lower sensitization response in comparison to morphine-treated rats housed with other

morphine-treated animals (Hofford *et al.* 2010). These studies emphasize the importance of exploring the impact of social interactions, as they might have drastic impacts on the development or prevention of harmful behaviors, such as drug abuse.

These studies demonstrate that adolescents are more sensitive than adults to the states and/or the communications of their peers. The alterations in the behavioral response to administration might be due to changes in adolescent social play. The social influences on behavioral sensitization were prevalent when exposure to different social partners occurred during the critical period of juvenile social play (postnatal day 28 to 33), but not during postnatal day (PND) 63 to 68 (i.e., adulthood). Juvenile mammals increasingly engage in social play behaviors, and in rodents these behaviors emerge around PND 18, peak during the peripubertal period (PND 30-40), and then decline after puberty (Laorden *et al.* 2003; Panksepp & Normansell 1984). Juvenile social play interaction is both rewarding, and is involved in the development of a healthy species-dependent adult social contact patterns. Social play has been demonstrated to play an important role in cognitive and emotional development, and facilitates the ability to cope with social conflicts during adulthood (Hellemans, Benge, & Olmstead 2004; Ibi et al 2008; McCormic, Smith, & Mathews 2008; van Den Berg *et al.* 1999; Weiss *et al.* 2004; Vanderschuren, Niesink, & Van Pee 1997). However, a lack of social play during the juvenile period results in the development of abnormal patterns of social, sexual, and aggressive behaviors (Arakawa, 2007; Einon, Morgan, & Kibbler 1978; Gerall, Ward, & Gerall 1967; Potegal & Einon 1989; Hellemans, Benge, & Olmstead 2004; Panksepp &

Normansell 1984; van Den Berg *et al.* 1999; Weiss *et al.* 2004). Morphine was demonstrated to alter social behaviors in both mice (Landauer & Balster 1982) and rats (Niesink & Van Ree 1989; Normansell & Panksepp 1990). Our recent studies seem to suggest that social communications among juveniles (in the form of social play or other forms) could modulate adaptive processes during brain development and as a result alter basic learning forms such as behavioral sensitization.

Modulation of behavioral sensitization requires changes of synaptic transmissions. Thus, our recent results suggest that exposure to different social partners during adolescence modulates the expression and functionality of neurotransmitters and/or their receptors. Moreover, our results suggest that adolescents are more sensitive than adults to neuroplastic modulations following exposure to different social partners. These studies also indicate that the exact nature of these neuroplastic modulations in adolescents are species and sex dependent. Oxytocin (OT) and arginine vasopressin (AVP) are neuropeptides that are known to be modulated by social and environmental conditions (Curley *et al.* 2009; Karelina & DeVries 2011; Karelina *et al.* 2009; Lukas *et al.* 2010; Oreland, Gustafsson-Ericson, & Nylander 2010; Pan *et al.* 2009; Ruscio *et al.* 2007; Tanaka, Osako & Yuri 2010; Veenema, Bredewold, & Neumann 2007; Vitalo *et al.* 2009) and to regulate social behaviors in a sex- and species-dependent manner (Choleris *et al.* 2009; Ferguson, Young, & Insel 2002; Insel 1997; Insel 2010; Young, Liu, & Wang 2008). As such, these neuropeptides may be involved in mediating the heightened social adaptability observed in adolescents. Opioids are known to regulate social play

behaviors (Vanderschuren, Niesink, & Van Pee JM 1997), and morphine-induced modulation of the OT and AVP system could result in changes in the behavior of the animals towards cage-mates, which in turn could modulate the expression levels and functionality of the OT and/or AVP systems in the cage-mates. Indeed, there are several mice and rat studies that support our speculation by demonstrating that morphine modulates the OT and AVP systems in various brain areas (Johnstone et al 2000; Kovács *et al.* 1987a; Kovács *et al.* 1987b; Laorden *et al.* 2003; You *et al.* 2000; Zhou *et al.* 2007). Moreover, OT has demonstrated, in general, to attenuate opioid-induced behaviors (Sarnyai & Kovács *et al.* 1994). Specifically, the activation of the OT system (Ibi *et al.* 2008) and inhibition of the AVP system (Insel 1997) were both demonstrated to reduce the development of opioid dependence and attenuate opioid self-administration in adult rodents.

Recent surveys have shown an increase in the use of nonmedical opioid prescription pain killers in both teenage and adult age groups (Partnership for a Drug-Free America 2009; SAMHSA 2009). In fact, nonmedical use of opioid prescription pain killers is only second to marijuana as the most used illicit drug group (SAMHSA 2009). The prevalence and increase of opiates as a drug of abuse warrants additional research, to better treat and combat the development of abuse, dependence, and addiction.

Project description

The aforementioned role that drug abuse might play in the formation and precipitation of mental disorders, in addition to recent increases in opiate abuse, warrants further inquiry into the interaction effects of social interaction and the dependence effects typical of opiate drugs. As the addictive properties of drugs are highly linked with their rewarding properties, a more direct measure of reward is appropriate for further inquiry.

Thus, the first study is designed to examine the effects of social housing conditions on the rewarding properties of morphine by using conditioned place preference (CPP) paradigm. CPP measures reward by associating a particular set of environmental cues in a location to an appetitive stimulus (i.e., addictive drugs, salient scents, food, etc.) and measuring the change in time spent in the location paired with the stimulus. Adolescent mice were group-housed in one of two conditions referred to as ‘only’ and ‘cage-mates’ (see figure on p. 10). In the mixed treatment condition, morphine- and saline-treated mice were housed together (i.e. 2 mice received morphine and 2 mice received saline per cage). These groups are referred to as ‘morphine cage-mates’ and ‘saline cage-mates’, respectively. In the separated treatment conditions, all 4 mice in the cage received morphine or saline and cages were visually separated from each other. These groups are referred to as ‘morphine only’ and ‘saline only’, respectively. All mice were subsequently individually tested for their ability to acquire morphine CPP after only one conditioning session with 10, 20 or 40 mg/kg morphine or saline.

The second study was designed to determine the role of OT in mediating the effects of social regulation on the expression of morphine CPP. Mice were housed and treated with saline or morphine in their home cages as described for the first study. Seven days after the last morphine/saline injection mice were tested for the acquisition of morphine CPP. In these experiments, mice were injected with specific OT antagonist or the corresponding vehicle 30 minutes prior to their conditioning session in the drug-paired chamber.

CHAPTER II

METHODS

Animals

Adolescent male C57BL/6 mice were purchased from Harlan Lab (Houston, TX) and housed 4 per cage with food and water *ad lib* in a temperature-controlled (21 ± 2 °C, humidity 45%) vivarium with a 12 h/12 h light/dark cycle (light on at 07:30). All mice were housed as cage-mates from the day of weaning (PND 21) in Harlan facilities, shipped as cage-mates, and remained cage-mates after arriving at our facility and for the entire experiment.

In our initial experiment, adolescent mice were purchased at PND 22, acclimated to the vivarium until PND 28, injected during PND 28-33, and behavioral testing was performed on PND 40-43.

In our OT antagonist experiments, mice were acclimated until PND 25, had intracerebroventricular (ICV) implantation on day 25 or 26, were allowed to recover for 4-5 days (PND 26 or 27-PND 30), injected with morphine PND 31-36, and behavioral testing was performed on PND 42-45.

