Morphine and Impulsivity: The Effect of Dependence and Withdrawal on Delay Discounting in Rats.

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For Amma and Max, so that your names can live on.

Dear future generations: Please accept our apologies. We were rolling drunk on petroleum.

-Kurt Vonnegut

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Abstract

In the opiate dependent population, the most consistently reported decision-making deficit is an increase in impulsivity compared to controls. The level of impulsivity observed in this population shows both reward-specificity and state-dependency. In particular, opiate dependent humans are more impulsive towards opiate rewards than monetary rewards. Similarly, opiate dependent individuals show increased impulsivity when they are tested in a state of withdrawal. On the other hand, extended abstinence and acute administration of opiates appear to decrease the level of impulsivity observed.

Based on this relationship, impulsivity may be a risk factor for opiate addiction; that is, impulsivity predates and predicts drug use and abuse. Alternately, it is possible that the physiological and psychological changes that accompany drug dependence result in a state of increased impulsivity. The goal of this thesis is to test the latter hypothesis in rats order to better understand the relationship between impulsivity and opiate dependence in humans. Impulsivity can be modeled with delay discounting – a method to determine the rate at which the value of future rewards is discounted when there is a delay before delivery. In this task, rats chose between pressing one of two levers for liquid sucrose reward. Pressing one lever yielded a small immediate reward (50µl 20% sucrose) and the other yielded a larger delayed reward (150µl 20% sucrose). Delay discounting sessions consisted of five blocks of fourteen forced trials. On the first four trials of each block rats were forced to sample each reward twice. On the following ten trials rats could choose either lever. Length of the delay was systematically increased (0s, 5s, 9s, 16s, 30s) between

successive blocks within session. Delay discounting curves were determined by plotting mean proportion choice for the larger reward against delay.

Across several experimental manipulations, this method was used to test the difference in impulsivity between morphine-dependent rats that received a nightly 30mg/kg subcutaneous dose of morphine and non-dependent rats that received a nightly saline injection. These experiments revealed that morphine dependence can cause an increase in impulsivity that is restricted to the period of exposure. Precipitating withdrawal in dependent rats also caused an increase in impulsivity. Dependent animals discounted morphine rewards at a faster rate than non-morphine rewards, whereas both rewards were discounted similarly in non-dependent rats. On the other hand acute morphine caused increased impulsivity in non-dependent animals whereas dependent rats showed tolerance to this effect. These results support the hypothesis that impulsivity in the human opiate dependent population can result from extended exposure to large doses of opiates and that the effects are transient and vary with both the physiological and motivational state of the organism

Résumé

Dans les populations dépendantes aux opiacés, le déficit au niveau des prises de décision le plus régulièrement observé est une augmentation d'impulsivité comparé aux groupes contrôles. Les niveaux d'impulsivité observés dans cette population sont à la fois spécifiques aux récompenses et dépendants de l'état de la personne. En particulier, les individus dépendants aux opiacés sont plus impulsifs envers des récompenses reliées aux opiacés qu'envers des récompenses monétaires. De même, les individus dépendants aux opiacés démontrent plus d'impulsivité lorsqu'ils sont observés en état de sevrage. Par contre, une abstinence prolongée et une consommation aigüe d'opiacés semblent diminuer les niveaux d'impulsivité observés.

Dû à ces associations, l'impulsivité peut être un facteur de risque pour la dépendance aux opiacés; ainsi, l'impulsivité est antérieure à, et prédit, la consommation de drogues et la dépendance. Alternativement, il est possible que les changements physiologiques et psychologiques qui accompagnent la dépendance entraînent un état d'impulsivité accrue. Le but de cette thèse est d'examiner cette dernière hypothèse chez les rats afin de mieux comprendre la relation entre l'impulsivité et la dépendance aux opiacés chez les humains. L'impulsivité peut être reproduite chez les rats en utilisant "delay discounting" - une méthode qui indique la vitesse à laquelle la valeur de récompenses futures est ignorée lorsqu'il y a un délai avant la distribution de la récompense. Dans cette tâche, les rats choisissent d'appuyer sur un de deux leviers pour une récompense de sucrose liquide. L'un des leviers donne une petite récompense immédiate (50µl 20% sucrose) tandis que l'autre donne une récompense plus grande, mais à retardement (150µl 20% sucrose). Les sessions de "delay discounting" étaient composées de cinq blocs de

quatorze essais forcés. Durant les premiers quatre essais de chaque bloc, les rats était forcés à choisir chaque type de récompense deux fois. Durant les prochains dix essais, les rats pouvaient choisir le levier qu'ils préféraient. La durée du délai avant la récompense augmentait de manière systématique (0 secondes, 5 secondes, 9 secondes, 16 secondes, 30 secondes) entre chaque bloc des sessions. Des courbes de "delay discounting" ont été créées en traçant la moyenne de la proportion de choix favorisant la récompense large à retardement.

Cette méthode a été utilisée avec plusieurs manipulations expérimentales pour déterminer la différence d'impulsivité entre des rats dépendants à la morphine qui recevaient une dose sous-cutanée à chaque soir (30mg/kg) et des rats non-dépendants qui recevaient une injection saline à chaque soir. Ces expériences révèlent que la dépendance à la morphine peut causer une augmentation d'impulsivité limitée à la période d'exposition à la substance. Précipiter le sevrage chez les rats dépendants peut aussi causer une augmentation d'impulsivité. Les animaux dépendants ignoraient les récompenses de morphine plus rapidement que les récompenses non-reliées à la morphine, tandis que les deux récompenses étaient ignorées à un taux similaire chez les rats non-dépendants. En revanche, la consommation aigüe de morphine causait une augmentation d'impulsivité chez les animaux non-dépendants, tandis que les rats dépendants démontraient une tolérance à ce niveau. Ces résultats soutiennent l'hypothèse que l'impulsivité chez les humains dépendants aux opiacés peut être le résultat d'exposition prolongée à des doses élevées d'opiacés, et que les effets sont éphémères et varient dépendamment de l'état physiologique et motivationnel de l'organisme.

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Statement of Original Contribution

The research presented in thesis is based on four original manuscripts, two of which have been published in *Psychopharmacology (Berl.)*, and two of which are currently in review at *Psychopharmacology (Berl.)* and *Behavioral Pharmacology* respectively. Although the effects of acute and chronic exposure to drugs on impulsivity have been previously examined, no literature has specifically investigated how the state of physical dependence and/or withdrawal affects impulsivity in animals. Manuscript one here, is the first published article to specifically document the effects of opiate dependence on delay discounting in rats. The fourth manuscript is also the first article to isolate the effects of opiate withdrawal on impulsivity in rats. As a series of articles, the four manuscripts presented in this thesis are the first to extensively investigate how morphine dependence contributes to reward- and state-specific impulsivity. Specifically, manuscript two is the first to document how morphine dependence affects impulsivity towards morphine and non-morphine rewards in rats. Manuscript three is the first to investigate whether morphine dependence can cause tolerance/sensitization to the acute effects of morphine on delay discounting. Finally, manuscript three contains and validates a novel method of operant oral morphine self-administration. This is both the first usage of an operant procedure to deliver oral opiate rewards and also the first study to directly compare discounting of drug and non-drug rewards in the animal literature.

Author Contributions

I designed all four experiments contained in this thesis through discussions with Keith Franklin. For all four manuscripts presented here I was the primary author. Keith Franklin contributed to writing and editing of all four manuscripts in this thesis.

For the experiments presented in manuscript one, Both Johnna Perdrizet and I ran the animals and collected data. Keith Franklin and I performed statistical analysis. Johnna Perdrizet also contributed to literature research for the manuscript and assisted in the editing.

Jenna Parnigoni collected data and ran rats in a pilot project that predated and led to the experiments presented in manuscript two. Johnna Perdrizet and I ran the animals and collected data for the experiments presented in manuscript two. Keith Franklin and I performed statistical analysis. Johnna Perdrizet also contributed to literature research for the manuscript and assisted in the editing.

For the experiments presented in manuscript three, I ran all the animals and collected all the data presented. Hyunchoong Yong assisted with data entry. Keith Franklin and I performed the statistical analysis.

For the experiments presented in manuscript four, Hyunchoong Yong, Allyson Brisebois and I ran the animals. Hyunchoong Yong performed the data entry. Portions of Allyson Brisebois' undergraduate honors thesis and the literature research therein were used as a template for drafting of the manuscript. Both Allyson Brisebois and Hyunchoong Yong contributed to editing of the manuscript.

Chapter 1: General Introduction

Contemporary models of drug dependence emphasize the contribution of deficits in decision making to the etiology of the disorder. Substance dependent individuals tend to make decisions that are risky, poorly planned, badly executed, and have low estimated value (for review see Bechara, 2005; Clark & Robbins, 2002). It is hypothesized that these deficits serve to facilitate the continued use of drugs despite negative personal, social and vocational consequences. The most frequently reported decision making deficit in opiate dependent individuals is impulsivity (de Wit, 2009; Pau, Lee, & Chan, 2002; Perry & Carroll, 2008). Impulsivity, or impulsive choice, can be defined as an exaggerated preference for small immediate rewards over larger delayed ones. It is primarily measured in humans and animals using a delay discounting paradigm. Studies using this paradigm reliably report opiate dependent individuals to be more impulsive that non-dependent controls (Kirby & Petry, 2004; Kirby, Petry, & Bickel, 1999; Madden, Petry, Badger, & Bickel, 1997). It is thought that impulsive choice may play an important role in opiate dependence but the nature of this relationship remains contentious.

1.1 An Introduction to Delay Discounting

In delay discounting paradigms subjects are given a series of choices between a small immediate reward and a larger delayed reward. Reward types vary between experiments, but money and food are the most commonly used in humans and animals respectively. In animals, two-lever operant tasks are used to determine choice preference

between a lever that is associated with the immediate reward and one that is associated with the delayed reward. This preference can be used as a proxy for relative subjective value in accordance with Herrnstein's matching law (Herrnstein, 1970). In the human analogue task, the relative subjective value of immediate and delayed options is usually ascertained through explicit questioning (Lagorio & Madden, 2005; Madden, Begotka, & Raiff, 2003; for overview of human exemplar methodology see Madden et al., 2004), but can be determined with operant tasks similar to those used in animals(e.g. Lane, Cherek, & Pietras, 2003). In both cases delay and/or reward size are systematically varied such that the relative preference for the large reward can be assessed across a wide range of delays and/or sizes of reward. The distribution of choice preference is then used to determine the rate at which the subjective value of a reward decays as a function of time until its delivery. Individuals discount delayed rewards in an exponential or hyperbolic manner such that a smaller immediate reward has higher subjective value than larger reward delivered after a long enough delay (Madden et al., 2003). However, the more impulsive an individual the faster the rate at which they discount delayed rewards, and thus the more quickly the subjective value of a reward decays with time.

In animals there are two main methods of evaluating delay discounting: adjusting and fixed (Mar & Robbins, 2007). In the adjusting methodology either delay or reward size is altered in conjunction with choice distribution while the other variable is kept constant. Taking the situation where amounts are kept constant (adjusted *delay* method): rats are allowed to sample between a lever that delivers an immediate small reward and a one that delivers a 3-5 times larger reward after a given delay. These sampling trials are called forced choice trials, as only one of the levers is active and must be selected by the rat. Once

the rat samples both rewards, it is given a small number of free choice trials to choose between the levers. Lever choice during these trials is used to determine the delay associated with the large reward in subsequent trials. That is, when the large reward is selected the delay to its delivery is increased on the following trial or during the following block of trials. If the small reward is selected, the delay to the delivery of the large reward is decreased on the following trial or block of trials. In this way the rat titrates its own indifference point; that is, the delay at which the large and small reward is selected with equal frequency. This indifference point, usually referred to as the mean adjusted delay, is the dependent measure of the experiment and is calculated by averaging the adjusted delay over all, or over the latter portion of trials. In an adjusted *amount* procedure the delay to delivery of the large reward and the size of the small reward are held constant whereas the size of the large reward is adjusted by choice behavior. When the large reward is selected its size increases on successive trials, and decreases if the small reward is selected.

The advantage of the adjusting methodology is that very few assumptions have to be made about the nature of the function relating time to subjective value. This means that the dependent variable is an untransformed variable. Among the drawbacks of this methodology is that a large number of forced choice trials are needed because rats must be alerted whenever choice parameters are changed, which happens every few trials. In turn this increases the overall length of individual sessions needed to evaluate delay discounting. In addition, though the task is holistically sensitive to changes in impulsivity, individual trials and small subsets of trials are largely insensitive (Cardinal, Daw, Robbins, & Everitt, 2002). These limitations specifically make this methodology less suitable for

time-sensitive pharmacological interventions using drugs with short half-lives, since a longer steady state is required for accurate measurement.

The alternate methodology is the fixed method wherein reward sizes are kept constant (at a 3-5:1 size ratio) and delays are systematically varied. In such a way the distributions of choice between the small immediate lever and the large delayed lever is evaluated over a predetermined range of discrete delays. The entire range of delays can be evaluated daily with delay varied between blocks, or evaluated over several days so that delay remains constant within a daily session and is varied between sessions. This method maintains the structure of the adjusting method (force choice trials followed by free choice trials) but with a higher ratio of free choice to forced choices trials. The average proportion choice of the large reward at each delay is determined and used to construct a delay discounting curve. This raw delay discounting curve and its descriptors (e.g. slope) are used as dependent measures.

A major limitations of the fixed method stems from order effects when delays are varied systematically. Randomly varying delays to account for order effects is also problematic as longer delays may have more powerful effects on subsequent choice behavior than shorter delays (Mar & Robbins, 2007). A further limitation is that descriptors derived from the delay discounting curve require assumptions about the mathematical form of discounting -- which remains contentious -- and relies on imperfect curve fitting ($r^2 < 1$). This limitation can be circumvented by describing delay discounting using area under the curve which requires no assumptions about the mathematical form of discounting (Myerson, Green, & Warusawitharana, 2001).