Experiment I: Social effect on the rewarding properties of morphine

Saline and morphine treatment regimen

The different experimental groups are summarized in the table on page 13. Mice were injected twice daily (9 a.m. and 5 p.m.) in their home cage for 6 consecutive days with increasing doses of morphine (10-40 mg/kg, 10 ml/kg, subcutaneously) or saline for a total of 12 injections. Specifically, on days 1 and 2, the mice were injected with 10 mg/kg morphine or saline. On days 3 and 4, they were injected with 20 mg/kg morphine or saline. On days 5 and 6, they were injected with 40 mg/kg morphine or saline. Morphine sulfate was purchased from Sigma (St. Louis, MO).

Housing

As illustrated in Figure 1, mice were group-housed in one of two conditions referred to as "only" and "cage-mates". "Morphine only" mice were morphine-treated animals housed physically and visually separated from saline-treated animals (i.e., all 4 mice in the cage received morphine). Similarly, "saline only" mice were saline-treated animals housed physically and visually separated from morphine-treated animals (i.e., all 4 mice in the cage received saline). Additionally, there were morphine-treated and saline-treated mice housed together (i.e., 2 mice received morphine and 2 mice received saline in each mixed cage), which represented two different treatment conditions (saline treated and morphine treated). The saline-treated animals of this group are referred to as "saline cage-mates," and each saline cage-mate mouse had 2 morphine-treated and 1 saline-treated cage-mate. The morphine-treated animals are referred to as "morphine cage-

mates," and each morphine cage-mate mouse had 2 saline-treated and 1 morphine-treated cage-mate.

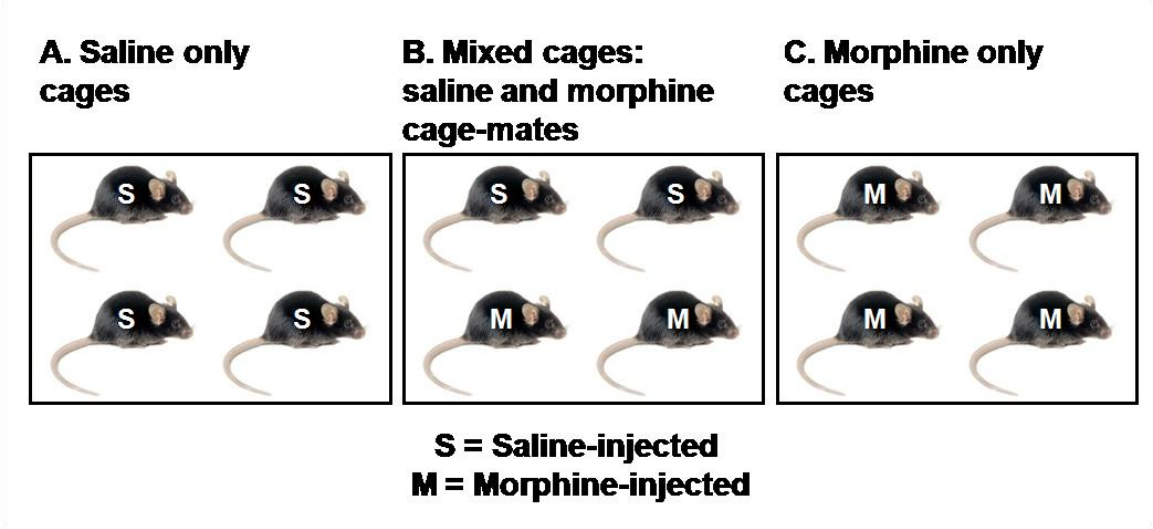

Figure 1 Housing conditions

Morphine conditioned place preference (CPP)

The assessment of morphine CPP was made in a set of 8 identical apparatuses. Each Plexiglas CPP apparatus contains three 20 × 20 × 30.5 cm square chambers. Two chambers were used as the conditioning chambers. One of these chambers had walls decorated with black and white "checkered" (2-cm squares) contact paper and contained almond extract (Adams Extract and Spices, LLC., Gonzales, TX) as an olfactory (scent) cue (200 µl on a strip of filter paper hung from the top corner of the chamber). The other chamber had walls decorated with black and white "cow" print contact paper and was accentuated with lemon extract (Adams Extract and Spices, LLC., Gonzales, TX) presented in the same fashion. The visual cues offered approximately equal amounts of

white and black. The third chamber, designated the neutral "start" chamber, had no decoration and will remain odorless. Two doors allowed access from the neutral chamber to each of the conditioning compartments. Animals were placed directly into this neutral chamber during habituation and test sessions.

Each CPP apparatus was located within automated optical beam activity monitors (Model RXYZCM-16; Accuscan Instruments, Columbus, OH, USA), which uses a multiplexor-analyzer updated the position of animals in the CPP apparatus every 10 ms using a 100% real-time conversion system.

Seven days following the final home cage treatment dose of morphine or saline (i.e., experimental day 13, see table on p. 13), mice were placed in the neutral chamber and allowed free access to the entire apparatus for 30 minutes. The time spent in each of the chambers was recorded to measure any initial bias. The following two days (i.e., experimental days 14-15, Table 1), each animal had two conditioning sessions, one session per day. The chamber that was less preferred in the initial recording was assigned to be the drug-paired chamber. For one conditioning session, animals were injected with 10, 20, or 40 mg/kg morphine or saline and confined for 60 minutes to the less preferred conditioning chamber. For the other conditioning session, animals were injected with saline and confined for 60 minutes to the other chamber. Animals from each treatment group were randomly assigned to receive the morphine conditioning session on experimental day 14 or 15, ensuring that from each treatment group and

conditioning dose half of the animals received the morphine conditioning session on experimental day 14, and the other half received the morphine conditioning session on day 15. The following day (i.e., experimental day 16, Table 1), mice in a drug-free state were allowed to freely explore the apparatus for 30 min and compared with the initial 30 minute exposure on experimental day 13. The time spent in each chamber was recorded to the nearest second (see Fig 2 for timeline).

Figure 2 Timeline of injection regimen and CPP behavior testing

Table 1 Summary of the experimental groups

Morphine conditioning dose (mg/kg)	**Treatment group**	**Housed in cage type (see Fig 1)**	**Exp days 1-6**	**Exp days 7-12**	**Exp day 13**	**Exp days 14-15**	**Exp day 16**
			PND 28-33	**PND 34-39**	**PND 40**	**PND 41-42**	**PND 43**
0	Saline only	A	Saline	No treatment	Habituation to the CPP apparatus/ Recording of baseline preference	One conditioning session per chamber with saline	Testing the post-conditioning preference to the different chambers in a drug-free state
	Saline cage-mates	B	Saline				
	Morphine cage-mates	B	Morphine (10-40 mg/kg)				
	Morphine only	C	Morphine (10-40 mg/kg)				
10	Saline only	A	Saline			One conditioning session with 10 mg/kg morphine in the less preferred chamber and one conditioning session with saline in the other chamber	
	Saline cage-mates	B	Saline				
	Morphine cage-mates	B	Morphine (10-40 mg/kg)				
	Morphine only	C	Morphine (10-40 mg/kg)				
20	Saline only	A	Saline			One conditioning session with 20 mg/kg morphine in the less preferred chamber and one conditioning session with saline in the other chamber	
	Saline cage-mates	B	Saline				
	Morphine cage-mates	B	Morphine (10-40 mg/kg)				
	Morphine only	C	Morphine (10-40 mg/kg)				
40	Saline only	A	Saline			One conditioning session with 40 mg/kg morphine in the less preferred chamber and one conditioning session with saline in the other chamber	
	Saline cage-mates	B	Saline				
	Morphine cage-mates	B	Morphine (10-40 mg/kg)				
	Morphine only	C	Morphine (10-40 mg/kg)				