1.2 Behavioral Economic Theory: Impulsivity and Drug Dependence

Behavioral economic theories of drug dependence (e.g. Bickel, Jarmolowicz, Mueller, & Gatchalian, 2011) posit that impulsivity can play a causal role in drug use. In impulsive drug-dependent individuals it is hypothesized that the immediacy of the rewarding effects of drugs may increase their relative desirability when compared to the deferred pro-social rewards that usually accompany abstinence (Bickel & Marsch, 2001). Furthermore, accelerated discounting may enhance the aversiveness of withdrawal, due to its quick onset following termination of a dosing regimen, and reduce the deterrent effect of the less immediate negative consequences of drug dependency (Bickel & Marsch, 2001; Odum, Madden, & Bickel, 2002). Devaluation of future health status has been shown to be prominent in addicts; health benefits and costs are discounted more steeply in drug users than controls (Odum et al., 2002). Together, these postulates predict that to a drug dependent individual, impulsivity would simultaneously increase the subjective value of continuing drug use while decreasing the value of abstinence. Consequently, when confronted with a decision between taking drugs and abstinence, the increased discrepancy in subjective value between these options due to impulsivity, would bias a drug dependent individual towards selecting drug use. In this framework, the exaggerated strength and immediacy of the aversive state of withdrawal in opiate dependency would serve to further bias decisions towards drug taking when compared to other non-opiate drug dependencies.

1.3 Impulsivity and Relapse

A further prediction of behavioral economic models is that impulsivity should foster drug dependence specifically through promoting relapse and impairing attempts at abstinence. MacKillop and Kahler (2009) showed that pre-enrollment delay discounting performance was correlated with smoking cessation treatment outcomes in dependent individuals, including relapse rate during abstinence. Impulsivity has been shown to be associated with relapse during cocaine-dependence treatment (Moeller et al., 2001). Similarly, low levels of impulsivity has been shown to be a predictor of positive rehabilitation outcomes in adolescents receiving treatment for marijuana dependence or abuse (Stanger et al., 2012). This relationship is also observed in opiate dependent individuals; impulsive decision making in the Iowa gambling task predicted relapse in opiate dependent individuals receiving outpatient treatment (Passetti et al., 2011). Furthermore, 65-74% of opiate addicts relapse within the first week following rehabilitation (Gossop, Green, Phillips, & Bradley, 1989; Smyth, Barry, Keenan, & Ducray, 2010) -- the period of time that opiate withdrawal persists (Loimer, Linzmayer, & Grünberger, 1991). In the animal literature, rats that show accelerated delay discounting prior to drug initiation, take longer to extinguish drug responding, and show greater response to cue induced relapse following nicotine and cocaine self-administration (Belin, Mar, Dalley, Robbins, & Everitt, 2008; Diergaarde et al., 2008).

If, as the human and animal literature suggests, impulsivity has a causal role in drug dependence and relapse, then increasing impulse control should have a protective effect against drug initiation and/or relapse. The use of pharmacological agents such as

methylphenidate (Ritalin, α-phenyl-2-piperidine-acetic acid methyl ester) to increase catecholaminergic neurotransmission is one method of increasing impulse control (K. S. Patrick & Markowitz, 1997). This method is primarily used in individuals with ADHD. A meta-analysis found that children diagnosed with ADHD whose impulsivity was treated with Ritalin or similar pharmacotherapy exhibit a 1.9-fold reduction in substance use disorders when compared to ADHD youths who did not receive pharmacotherapy (Wilens, Faraone, Biederman, & Gunawardene, 2003). Independent of ADHD, methylphenidate also has effects on drug taking; Collins et al. (2006) found that acute doses of methylphenidate in drug users decreased the positive reinforcing effects of acute cocaine and participants' preference for cocaine reward over monetary reward. Economidou et al. (2011) found that pretreatment with atomoxetine (a selective norepinephrine reuptake inhibiter that is used to treat impulsivity) attenuated cue-induced relapse to cocaine and heroin in rats. This limited literature suggests that pharmacotherapy that increases impulse control could possibly improve the treatment of drug dependence. However in order to target such medication, a better understanding of the way in which drug dependence modulates impulsivity is necessary.

1.4 Drug Dependence and Impulsivity: Hypotheses

The observation that individuals dependent on drugs (Bickel, Odum, & Madden, 1999; Bjork, Hommer, Grant, & Danube, 2004; Coffey, Gudleski, Saladin, & Brady, 2003; Kirby & Petry, 2004) – including, but not limited to opiates – are more impulsive than the general populations suggests two plausible hypotheses. Firstly, impulsivity may represent

a risk factor for drug addiction; that is, impulsivity predates and predicts drug use and abuse (for review see Perry & Carroll, 2008). It is also feasible that the physiological and psychological changes that accompany drug dependence result in a state of increased impulsivity. In other words, drug dependence may either cause impulsivity, result from it, or some combination of both. It must be noted that these convergent hypotheses have divergent clinical implications. If impulsivity predates drug dependence a preventative approach targeting impulsivity in high-risk individuals prior to drug initiation would be most efficacious – treat impulsivity, prevent drug dependence. On the other hand if impulsivity is a result of drug dependence, then intervention would have to be applied after the development of impulsivity in the dependent individual – treat impulsivity, booster rehabilitation.

While this problem is of a clinical nature, investigating the directionality of the link between impulsivity and drug dependence in human populations is problematic. First and foremost, the previously mentioned human studies are all correlational and thus cannot be used to prove whether impulsivity causes drug dependence or vice versa (de Wit, 2009). Prospective longitudinal studies of drug-naive high and low impulsive individual could address whether impulsivity predicts drug dependence; but, no such study has yet been executed likely due to the cost (Verdejo-García, Lawrence, & Clark, 2008). On the other hand, it would be impossible to accurately determine if drug dependence can cause impulsivity in humans since experimental ethics would not allow random assignment of individuals to a dependence-inducing dosing regimen. Due to these issues in determining causality with human studies, animal experiments are better suited to disentangle the nature of the impulsivity dependence relationship.

1.5 Drug Dependence and Impulsivity: Non-opioid Drugs

As it pertains to non-opioid drugs of abuse, animal experiments have supported both hypotheses of the dependence/impulsivity relationship. In rats, chronic exposure to moderate or high doses of cocaine increases impulsivity in delay discounting paradigms during the dosing regimen (Paine, Dringenberg, & Olmstead, 2003), and 3-months after exposure (Roesch, Takahashi, Gugsa, Bissonette, & Schoenbaum, 2007; Simon, Mendez, & Setlow, 2007). However some studies have reported that cocaine has no effect on impulsivity at shorter abstinence intervals (Winstanley et al., 2007). Thus, cocaine may increase impulsivity during dependence, but long-term effects on discounting require an incubation period. This fits with previous models of cocaine-sensitization which have shown that an incubation period is necessary for other long-term behavioral effects of cocaine (Setlow, Mendez, Mitchell, & Simon, 2009). Similar increases in impulsivity have also been shown following chronic nicotine (Dallery & Locey, 2005) and methamphetamine (Richards, Sabol, & de Wit, 1999) exposure. Thus, the impulsivity observed in human drug dependent individuals might partially result from drug use, since chronic exposure to large doses of psychostimulant drugs in animals can cause increases in impulsivity.