Plasma corticosterone

To determine whether stress levels play a role in social interaction, nine days following the final treatment dose of morphine or saline (corresponding to experimental day 15 of the CPP test), mice (n=16 per group) were anesthetized with pentobarbital (100 mg/kg, i.p.) and their blood collected via intra-cardiac puncture. Plasma was stored at -80°Cafter being separated via centrifugation (15 min, 1000g, 4°C). Levels of corticosterone were determined using the Corticosterone EIA Kit (Cayman Chemicals, Ann Arbor, MI). Plasma samples were diluted with EIA buffer to fall within the detection range. The detection range for the Corticosterone EIA Kit is 0.04-10 ng/ml with an interassay coefficient of variation of 0.036 ng/ml.

Experiment II: The involvement of oxytocin in the social effects on drug reward

Housing

The housing of animals to be used for this study was similar to experiment 1 (see Fig 1). Briefly, mice were housed 4 per cage, with treatment groups of morphine only, morphine cage-mates, saline cage-mates, and saline only.

Stereotaxic manipulation

Surgeries took place on either PND 25 or 26. Cannulas (23 gauge) were inserted into the lateral cerebroventricle using Kopf's 1900 Stereotaxic Alignment instrument (Tujunga, CA). Mice were anesthetized and Lubricant Opthalamic Ointment was used to keep their eyes lubricated throughout the surgery. The hair was shaved off the mouse's head, and

the area was sterilized with 70% alcohol. The head was then immobilized in a stereotaxic apparatus by inserting the two knobs into the ears. Blunt ear bars were used to avoid damage to the ear. A cut was made caudally-medially to the eyes with a scalpel and the skin was separated with a spatula to expose the skull. Precise measurements of the bregma and lamba were taken followed by measurements of the appropriate location for cannula insertion. Small burr holes (2-mm diameter) were drilled in the skull for the placement of 23-gauge cannula, and the cannulas were inserted slowly. Cannulas were 7.5mm in total length, with 1.5mm of the cannula inserted past the skull. An additional small burr hole was drilled for screw insertion. In the other position, a sterile screw in inserted just to the length of the bone to hold the bone tight. Dental cement was applied around the cannula and the screw. The dental cement applied to the cannula and the screw were sterile and the site was monitored for infection and periodically cleansed with alcohol. Until mice regain consciousness, warm water, blankets, and bubble wrap will be used to provide supplementary warmth (this method prevents burns from occurring). Respiration and motor movement was monitored during recovery from anesthesia. Animals were given 5 days to recover. Antibiotics were mixed with mice drinking water to prevent infection.

To keep the cannulas open and flowing, mice received intra-cannula saline injections during recovery. All intra-cannula injections were performed using 30 gauge inserts 2.5mm (8.5mm in total length) past the skull to reach the ventricular region. At the end

of the experiment, the placement of the cannulas was confirmed via dye injections and any mice with incorrect cannula placement were excluded.

Saline and morphine treatment regiment

Five days after the stereotaxic manipulations, mice were injected twice daily (9 a.m. and 5 p.m.) in their home cage for 6 consecutive days with increasing doses of morphine (10-40 mg/kg, 10 ml/kg, s.c.) or saline for a total of 12 injections. Specifically, on days 1 and 2, the mice were injected with 10 mg/kg morphine or saline. On days 3 and 4, they were injected with 20 mg/kg morphine or saline. On days 5 and 6, they were injected with 40 mg/kg morphine or saline.

CPP procedures

Mice were examined using the CPP paradigm from the initial study. Seven days following the final home cage treatment mice were examined for the effect on OT antagonist on the expression of morphine CPP. Expression testing is where a pharmacological agent is given during the conditioning period and differences between these animals are compared against mice not receiving the agent (i.e., receiving vehicle). This is in contrast to development testing, where a pharmacological agent would be given during the initial 6 day treatment in their home cage. On the first day (i.e., habituation/experimental day 13), mice were placed in the neutral chamber and permitted free access to the entire apparatus for 30 minutes. The time spent in each of the chambers was recorded to measure any initial bias. The following two days (i.e.

experimental days 14-15), each animal had two conditioning sessions, one session per day. The chamber that was less preferred in the initial recording was assigned to be the drug-paired chamber. Prior to the morphine conditioning session, animals were injected into the lateral cerebroventricle with 20 µg tocinoic acid (OT antagonist) or vehicle. Thirty minutes later the mice received 20 mg/kg morphine and were confined for 60 minutes to the drug-paired chamber. For the other conditioning session, animals were injected with saline and confined for 60 minutes to the other chamber. Animals from each treatment group were randomly assigned to receive the morphine conditioning session on experimental day 14 or 15, ensuring that from each treatment group and conditioning dose half of the animals received the morphine conditioning session on experimental day 14, and the other half received the morphine conditioning session on day 15. The following day (i.e., experimental day 16, see Fig 2), mice in a drug-free state were allowed to freely explore the apparatus for 30 minutes and compared with the initial 30 minute exposure on experimental day 13. The time spent in each chamber was recorded to the nearest second.

Data analysis

For each mouse, the percent time spent in each chamber during the pre- and post-conditioning sessions was computed using the formula: [(time spent in the chamber in seconds/total time recorded in seconds) X 100]. Conditioned-place preferences are reported as the differences in the time spent in a compartment between post- and pre-conditioning sessions. The conditioned-place preferences of the control mice (receiving

only saline in the CPP apparatus) represents a drug-independent adjustment in time spent in the various chambers. Thus, conditioned-place preferences were normalized to the conditioned-place preferences of the control mice using the formula: [(conditioned-place preferences /average conditioned-place preferences of the control saline conditioned animals for this treatment group X 100)-100]. For instance, the amount of time spent in the drug-paired chamber on test day would be subtracted from the amount of time spent in the drug-paired chamber on habituation day for morphine only mice conditioned with morphine, and then divided by the average of drug-independent adjustment seen in morphine only mice who received saline in both chambers on both conditioning days. The overall design of the analysis of variance (ANOVA) for experiment 1 consists of between-group factors of treatment (saline vs. morphine), housing condition (only vs. cage-mates), and conditioning dose (0, 10, 20 and 40 mg/kg morphine). The overall designed of the analysis of variance (ANOVA) for experiment 2 consists of between-group factors of housing (morphine only vs. morphine cage-mates) and OT antagonism (OT antagonist vs. vehicle). The overall design of the plasma corticosterone analysis was a factorial consisting of between-group factors of treatment (saline vs. morphine) and housing condition (only vs. cage-mates). Additional post-hoc contrasts between each treatment group were computed using Tukey's post-hoc procedure (Kirk 1982). Differences less than 0.05 were deemed statistically significant. Results are presented as mean ± SEM.