On the other hand, preexisting levels of impulsivity have been shown to predict aspects of drug self-administration in animals. For example, highly impulsivity rats have been shown to acquire IV cocaine self-administration faster than low impulsivity rats (Anker, Perry, Gliddon, & Carroll, 2009; Perry, Nelson, & Carroll, 2008). Likewise, high impulsivity rats showed increased cocaine-primed reinstatement of conditioned responding (Perry et al., 2008). A similar study showed that impulsive choice predicted responding for a lever previously paired with nicotine during the extinction phase of a self-

administration, but did not predict rate of drug-response acquisition (Diergaarde et al., 2008). Mice bred for high alcohol intake also show accelerated delay discounting compared to those bred for low alcohol intake (Oberlin & Grahame, 2009). These results suggest that pre-existing impulsivity can make animals more likely to initiate and/or continue drug usage. Taken as a whole, the non-opioid drug literature suggests that both preexisting impulsivity, and impulsivity due to chronic exposure, increase the likelihood of drug taking and/or relapse. Specifically, impulsivity appears to disproportionately impair the ability of an individual to initiate and maintain abstinence. However, due the unique nature of opioid dependence, the extent to which this relationship holds true for opioid dependence cannot be directly surmised.

1.6 Opiate Dependency

Prescription opiates and heroin are increasingly used recreationally in the general populations with 2012 usage rates estimated at 5% and 0.8% respectively (Fischer, Keates, Bühringer, Reimer, & Rehm, 2013). Thus, the acute effects of opiates, including their effects on decision-making, are relevant to a large segment of the population. Additionally, opioid dependence is widespread; it is estimated that about 4.5% and 1% of Americans meet DSM-IV criteria within their lifetime, for prescription opiate and heroin dependence respectively (Mendelson, Flower, Pletcher, & Galloway, 2008). Opioid dependence manifests both psychologically, evidenced by the euphoria achieved by their use, and physiologically, evidenced by the physical withdrawal experienced upon discontinuation of a dosing regimen (Koob, 2006). In such a way, the study of opioid agonists gives us insight

into multiple factors that contribute to the complex pharmaco-psychosocial phenomena of drug dependence.

Of these factors, the strong withdrawal that accompanies opiate dependency is particularly pertinent to the study of impulsivity and drug dependence. In humans this aversive state can persist up to two weeks after termination of drug use and is associated with a wide range of state-dependent changes in behavior and neurochemistry (Loimer et al., 1991). Withdrawal is associated with decreased dopamine transmission in the nucleus accumbens (Pothos, Rada, Mark, & Hoebel, 1991). Previous studies have shown that dopaminergic lesions (unrelated to withdrawal) in the nucleus accumbens increase impulsive choice in rats (Cardinal, 2001). From a motivational standpoint, withdrawal can increase the incentive salience of drug rewards and concurrently decrease the salience of non-drug rewards (Cooper, Shi, & Woods, 2010; G. C. Harris & Aston-Jones, 2002; Rouibi & Contarino, 2011). State-dependent motivational increases have been shown to cause accelerated discounting in non-dependent rats (Eisenberger, Masterson, & Lowman, 1982). Thus, behavioral and neurochemical changes due to withdrawal might be responsible for impulsive choice in dependent individuals.

In addition, repeated exposure to opiates has been shown to cause both sensitization and tolerance to the effects of acute doses on a wide variety of behavioral measures (Stewart & Badiani, 1993; for review see Stewart, de Wit, & Eikelboom, 1984). Tolerance is more often observed than sensitization, especially with direct physiological effects. For example, pain thresholds in the tail-flick test, hot-plate test and acetic-acid writhing test decrease with repeated exposures to morphine (Fernandes, Kluwe, & Coper, 1977; G. A. Patrick, Dewey, Huger, Daves, & Harris, 1978; Roerig, O'Brien, Fujimoto, &

Wilcox, 1984). Similarly, after repeated exposure rats show decreased sedation and decreased respiratory depression in response to acute morphine (LeBlanc & Cappell, 1974). Animals dependent on morphine show decreased aversion to novel solutions paired with morphine injections when compared to naïve animals (Domjan & Siegel, 1983; Hunt, Spivak, & Amit, 1985). It must be noted that although the nature of the aversive processes associated with acute morphine are poorly understood, they are independent of, and dissociable from the positive rewarding effects (Sherman, Pickman, Rice, Liebeskind, & Holman, 1980). Tolerance to the depressive, aversive and analgesic effects of morphine appear to be frequently mediated peripherally rather than centrally (Bechara, Zito, & van der Kooy, 1987). Underlying tolerance is a wide range of physiological effects including desensitization of all subtypes of opiate receptors and their decoupling from effector g-proteins (Collin & Cesselin, 1991).

On the other hand some behavioral processes -- in particular dopamine mediated processes -- show sensitization to opiates. For example, after repeated exposure to opiates rats show increased locomotive and exploratory behavior in response to acute doses (Bartoletti, Gaiardi, Gubellini, Bacchi, & Babbini, 1983; Hoffman et al., 2006). Similarly, morphine-experienced animals show increased magnitude of conditioned place preference when compared to naïve rats receiving similar doses and exhibit conditioned place preference to doses of morphine that have no effect in naïve animals(Lett, 1989). Sensitization to conditioned reward processes and locomotive response has been shown to involve the mesolimbic dopaminergic system (Stewart & Badiani, 1993).

One hypothesis of the processes underlying sensitization is a switch in the mechanism of morphine reward during opiate dependence (for review see Ting-A-Kee &

van der Kooy, 2012). It is proposed that in naïve animals, opiate reinforcement is moderated through a dopamine-independent pathway wherein opiates bind to μ-opiate receptors on GABAa channels -- pre-synaptic to GABA neurons -- inhibiting them. This inhibition causes depolarization of GABA neurons causing GABA release, which then inhibits dopamine neurons through binding to GABAb channels located on them. Simultaneously, GABA causes activation (through an unknown mechanism) of a descending pathway to the brainstem tegmental pedunculopontine (TPP) nucleus. This TPP pathway is responsible for opiate motivation in naïve animals since dopaminergic release in the VTA and nucleus accumbens is inhibited. In opiate dependent animals, a change in ion gradient in GABA neurons reverses the direction of chloride ion influx through the GABAa receptor and renders the formerly inhibitory receptor excitatory. Opiates maintain their binding site, but through this switch, now their binding leads to hyperpolarization and decreased GABA release. In turn, this causes disinhibition of the ascending dopaminergic pathway from the VTA to the NAcc, resulting in increased dopamine release in the NAcc. This dopaminergic release in the NAcc is responsible for opiate motivation in dependent rats. It would follow that in dependent rats the increased dopamine release (compared to non-dependent rats) would cause increased activation of dopamine-related processes and thus sensitization.