CHAPTER III

RESULTS

Experiment I: Social effect on the rewarding properties of morphine

Examining differences in the degree of bias against the drug-paired chamber during habituation day.

In our CPP experiment, we used a biased design. A biased design means that the animals are conditioned to morphine in the chamber that they spent the least amount of time in on the habituation day (i.e., the chamber they less preferred during habituation day is designated as the drug-paired chamber). It was demonstrated that the amount of time spent in the drug-paired chamber (or the bias against the less preferred chamber) during habituation could impact the degree of subsequent conditioning to morphine. This is why it is important to determine whether there are differences between the experimental groups in the bias against the drug-paired chamber during the habituation day. A major difference between experimental groups would make it hard to conclude whether differences in conditioning to morphine between experimental groups are in fact due to the effect of treatment and/or housing conditions on conditioning to morphine or merely their effects on initial preferences to the drug-paired chamber during the habituation day. If significant bias exists, both our analysis and subsequent conclusions would be considerably confounded.

In order to determine if there were any initial differences in the degree of bias against the drug-paired chamber of CPP apparatus during habituation day, differences between treatments (i.e., saline treated versus morphine treated mice), housing conditions (i.e., mixed cages versus all morphine or all saline cages), and conditioning dose (10 mg/kg, 20 mg/kg, or 40 mg/kg) were analyzed. We found that there were significant differences in chamber preference between mice receiving saline (saline only and saline cage-mate mice) and morphine (morphine only and morphine cage-mate mice). Saline treated mice displayed a slightly larger bias against the least preferred/drug-paired chamber relative to morphine-treated mice, meaning that during the habituation day the saline-treated mice liked the drug-paired chamber less than morphine-treated mice. However, there were no differences in the initial bias against the drug-paired chamber between mice receiving different morphine conditioning doses (e.g., a saline cage-mate mouse conditioned with a 20 mg/kg dose of morphine showed a similar level of initial bias as a saline cage-mate conditioned with a 40 mg/kg dose). Importantly, there were no significant differences between saline only and saline cage-mates and between morphine only and morphine cage-mates in their initial bias against the drug-paired chamber. These findings indicate that while there is a small bias difference between the saline groups and morphine groups, comparisons can be drawn between animals treated similarly but housed in different social conditions (e.g., morphine only vs. morphine cage-mates), and without significant interference from initial differences in bias against the drug-paired chamber.

Results for drug-independent adjustment mice

In order to determine whether the difference in chamber preference between habituation day and test day was due to morphine reward or other processes (i.e., drug-independent adjustment), we measured whether mice shifted the amount of time spent in the least preferred chamber (designed as the drug-paired chamber) without receiving a morphine pairing. These control measures were necessary, as any drug-independent adjustment which took place could lead to overestimation or underestimation of the amount of morphine CPP and our interpretation of the degree to which reward is affected by exposure to different social environments. For instance, if a mouse was to increase preferences towards a chamber, independently of morphine's effect, our results would show a higher level of conditioning than what actually occurred.

The results for the control mice that received saline pairings in both test chambers (rather than a morphine pairing in their least preferred chamber) are presented in Fig 3. In order to determine whether significant drug-independent adjustment took place between the habituation day and test day, differences between treatments (i.e., saline treated versus morphine treated mice) and differences between housing conditions (i.e., mixed cages versus all morphine or all saline cages) were analyzed. We found that there was a significant difference in the amount of drug-independent adjustment between mice housed in mixed conditions and "only" conditions. Specifically, saline only, saline cage-mate, and morphine cage-mates increased the amount of time spent in the drug-paired (i.e., least preferred) chamber on test day. As these mice were only conditioned with

saline and not morphine, these results represent a drug-independent adjustment in the time spent in the chambers of the apparatus, and not an increase in preference due to morphine reward. In contrast, morphine only mice did not show significant adjustment in chamber preferences, meaning that they did not spend more time in the drug-paired (i.e., least preferred) chamber on test day relative to initial preferences on habituation day.

Normalization of morphine CPP

The percent of morphine place conditioning of the mice conditioned with 10, 20 or 40 mg/kg morphine was normalized with the drug-independent adjustment of the corresponding control group. For example, morphine cage-mate control mice, given saline in the drug-paired chamber, were used to normalize the data from morphine cage-mates conditioned with morphine. Normalization is a process by which data is altered in order to negate the effect a variable (drug-independent adjustment) on multiple sets of data and allow a comparison of data on a common scale. Normalization was necessary as any analysis of the results without normalization would overestimate morphine reward in the saline only, saline cage-mates, and morphine cage-mate groups (those in which we found drug-independent adjustment) and underestimate drug effects in the morphine only group relative to the other groups (where there was little drug-independent adjustment). This normalization allows us to distinguish between an effect of morphine reward and a drug-independent shift in chamber preference.

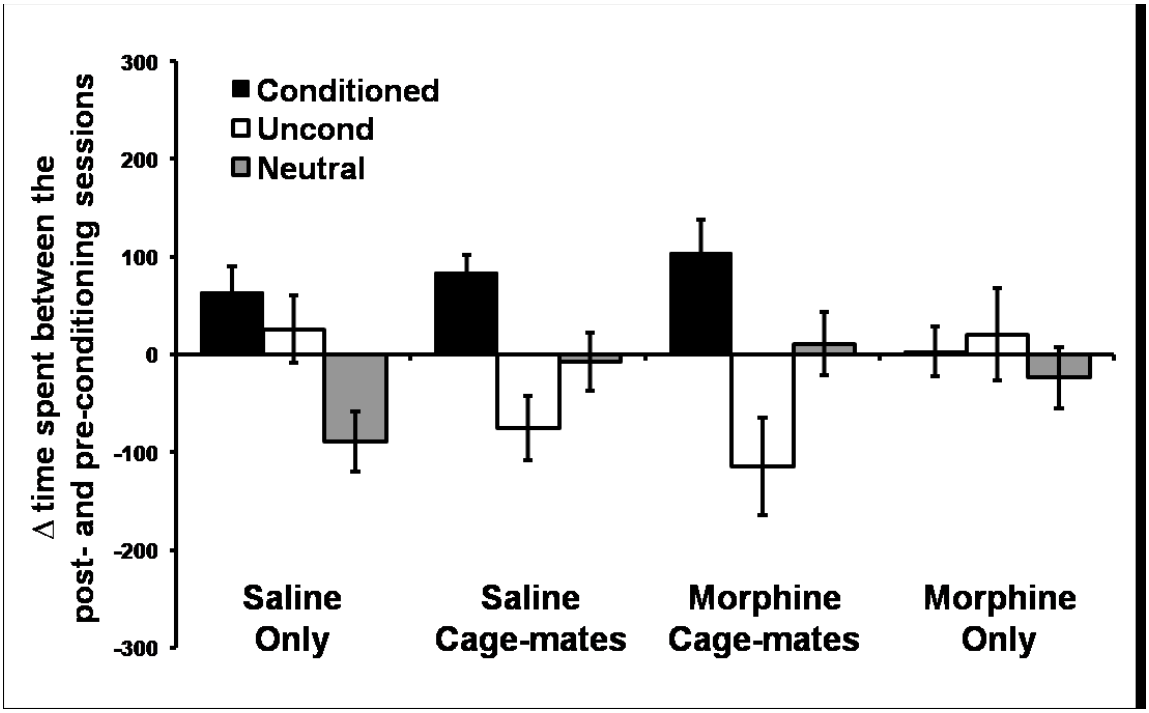

Figure 3 Drug-independent adjustment in time spent in the various chambers

The differences between the time spent in the post- and pre-conditioning sessions in each of the chambers of the CPP apparatus for the saline only, saline cage-mates, morphine cage-mates, and morphine only animals which were conditioned only with saline (i.e., one conditioning session with saline in the less preferred chamber – this chamber designated as the conditioning chamber; and one conditioning session with saline in the other chamber). Black bars – conditioning chamber (i.e., chamber that was less preferred during the pre-conditioning session); White bars – non conditioning chamber; Grey bars – neutral chamber. Results are presented as mean ± SEM.