As with other behavioral processes, it is possible that individuals may exhibit either tolerance or sensitization to the effects of acute opiate doses on impulsivity. Whether opiates effects on impulsivity shows tolerance or sensitization in dependent individuals, should inform on the neurochemical processes underlying impulsivity. Specifically, observed tolerance or sensitization would give an indication of the extent to which opiate-

related impulsivity is centrally or peripherally mediated and dopamine dependent or independent.

1.7 Morphine: Rationale

One can argue that morphine is a preferable opiate exemplar to heroin. From an epidemiological standpoint, prescription opiate usage and dependence is more wide spread than heroin usage (reported life-time non-medical usage of 13.6% and 1.5% respectively; lifetime incidence of drug dependence 4.5% and <1% respectively) (Mendelson et al., 2008). Morphine is widely prescribed as a pain medication and has a high abuse liability. Additionally, the psychopharmacological effects of morphine can inform about the psychopharmacological effects of heroin since heroin is converted to morphine in the brain.

Morphine is most frequently abused via oral administration in the dependent population (Butler et al., 2013). This validates using an oral self-administration model in animals, which is particularly useful for testing reward-type specific effects (as will be explored later). Additionally, the link between impulsivity and opiates has largely been found in samples of individuals who were currently or previously dependent on opiates, but with a preponderance of prescription opiate users as opposed to heroin users. Thus, to model the effect of human opiate addiction on impulsivity, morphine maybe preferable to heroin as an experimental drug.

1.8 Opiates and Impulsivity: Human Literature

As previously mentioned, opiate dependent individuals are consistently reported to be more impulsive than non-dependent and naïve controls (Kirby et al., 1999; Kirby & Petry, 2004; Madden et al., 1997). This relationship has been documented with individuals dependent on heroin (Kirby et al., 1999; Kirby & Petry, 2004), individuals being maintained on buprenorphine (Giordano et al., 2002; Madden et al., 1997) and samples of individuals dependent on a variety of opiates (Madden, Bickel, & Jacobs, 1999). What is particularly unique about opiate addiction when compared to other drugs is the extent to which impulsivity in the opiate dependent population varies with the states of dependence and withdrawal. For example, formerly dependent heroin users show decelerated discounting of monetary rewards when compared to currently dependent individuals (Kirby et al., 1999) whereas this difference is not observed in former and current cocaine addicts (Kirby & Petry, 2004). This suggests that the increased impulsivity in opiate-dependent individuals may be restricted to the period of dependence. Opiate dependent individuals show accelerated discounting of opiate rewards when compared to non-drug rewards (Madden et al., 1997). Thus, impulsivity in opiate dependent individuals may be reward-specific. In addition, dependent individuals show accelerated discounting when tested while drug-deprived -- just prior to receiving their daily maintenance dose -- than when tested after receiving opiates (Giordano et al., 2002). That is, currently dependent individuals show more impulsivity during periods of mild withdrawal than when satiated. This is particularly interesting since opiates are reported to have no effect on discounting in naïve individuals (Zacny & de Wit, 2009). This would suggest the effects of acute opiates on impulsivity differ in dependent and non-dependent individuals due to differences in

either direct effects (sensitization/tolerance as outlined above) or through a secondary pathway such as alleviating withdrawal in dependent individuals.

Due to the aforementioned issues with establishing causality in the human literature, the extent to which chronic and acute exposure, dependence and withdrawal contribute to impulsivity are hard to establish in the human literature. Besides the issue of causality, withdrawal requires concurrent physical dependence (that is, they co-occur) and thus their individual impact on impulsivity is hard to disentangle. Though complicated, the individual impact of each of these factors can be better ascertained using animal models. Specifically, animals can be randomly assigned to dependence or non-dependence dosing regimens, both chronic and acute dosages can be controlled and withdrawal precipitated in a deliberate manner; all these manipulations are impossible or impractical in humans.

1.9 Opiates and Impulsivity: Animal Literature

Studies that investigate impulsivity as a determinant of opiate self-administration in the animal literature are limited, and their findings mixed. Garcia-Lecumberri and colleagues (2011) reported that Lewis rats show innately accelerated delay discounting and subsequently self-administer more morphine and reach higher breakpoints in order to acquire additional doses than the less impulsive Fischer strain. However, no intra-strain relationship between impulsivity and self-administration was reported. On the other hand, differences in delay discounting within Fischer rats did not predict initiation or escalation of heroin self-administration or the propensity to reinstate following drug cues (Schippers, Binnekade, Schoffelmeer, Pattij, & Vries, 2011). Taken together, these results might suggest

that the increased impulsivity and increased self-administration in Lewis rats, and the opposite relationship in Fischer rats, may be coincidental rather than causal. If this is the case, then the animal literature does not support impulsivity as a determinant of self-administration in rats, and exploring the alternate hypothesis -- that opiates increase impulsivity -- might be more promising.

The majority of studies on opiate-related increases in impulsivity have investigated the effects of acute doses of morphine on delay discounting in rats. García-Lecumberri et al., (2011) reported no effect of acute doses of morphine ranging from 0.25mg/kg to 2 mg/kg on delay discounting in two rat strains. Pattij and de Vries (2013) reported that moderate doses of morphine (6 mg/kg) did not affect the slope of delay discounting but did decrease zero delay preference for the large reward. On the other hand, Kieres et al. (2004) reported acute morphine (.5mg/kg – 2.0 mg/kg) caused a dose-dependent increase in impulsivity. Similarly, Tanno, Maguire, Henson, & France (2014) found that morphine produced a dose-dependent decrease in preference for the large reward at all delays and when immediately delivered, suggesting both an increase in impulsivity and decreased sensitivity to reward size. The variability in the results of these studies reflects the variability in their respective methodologies. The most pertinent methodological differences between these studies is the length of exposure to morphine, which ranged from 2 exposures at each dose (Tanno et al. 2014), to 6 weeks of total exposure (Kieres et al. 2004). Thus, the literature suggests that the effects of acute doses of opiates vary with prior history and the resulting tolerance or sensitization that exposure might produce. In addition all experiments exposed rats to multiple doses of the drug, either in ascending or random order. This may make interpretations of dose-response curves within experiments

problematic, since tolerance or sensitization effects may disproportionately affect later doses in the testing sequences.

To date, two studies have examined the effect of chronic opiate exposure on delay discounting in animals. Schippers and colleagues (2011) found that self-administration of approximately 1.5mg/kg/day heroin produced increased impulsivity for sucrose rewards during the period of self-administration. Animals were tested 1 hour before self-administration sessions, a period during which heroin would be eliminated from their system. The effect dissipated after a 5-week abstinence period. This would suggest that chronic heroin exposure causes increased impulsivity restricted to the period of administration, but independent of the acute effects of the drug. However, Harty et al. (2011) found that immediately after cessation of a 10-day, experimenter-administered 2mg/kg per day heroin dosing regimen, delay discounting in heroin-sensitized rats did not differ significantly from saline controls. This result suggests that chronic opioid exposure is not sufficient to produce protracted increases in impulsivity.