To highlight the necessity of normalization, the results for the mice conditioned with 20 mg/kg prior to normalization are presented in Fig 4, which can be compared with the drug-independent adjustment mice in Fig 3. The saline only, saline cage-mate, and morphine cage-mates all drug-independently showed an increase in their least preferred chamber on test day. This increase indicates that the amount of true morphine conditioning and response to morphine reward seen in these groups was actually less than the raw data in Fig 4 shows. Morphine only mice which received saline in their least preferred chamber did not adjust much, which indicates that the morphine CPP

observed in Fig 4 was primarily due morphine reward. Thus, we used the data presented in Fig 3 of the mice receiving only saline in the CPP apparatus to normalized the data for mice receiving 10, 20, 40 mg/kg morphine pairings in the CPP apparatus. The result of this normalization is presented in Fig 5.

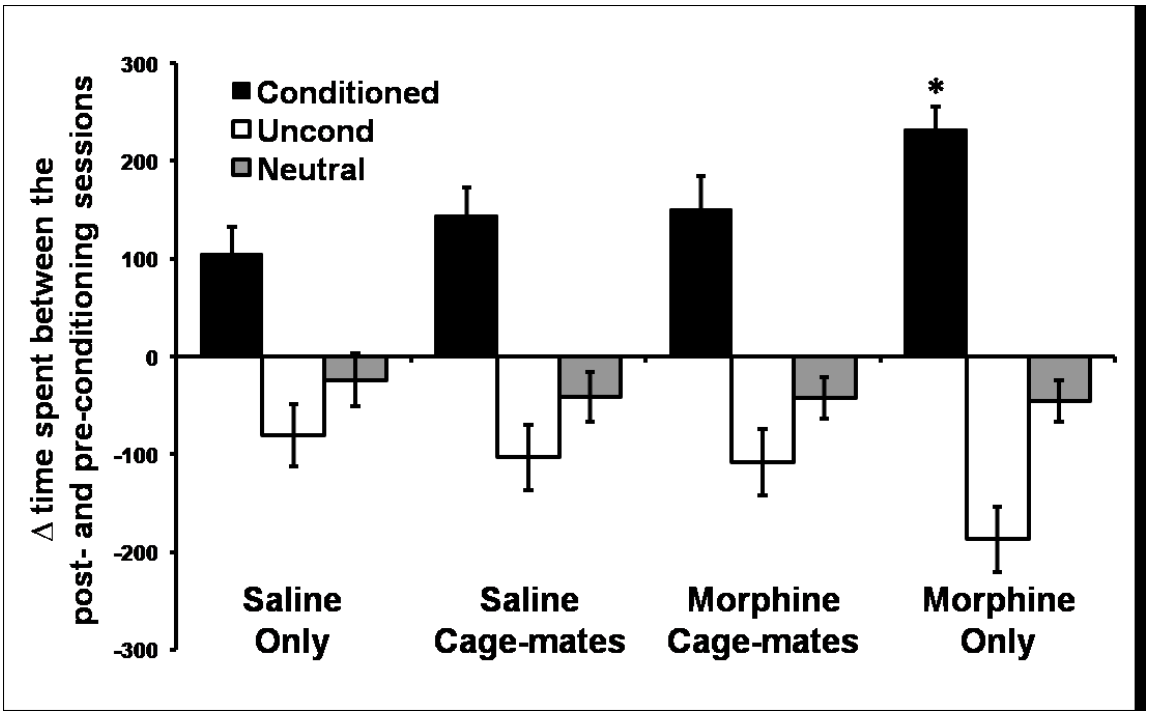

Figure 4 Non-normalized data of the conditioned-place preference with 20 mg/kg morphine

The differences between the time spent in the post- and pre-conditioning sessions in each of the chambers of the CPP apparatus for the saline only, saline cage-mates, morphine cage-mates, and morphine only animals which were conditioned with 20 mg/kg morphine. Black bars – drug-paired chamber; White bars – saline-paired chamber; Grey bars – neutral chamber. (*) indicates a significant difference from saline only animals ($p<0.01$). Results are presented as mean ± SEM.