Due to the fundamentally different methods of drug exposure in these two studies, the discrepancy between these findings could be a result of any of the following: (1) the method of drug administration (self-administration versus passive administration), (2) the period of exposure (10 days versus 15 days), (3) the point at which impulsivity was assessed (during the dosing regimen or immediately following), and (4) the dose of drug received (1.5mg/kg v 2.0 mg/kg). Also, neither paper purports or provides evidence of dependence in their sample. The opiate literature shows that behavioral and neurochemical changes that accompany dependence – for example patterns of drug seeking and reinstatement – can be dissociable from behavioral changes resulting from

opiate sensitization (Lenoir & Ahmed, 2007). Furthermore, the lack of an extended effect in both studies may be due to low doses not sufficient to cause lasting changes. Previous studies of non-opioid drugs have shown that high dose regimens produce protracted impulsivity (Paine et al., 2003) whereas low dose regimens may not (Winstanley et al., 2007).

1.10 Project Overview

To date, the effects of opiate dependence on impulsivity have not been directly examined in the animal literature. Despite its relevance to the human dependent population and opiate replacement therapy, the extent to which opiate dependence further modulates reward-specific and state-dependent impulsivity has also not been investigated. This thesis considers a series of experiments to evaluate how opiate dependence affects both general impulsivity and further modulates state- and reward-specific impulsivity in rats. Herein, these issues are investigated using a fixed delay discounting paradigm in rats. The main questions to be addressed in this series of experiments are as follows: (1) Can morphine dependence cause impulsivity? (2) Are dependent animals more impulsive towards drug rewards than non-drug rewards? (3) Does dependence cause tolerance or sensitization to the effect of acute morphine on impulsivity? (4) Does withdrawal specifically contribute to impulsivity in dependent animals?

Chapter 2: The effect of morphine dependence on impulsivity

2.1 Transition to Manuscript 1

The initial experiments, Experiment 1a and 1b, serve to directly test the first and main question of the thesis: Can morphine dependence cause impulsivity? As opposed to similar human literature, here, animals can be randomly assigned to morphine dependent and naïve groups to establish a causal relationship. The experiments are designed in such a way as to eliminate several alternative explanations for a dependence-induced increase in impulsivity. In Experiment 1a, animals are tested 22.5h after their daily maintenance dose when all morphine would have been eliminated from their system. This removes the possibility that the acute effects of morphine are responsible for the observed changes in impulsivity. In Experiment 1a, a group of rats was fed ad libitum to mirror the decrease in motivation to acquire sucrose reward observed in dependent rats. This manipulation was to control for the possibility that decreased motivation to acquire sucrose rewards in dependent rats was solely responsible for changes in impulsivity. Finally, in both experiments employing naïve control groups eliminated the possibility that observed differences in impulsivity are caused by non-specific effects or experimental artifacts.

2.2 Manuscript 1: The effect of morphine dependence on impulsive choice in rats

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Abstract

Rationale: In the human opiate dependent population, the most consistently reported deficit in executive functioning is impulsivity. Previous research has shown that acute and chronic exposure to drugs of abuse can increase impulsive choice; however the extent to which opiate dependence contributes to increased impulsivity has not been examined.

Methods: We assigned rats randomly to either a dependent group that received a nightly 30mg/kg subcutaneous dose of morphine, or a morphine-naïve group that received a nightly saline injection. Delay discounting of a sucrose reward was examined in rats prior to initiation of the dosing regimen, 22.5 h after the daily maintenance dose, and after a 14-day abstinence period.

Results: The groups did not differ at baseline but rats showed accelerated delay discounting while dependent on morphine. After withdrawal from morphine, in previously dependent rats delay discounting was not significantly different from that of naïve rats. Since dependent rats also showed reduced motivation to acquire the sucrose reinforcer we performed a separate experiment to test whether such a decrease in motivation could cause an increase in impulsivity. We found that food-deprived rats switched to a free feeding diet did not differ in delay discounting from rats maintained at 85% of free feeding weight.

Conclusions: An increase in impulsivity can result from physical dependence on morphine, and cannot be attributed to changes in motivation to acquire sucrose-reinforced responses.

Keywords: Delay Discounting · Morphine Dependence · Impulsive Choice · Impulsivity · Opiate Withdrawal

Introduction

Contemporary models of drug dependence emphasize the contribution of deficits in executive functioning to the etiology of the disorder (George & Koob, 2010). Substance dependent individuals show impairments in working memory, attention, planning and impulsivity compared to controls (for review see Crews & Boettiger, 2009). It is hypothesized that these deficits facilitate the continued use of drugs despite negative personal, social and vocational consequences. In the opiate dependent population, impulsive choice is the most consistent and frequently reported deficit (de Wit, 2009; Pau, Lee, & Chan, 2002; Perry & Carroll, 2008). Impulsive choice or impulsivity can be defined as an exaggerated preference for small immediate rewards over larger delayed ones and can be measured in humans and animals using a delay discounting (DD) paradigm. In this paradigm subjects are given a series of choices between a small immediate reward and a larger reward at various delays in order to determine the rate at which the subjective value of a reward decays as a function of time until its delivery. The more impulsive an individual the faster the rate at which they discount delayed rewards. In the context of drug dependence, it is possible that the immediacy of the rewarding effects of drugs may increase their desirability to impulsive individuals when compared to deferred pro-social rewards (Bickel & Marsch, 2001). Likewise, accelerated discounting may reduce the deterrent effect of the delayed negative consequences of drug dependency (Bickel & Marsch, 2001). In such a way impulsivity may promote continued use and abuse despite negative consequences

In the human literature, individuals dependent on prescription opioids or heroin show accelerated DD of monetary and drug rewards when compared to drug naïve individuals (Kirby, Petry, & Bickel, 1999; Madden, Petry, Badger, & Bickel, 1997). This interaction seems to be modulated by the level of opioid deprivation such that opioid withdrawal further increases the rate of discounting of delayed reward (Giordano et al., 2002). These findings support a relationship between impulsivity and opiate dependence. However, the nature of this interaction is unclear. It is possible that impulsivity may be a risk factor for opiod addiction; that is, impulsivity predates and predicts drug use and

abuse (for review see Perry & Carroll, 2008). It is also feasible that the physiological and psychological changes that accompany drug dependence result in a state of increased impulsivity. In other words, opiate dependence may cause impulsivity.

As it pertains to non-opioid drugs of abuse, experiments in animals have supported both hypotheses. Chronic cocaine exposure has been shown to increase impulsivity in DD paradigms in rats during the dosing regimen (Paine, Dringenberg, & Olmstead, 2003), and 3-months after exposure (Simon, Mendez, & Setlow, 2007). Similar results have been shown following chronic nicotine (Dallery & Locey, 2005) and methamphetamine (Richards, Sabol, & de Wit, 1999) exposure. On the other hand, Anker and colleagues (2009) showed that high impulsivity rats, as determined by a DD procedure, acquired IV cocaine self-administration faster than low impulsivity rats. A similar study showed that rats bred for high impulsivity self-administer more alcohol than rats bred for low impulsivity (Poulos, Le, & Parker, 1995).