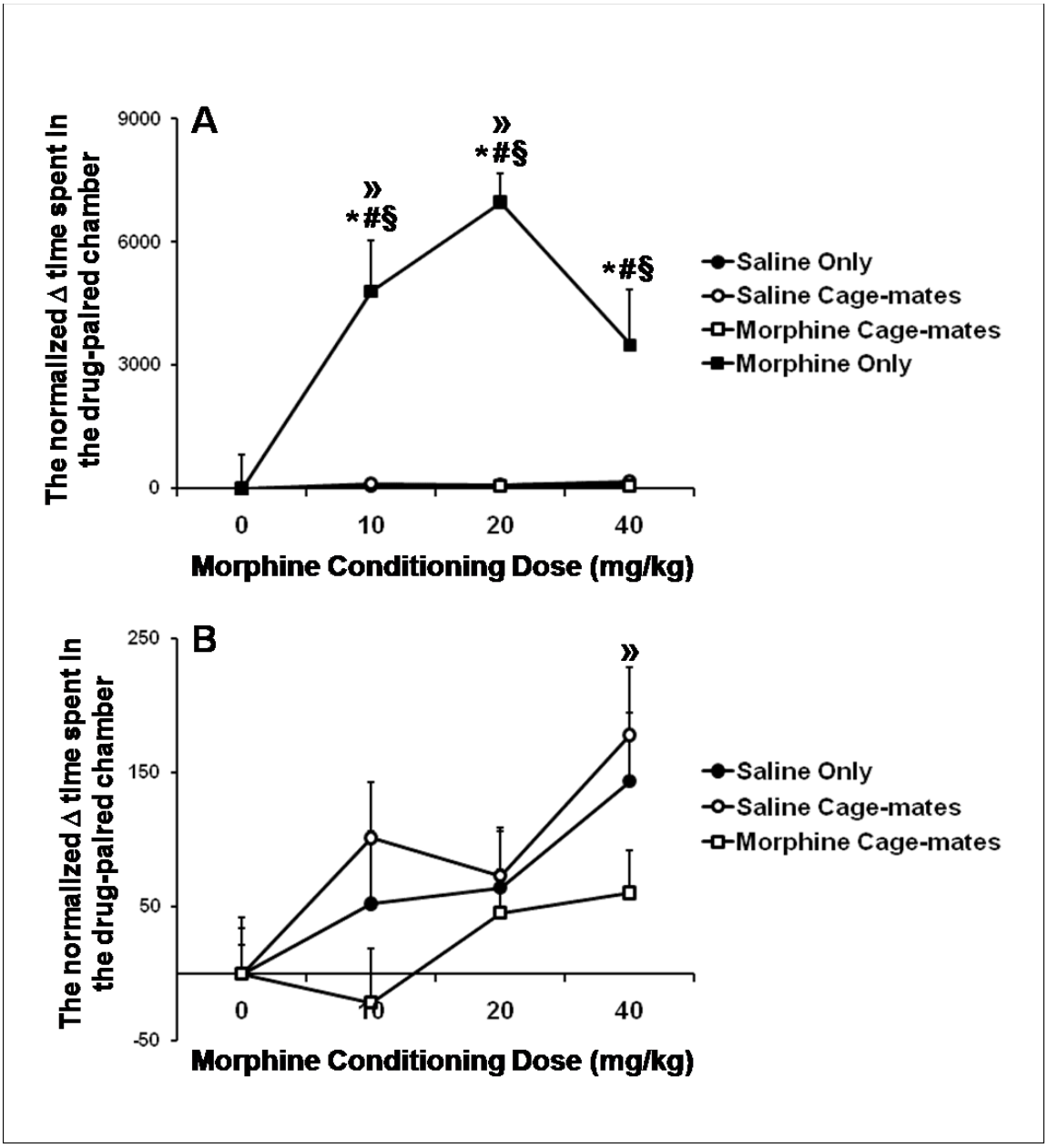

Figure 5 Effect of social environment on the acquisition of morphine CPP

The normalized Δ time spent in the drug-paired chamber of the CPP apparatus for the saline only, saline cage-mates, morphine cage-mates, and morphine only animals which were conditioned with 10, 20, 40 mg/kg morphine or saline. (**A**) The results in all 4 experimental groups. Given that differences between the saline only, saline cage-mates and morphine cage-mates animals could not be depicted when using the y scale required to include the results from the morphine only animals, (**B**) represents the results for the saline only, saline cage-mates and morphine cage-mates animals. (») indicates a significant difference from the control animals (conditioned with only saline) for the corresponding experimental group ($p<0.01$); (*) indicates a significant difference from saline only animals ($p<0.01$); (#) indicates a significant difference from saline cage-mates ($p<0.01$); (§) indicates a significant difference from morphine cage-mates ($p<0.01$). Results are presented as mean ± SEM.

Results of normalized data for social influences on morphine CPP

In order to measure the effects of social influences on morphine reward, we measured the differences between the amount of time spent in the drug-paired chamber on habituation day and test day, following a pairing of morphine in the animal's least preferred chamber. This data was normalized to the control drug-independent adjustment as explained above. The data for all three morphine doses normalized by saline controls are presented in Fig 5. We found that there were significant differences in the amount of time spent in the drug-paired chamber based on treatment received in their home cage (i.e., morphine vs. saline), housing conditions (mixed vs. only), and conditioning dose (0, 10, 20, or 40 mg/kg morphine).

As expected, saline only animals did not condition to morphine after only one pairing, i.e., they did not spend significantly more time in the drug-paired chamber on test day. Although there was a trend of increase time spent in the drug-paired chamber as the dose of morphine increased, at none of the doses tested did they show a significant preference for the morphine-paired chamber compared to saline only counterparts that did not receive a morphine pairing (and only received saline in the CPP apparatus). In contrast, saline cage-mate mice were conditioned to 40 mg/kg morphine, i.e., they spent significant more time in the morphine-paired chamber when receiving in that chamber the 40 mg/kg dose as compared to saline only counterparts that did not receive a morphine pairing. In other words, they found the 40 mg/kg morphine dose rewarding after one pairing.

In line with previous findings, morphine only animals showed a significant conditioning to morphine at 10 and 20 mg/kg doses. Morphine was significantly more rewarding to morphine only animals as compared to saline only, saline cage-mate animal, and morphine cage-mate animals. These differences between morphine only and saline only mice is in line with literature demonstrating that previous exposure to morphine can result in a modulation of the abuse potential/rewarding properties of morphine.

In contrast to morphine only animals, morphine cage-mates animals did not condition to any of the doses tested. Although there was a trend of increase time spent in the drug-paired chamber as the dose of morphine increased, at none of the doses tested did they show a significant preference for the morphine-paired chamber compared to morphine cage-mates counterparts that did not receive a morphine pairing (and only received saline in the CPP apparatus). In other words, although receiving the same morphine treatment in their home cage, morphine only animals found morphine rewarding and morphine cage-mates did not. These differences between the morphine only and the morphine cage-mate animals provides evidence that exposing an animal to various peers (peers administered vs. not administered with morphine) can shift the rewarding properties of morphine.

Stress and plasma corticosterone levels

Plasma coricosterone levels were measured to determine whether stress plays a role in the social effects on morphine CPP or morphine reward (see Fig 6 for results). If stress

levels were seen to be significantly different between different groups of mice, this could provide a mechanism by which differences in responses to morphine are shifted. Plasma corticosterone was measured nine days following the last administration of morphine, which corresponds to the conditioning period in the CPP apparatus. No significant differences were observed between saline only, saline cage-mates, morphine cage-mates, or morphine only animals in plasma corticosterone levels, which indicates similar levels of stress in all treatment groups during the conditioning period.