To date, only one study has examined the effect of chronic opiate exposure on DD in animals. Harty and colleagues (2011) found that immediately after cessation of a 10-day, 2mg/kg per day heroin dosing regimen, DD in heroin-sensitized rats was not significantly different from saline controls. This result suggests that chronic opioid exposure is not sufficient to produce protracted increases in impulsivity. However, even though rats in this experiment were sensitized to heroin, the doses administered may not have been large enough to produce physical dependence (Tjon et al., 1995). Previous studies have shown that drug-induced impulsivity changes may be transient (Paine, et al., 2003) and that high dose regimens produce impulsivity (Paine, et al., 2003) whereas low dose regimens may not (Winstanley et al., 2007). In addition, the opiate literature shows that behavioral changes that accompany dependence – for example patterns of drug seeking and reinstatement – can be dissociable from behavioral changes resulting from opiate sensitization (Lenoir & Ahmed, 2007). In humans, the link between impulsivity and opioids has been found in samples of individuals who were currently or previously dependent on opioids. Thus, to properly model the effect of human opiate addiction on impulsivity, it is necessary to induce dependence in subjects. We have, therefore, re- examined the changes in impulsive choice in a DD paradigm in rats before exposure to morphine, during opioid dependence and after withdrawal. In addition, we investigated whether decreased

motivation to acquire sucrose-reinforced responses could account for DD changes observed in morphine dependent rats. We report that inducing physical dependence in rats will cause an increase in impulsive choice that is ameliorated after a period of abstinence. This shift in DD is dissimilar from changes in DD caused by decreased motivation for sucrose.

Methods

Experimental Design Overview

Two experiments were conducted; Experiment 1a tested the effects of morphine dependence on DD; and Experiment 1b tested the effects of motivation for sucrose on DD. In Experiment 1a (figure 1a), baseline DD curves were determined, and then rats were randomly assigned to either a dependent group that received a nightly 30mg/kg subcutaneous dose of morphine, or a naive group that received a nightly saline injection. DD were then redetermined during the dosing regimen (22.5h after nightly injections), and again after a 14-day abstinence period following withdrawal precipitated with 1mg/kg naloxone. In Experiment 1b (Figure 1b), baseline DD curves were determined while all rats were maintained at 85% free feeding weight, and then rats were randomly assigned to either a "free-fed" group that was given unrestricted access to food, or a control group that continued to be maintained at 85% free feeding weight. DD curves were then redetermined 14-day after the change in feeding regimen.

Animals

This research was reviewed by the Animal Ethics Committee of McGill University and carried out in accordance with the guidelines of the Canadian Council on Animal Care.

Subjects were 23 male Long Evans rats received at weights between 150g and 225g (Charles Rivers Laboratories, Montreal, Que, Canada). Rats were individually housed in a colony maintained on a 12h light: 12h dark light cycle with (light phase: 7am to 7pm). After

7 days of acclimatization/ handling, food access was restricted to maintain rats' weight at 85% of free-feeding values.

Operant Conditioning Apparatus

Testing took place in clear Plexiglas boxes (29.5cm wide, by 28cm deep, by 27.5cm high). One wall of the box had metal siding with two metal levers (Coulbourn Instruments, Allentown, PA) mounted 3cm apart, 6.5cm above the metal rod floor. Beneath each lever was a small metal sipper cup. The sipper cup had an overflow capacity of approximately 200 µl. Liquid rewards were supplied to the sipper cup using an automated syringe pump (Model 22, Harvard Apparatus, St. Laurent, Que., Canada) at a flow rate of 100µl per second. Above each lever was a metal panel containing 3 small LED stimulus lights (Coulbourn Instruments, Allentown, PA). Each operant conditioning box was housed in a sound- and light- attenuating chamber (65cm by 50cm by 52cm) that contained a small house light 41cm above the floor (Coulbourn Instruments, Allentown, PA). Programming of events and recording of data was controlled by a personal computer.

Nociceptive Testing Apparatus

An Isotemp 3016 (Fischer Scientific, Ottawa, Ont., Canada) circulation bath was used to heat water to a temperature of 54 +/- 0.1˚C. Latencies were timed using a foot-pedal operated Timer (Model 54519-A, Lafayette Instruments, Lafayette, IN)

Delay Discounting Procedure

Training

The DD paradigm was modified from Mar and Robbins (2007). Rats were first trained to drink 20% sucrose solution from both sipper cups. Sucrose liquid (150µl 20% sucrose) was presented at random to each sipper cup. Rats were then trained to press both levers on a fixed ratio one (FR-1) schedule of reinforcement. Only one lever was present during each

session (50 trials of 150 µl rewards). Next, both levers were made available during sessions (50 trials), but only one lever was active during each trial. Between trials the active lever changed randomly. The active lever was signaled by illumination of the corresponding cue light.

When rats were pressing both levers reliably they were trained to discriminate the size of rewards on a discrete trial procedure. Reward size discrimination and DD sessions had the same structure. Each daily session contained 5 blocks of 14, 55s trials. Each block began with 4 forced trials in which only the left or right lever (2 trials on each, randomly distributed) was active. The active lever was signaled by illumination of the corresponding cue light. On the 10 free choice trials that followed, both levers were active and both cue lights were illuminated. A response on either lever caused the cue lights to be extinguished and a liquid reward to be delivered in the sipper cup under the selected lever after a programmed delay. The house lights were extinguished 35s after trial initiation for a time out period of 20 s. If the rat had not responded in the 35s choice period then the cue lights extinguished simultaneously with the house lights and the trial was scored as an omission. Immediately after the time out period, the house lights and the appropriate stimulus lights were illuminated and the following trial began.

During reward size discrimination, pressing on one lever resulted in immediate delivery of a small reward (50µl 20% sucrose) and on the other resulted in immediate delivery of a large reward (150µl 20% sucrose). The location of the large and small reward (right versus left lever) remained constant within session but alternated randomly between sessions. Rats continued this discrimination task until they selected the large reward lever on at least 70% of free choice trials on 2 successive days – one of which the large reward was associated with the left lever, and for the other the large reward was associated with the right lever. After criterion was achieved rats were given 14 days of DD training. To assure that only rats that were competent at the task were included in the experiment, rats that exhibited no change in large reward preference regardless of delay were excluded. Five rats failed to meet performance criteria and did not participate in further testing. After baseline testing began, no further rats were excluded.

DD sessions had the same trial and block structure as reward discrimination training. Pressing on one lever resulted in the delivery of an immediate small reward (50µl

20% sucrose), and on the other lever resulted in the delivery of a large reward (150µl 20% sucrose) delivered after a specified delay. Delays remained constant within a block but increased between blocks in ascending order (0s, 5s, 9s, 16s, 30s). The location of the immediate and delayed lever remained constant for all DD sessions for each rat but was counterbalanced between rats.