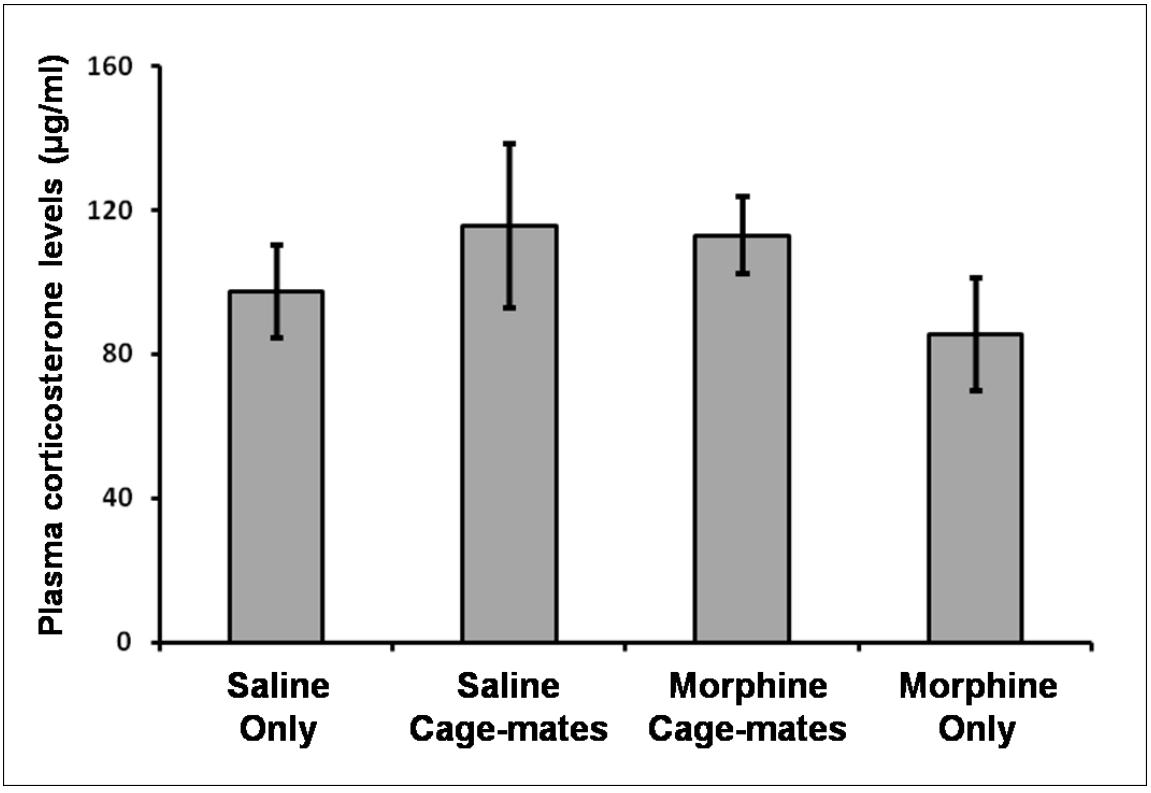

Figure 6 Plasma corticosterone levels in socially-housed saline- and morphine-treated adolescent mice

Experiment II: The involvement of oxytocin in the social effects on drug reward

We examined the involvement of OT in the social effect on morphine reward. Specifically, we examined whether the lack of conditioning to morphine in the morphine cage-mates is mediated via the OT system. In order to determine whether the OT receptor plays a role in social effect on morphine CPP we examined how administration of OT antagonist during the morphine-pairing period alters the conditioning to morphine in the morphine only and morphine cage-mate animals.

Morphine only mice were observed not to be significantly affected by OT antagonism and showed comparable levels of CPP relative to morphine only mice receiving vehicle, indicating that the OT antagonist had no effect on the degree of morphine reward experienced by morphine only mice. Importantly, OT antagonism had an effect on the rewarding properties of morphine in morphine cage-mate animals. Morphine cage-mate mice treated with OT antagonist found morphine rewarding. In other words, morphine cage-mate mice treated with an OT antagonist during the morphine paring in the CPP apparatus exhibit increased conditioning to morphine compared to control morphine cage-mates mice (i.e., receiving vehicle). These findings indicate that differences in the function of the OT system plays a role in the social effect on morphine CPP and the potential for morphine abuse. The results for the effects of OT antagonism on morphine CPP can be seen in Fig 7.

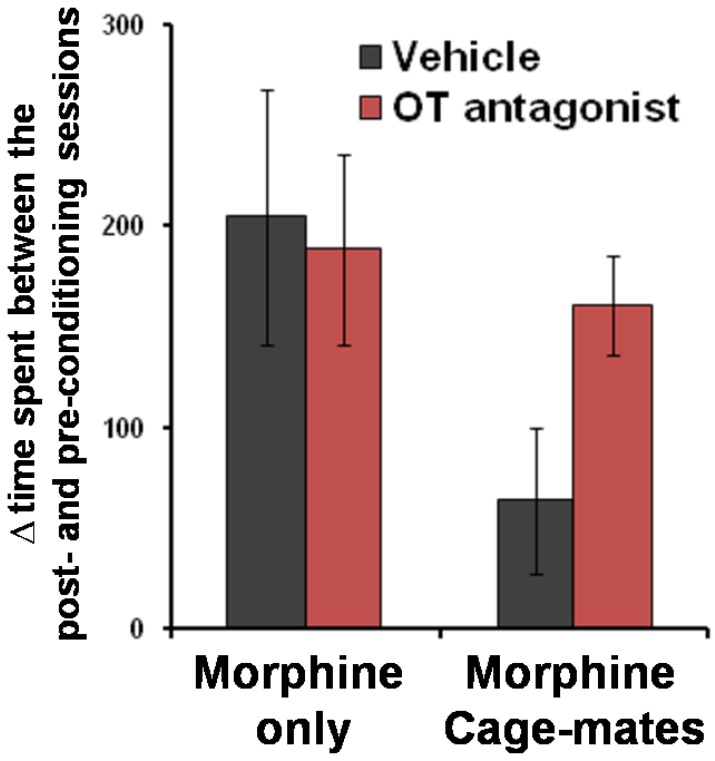

Figure 7 Effects of OT antagonism on morphine CPP in morphine only and morphine cage-mate mice

The differences between the time spent in the drug-paired chamber during the post- vs. pre-conditioning sessions. Thisis non-normalized data of CPP with 20 mg/kg morphine, similar to Fig 4. Results are presented as mean ± SEM.

CHAPTER IV

DISCUSSION AND CONCLUSIONS

Discussion

Social effect on morphine CPP

These findings provide evidence that exposure to different social environments modulate the rewarding properties of morphine. Saline only mice did not show a major preference for morphine and did not acquire morphine CPP following one conditioning session. However, saline cage-mate mice (i.e., drug-naïve mice housed with drug exposed mice) acquired morphine CPP after one conditioning session at the 40 mg/kg dose, and thus, showed an increase in preference for morphine. Interestingly, morphine cage-mate mice failed to acquire morphine CPP at any of the doses tested, whereas morphine only mice conditioned both at 10 and 20 mg/kg doses. The morphine only group's acquisition of morphine CPP is in line with previous literature showing that previous exposure to morphine has a sensitizing effect on the rewarding properties of morphine (Lett 1989; Gaiardi *et al.* 1991; Shippenberg *et al.* 2009). These findings highlight the importance of social interaction during adolescence, as these mice were injected, handled, and cared for identically, and yielded differing responses in the ability of morphine to induce CPP depending on the exposure (or lack of exposure) of peers to morphine.

Morphine cage-mate mice

Morphine cage-mate mice did not acquire morphine CPP for any of the doses tested, and responded the least to morphine out of all four treatment groups. The lack of acquisition of morphine CPP in this group as compared to morphine only mice suggest that exposure to drug naïve animals is protective against the abuse potential of opioids. It is somewhat counterintuitive that the morphine cage-mates would condition even less than saline treated mice, given that previous exposure to opiates has been shown to increase later morphine reward (i.e., sensitization). However, it is possible that tolerance and sensitization were modulated simultaneously in these mice. Interactions with drug-naive mice may have diminished the sensitization of morphine cage-mates to morphine while also increasing morphine tolerance, resulting in the reduction of morphine reward seen. Saline cage-mates may not have developed this tolerance due to a lack of drug exposure in the home cage and were sensitized to morphine due to peer influences. These adaptations could then result in a higher level of response to morphine in saline cage-mates relative to morphine cage-mates.

Morphine only mice

Notably, morphine only mice acquired morphine CPP at the 10 and 20 mg/kg doses but not at the 40 mg/kg dose. This may be due to a heightened susceptibility to a drug-induced state, as these mice are much more sensitized to morphine's effects relative to the other three treatment groups. Conditioned place preference is an associative learning paradigm, and drug-induced euphoria may have impeded the ability of the mice to

associate the environmental cues (patterns/scents) of the CPP apparatus with morphine reward. Additionally, this decrease in morphine CPP might be explained by an increase in the aversive properties of morphine brought on by the highly sensitized state of the mice coupled with the relatively high dose (Bechara & van der Kooy 1987; Bechara, Zito & van der Kooy 1987). At lower doses these aversive properties may have been negligible or non-existent, and thus, did little to affect morphine CPP. While the rewarding properties of morphine might still have increased at the 40 mg/kg dose, the aversive properties may have also been much more noticeable, leading to a decrease in the amount of time spent in the drug-paired chamber.

Impacts of social influences in drug-independent adjustment mice

Further evidence of the impact of social environments was found from saline controls. Data from the mice who received saline in both chambers on both conditioning days revealed a remarkable difference between the morphine only mice and the other three treatment groups. While the saline only, saline cage-mate, and morphine cage-mate groups drug-independently increased the amount of time spent in least preferred chamber, the morphine only treatment group did not. This inability or unwillingness of the morphine only group to adjust to the CPP apparatus is particularly interesting, as the morphine cage-mates (i.e., the mice administered morphine in an identical fashion as the morphine only mice, but housed with drug-naive mice) did not show this lack of adjustment. Apparently, the social dynamic of the morphine only group produces a lack of adjustment which is negated or retarded in development through the morphine cage-

mates' exposure to drug naive peers. One potential source of this disparity is a greater level of anxiety from the initial exposure to the CPP apparatus in morphine only mice. The saline only, saline cage-mate, and morphine cage-mate groups most likely experienced initial anxiety during baseline testing, but after repeated exposure to the CPP apparatus anxiety levels may have diminished, resulting in the adjustment observed. However, the morphine only group mice may have been experiencing such a high degree of anxiety that four exposures to the CPP apparatus was insufficient for their anxiety to dissipate, resulting in a subsequent lack of adjustment.

Another potential source for the lack of adjustment, albeit somewhat speculative, is a reduction in learning flexibility within the morphine only group. The morphine only mice may display a lack of adjustment brought on by a shift from goal-directed learning to more habitual learning. It has been suggested that compulsive substance abuse may caused by shifting from goal-directed to habitual drug seeking (Everitt, Dickinson, & Robbins 2001; Everitt & Robbins 2005). Goal-directed learning draws associations between responses and the value or salience of outcomes. Habitual learning draws associations between stimuli and responses without linking the response to the outcome it generates. Outcomes generated by responses do not instrumentally reinforce shifts in responses to stimuli, and thus, fixation and a lack of adaptation occur following a shift to habitual learning. While the initial anxiety when exposed to the CPP apparatus eventually may have subsided in all treatment groups, including morphine only animals, the inability to shift from the established initial preference (unless a more salient

motivator is presented, such as morphine) represents an increase in habitual learning and reduced cognitive flexibility in the morphine only animals. Perhaps, interacting with drug-naive peers within the morphine cage-mate group stymies the progress of this shift, resulting in the retention of goal-directed learning and reduction in the potential to abuse morphine. Alternatively, the exposure to drug-naive mice may not have diminished or retarded the shift, but rather, the lack of exposure to drug naive peers may have precipitated the development of habitual learning.

Potential mechanisms explaining the social effect on morphine CPP

The potential roles of stress in morphine CPP

The major differences observed between the morphine only and morphine cage-mate groups may be explained in part by stress exposure in the home cage. Exposure to stressful social interactions has been shown to enhance morphine sensitization (Frances *et al.* 2000). Perhaps morphine only mice experienced higher stress than morphine cage-mates. As all four mice received morphine in the morphine only cage, there may have been a greater disruption of normal feeding, grooming, sleep, and communication habits. Additionally, aggressive behavior has been demonstrated to increase during morphine withdrawal (Rodríguez-Arias *et al.* 1999; Felip *et al.* 2000; Sukhotina 2001), and increases in aggressive behavior may be responsible for higher stress levels within the home environment. Alternatively, morphine cage-mates were housed with two drug-naïve peers, which may have ameliorated some of the stress brought by repeated

morphine injections and withdrawal within the home cage, leading to diminished morphine preference.

Additionally, saline cage-mate mice acquired significant CPP at the 40 mg/kg dose. The exposure of drug-naïve mice to drug exposed cage-mates appears to increase the rewarding properties of morphine, and thus the potential for abuse. Perhaps the exposure to morphine cage-mates resulted in a heightened state of stress in saline cage-mate mice, which in turn resulted in greater morphine CPP.

Our analysis of plasma corticosterone levels revealed no significant differences between the different treatment groups. Plasma coricosterone was taken nine days following the last treatment injection of either morphine or saline at their home cage, and thus, corresponds to stress levels during the conditioning period. While stress levels were not seen to be significantly different, these findings do not necessarily reflect stress levels during the treatment period in their home cage and/or during the withdrawal period. There may still be varying amounts of stress prior to the CPP testing that explain the different responses to morphine between the treatment groups.

Effects of oxytocin on social influences and morphine reward

The administration of an OT antagonist 30 minutes prior to conditioning appeared to facilitate the acquisition of morphine CPP in morphine cage-mate mice. In contrast, morphine only mice did not appear to be significantly affected by the administration of

an OT antagonist. These findings suggest that the tonic level of OT in morphine only mice is lower than morphine cage-mates. It is possible that a depressed OT system is not significantly affected by OT antagonism due to the already relatively little activation of this system. If there is little or no function to decrease, the behavior would not be expected to change significantly. On the other hand, antagonism of the OT system in morphine cage-mates resulted in increase acquisition of morphine CPP. These results suggest that low tonic activity within the OT system due to drug use (i.e., morphine only) or antagonism (i.e., morphine cage-mates) facilitates the acquisition of morphine CPP. Additionally, higher activity levels of OT in the morphine cage-mate group might be responsible for the diminished conditioning to morphine. Thus, these results suggest that the exposure to drug-naive mice by morphine-treated mice may increase the activity levels of OT system and in turn lower response to the rewarding properties of morphine. In contrast, the exposure to 3 other mice receiving morphine (i.e., morphine only mice) may result in a reduction of OT system functioning, leading to a larger response to morphine reward. This data is congruent with current literature showing that activation of the OT system decreases the development of opioid dependence and attenuates self-administration in adult rodents (Ibi *et al.* 2008).

Conclusions

These results are in line with our previous findings that social interaction with drug-naïve peers reduced the development of morphine locomotor sensitization in morphine-treated animals (Hofford *et al.* 2010; Hofford *et al.* 2011), and that exposure to mice

treated with morphine enhanced morphine-induced hyper-locomotion in drug-naïve animals (Hodgson *et al.* 2010). It appears, that exposure to different social partners can modulate the acquisition of morphine conditioned place preference. The capacity of a drug to induce CPP is an indicator of its abuse potential (Bardo & Bevins 2000). As such, it appears that exposure to drug-naïve peers has a protective effect for opioid administered adolescents (i.e., exposure reduces the abuse potential of morphine) and that exposure to opioid treated peers has a detrimental effect for drug-naive adolescents (i.e., exposure increases the abuse potential of morphine). Additionally, while stress may yet play a role in the social effects observed, our analysis of plasma corticosterone levels suggest that stress during CPP testing does not solely explain the effects of social influences on the acquisition of morphine CPP. Furthermore, it appears that the neuropeptide oxytocin (OT) might serve as a molecular mechanism by which social environments manipulate the rewarding properties of morphine and potentially other opioid compounds.

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