Delayed discounting experimental testing

After training rats were given 5 days of baseline DD testing on the same procedure. Individual baseline curves were calculated by averaging proportion choice for the large reward (total large reward choices in choice trials/ total completed choice trials) at each delay for this 5-day period. Rats were then randomly assigned to dependent/naive groups (Experiment 1a) or control/free fed groups (Experiment 1b).

14-days after baseline testing, approximately 22.5-h after rats received their 5th 30mg/kg maintenance dose (Experiment 1a) or 14 days after dietary switch (Experiment 1b), a 6-day DD test period began. Day 1 was treated as re-acclimatization session, and thus not included in the final analysis. Each rat's dependence (Experiment 1a) or test (Experiment 1b) DD curve was determined using the remaining 5-day mean proportion preference for the large reward at each delay.

Additionally in Experiment 1a, 14-days after cessation of the dosing regimen, another 6-day DD test period began. Again the day 1 data was not included in the final analysis. Each rat's abstinence delay-discounting curve was determined using the remaining 5-day mean proportion preference for the large reward at each delay.

Free sucrose consumption task

Rats were transported in their home cages to the testing room and allowed to habituate for 1-hr. A sipper bottle containing 20% sucrose solution was placed in the home cage. Rats were allowed to freely drink from this bottle for one hour and five minutes at the time that DD testing would usually occur. During the acclimatization and test period their normal water bottle was not available. The total volume of sucrose solution consumed during this period was determined for each rat. The baseline-testing period consisted of 3 consecutive

testing days, wherein the first served as a training session. All other sessions consisted of 2 consecutive testing days. The mean sucrose consumption was determined by averaging the 2 testing days. Sucrose consumption was tested on the 3 days prior to initiation of the morphine-dosing regime (baseline), on the days following the 3rd and 4th 30mg/kg maintenance dose (dependence), on the 2 days following completion of the abstinence DD testing (abstinence).

Nociceptive and withdrawal testing

Analgesia and hyperalgesia were measured using a modified tail withdrawal test (Franklin & Abbott, 1989). Rats were allowed to acclimatize to the testing room for 30-min prior to testing. The test consisted of immersing the distal 5cm of the rat's tail into a 54°C warm water bath. Latency from immersion to withdrawal (or sufficient squirming to indicate intention to withdraw the tail) was measured to the nearest 0.1s. The upper limit of withdrawal latency was 10s, after which the rat's tail was removed from the water bath by the experimenter. Each testing session consisted of 3 test trials at 5-min intervals. The first trial served as an acclimatization trial and was not analyzed. The mean withdrawal latency was determined by averaging the final 2 trials. Baseline drug-free latency was determined 1 day before initiation of the morphine-dosing regimen. Morphine analgesia was determined 60 min after the initial 10mg/kg dose and 60 minutes after 10mg/kg morphine as part of the 5th 30mg/kg maintenance dose (the remaining 20mg/kg was given immediately following the pain test). Data were represented as percentage of maximal possible effect (%MPE) using the equation:

(1)%MPE = (upper limit – test latency) *100 / (upper Limit – baseline latency)

Withdrawal signs and hyperalgesia were assayed the day after dependence testing was completed. Fifty min after the final (30mg/kg) maintenance dose, dependent rats and controls were placed in a clear Plexiglas container (dimensions 24cm wide, by 46cm deep, by 20cm high) for 10 minutes to acclimatize. Rats then received a 1mg/kg subcutaneous dose of naloxone. Over the 10-minute period following injection, the number of

occurrences of the following signs was scored for each rat; rearing, wet dog shakes, teeth chattering and writhing. At the 10-minute mark, rats were tested for presence or absence of; scream on touch, piloerection and ptosis. One hour thereafter naloxone tail withdrawal test of nociception was administered. Hyperalgesia was presented as percentage of baseline latency (%baseline). The weight change 10-min and 24-h after naloxone injection was recorded.

Drugs and dosing regimens

Morphine sulphate (Sigma-Aldrich, St. Louis, MO) was dissolved in 0.9% saline. Injections were administered subcutaneously by the experimenter in a volume of 1mL/kg. Delay-discounting testing occurred in a drug-free state, approximately 22.5-h after the previous daily maintenance dose. Approximately 30-min after the daily test session, morphine-treated rats received a maintenance injection of morphine while controls received an injection of saline. The maintenance dose was escalated over 7 days (10, 10, 15, 15, 20, 25, 30mg/kg Morphine/Saline). Thereafter, dependent rats were maintained on 30mg/kg morphine. The initial dose was administered 4-days after the final baseline-testing day. Once withdrawal was precipitated by naloxone rats received no further injections of morphine or saline.

Feeding regimen

For Experiment 1a, all rats were maintained at 85% of free feeding weight for the duration of the experiment. For Experiment 1b, after DD baseline testing, “free fed” rats were switched to unrestricted feeding for the remainder of the experiment. Control rats were maintained at 85% of free feeding weight throughout the experiment.

Data Analysis

Mean DD curves were derived for all groups at each relevant experimental time point. In addition, area under the curve (AUC) was used to measure discounting adjusted for any

differences in y-intercept. AUC is a theoretically neutral method used to measure rate of discounting. It is similar to the commonly used discounting rate parameter (k), except that it requires no assumptions about the mathematical form of DD function (Myerson, Green, & Warusawitharana, 2001).

The method used to calculate AUC was derived from Myerson, et al. (2001). In brief, each data point was expressed as a proportion of the maximal delay and proportion preference was represented as a proportion of the zero delay preference (ZDP) using these equations:

(1) Normalized Delay = (Maximum delay – delay)/ (maximum delay)

(2) Normalized Preference =

(ZDP – proportion preference for large reward$_{delay=n}$)/ ZDP

Normalized DD curves plotted normalized preference against normalized delay. Normalized curves were subdivided into a series of trapezoids (Myerson, et al., 2001). The area of each trapezoid was calculated using the equation

$$\text{Area} = [(x_2-x_1)[(y_1+y_2)/2]$$

Where x_2 and x_1 are successive delays and y_1 and y_2 are the preference values associated with these delays. Test areas were subtracted from baseline area to calculate Δarea. The negative of this measurement (-Δarea, hereafter referred to as AUC) represents the change in impulsivity from baseline due to the associated test manipulation.

Statistics

Statistics as indicated in text were calculated using SPSS version 16.0 (SPSS Inc). For both experiments a 3-way mixed model ANOVA was performed to analyze raw DD data with group (naïve versus control) as a between groups factor and delay (0,5,9,16 or 30) and state (baseline, dependence versus abstinence or baseline versus test) as repeated

measures. Significant 2 and 3-way interactions were observed for both Experiment 1a and 2, thus simple main effect analysis was used to compare the effects of delay (repeated measure) and group (between groups) at each level of state (For Experiment 1a: at baseline, during dependence and after abstinence. For Experiment 1b: at baseline and during test). Zero delay preference, AUC, response latencies, trial omissions and sucrose consumption were analyzed using a 2-way mixed model ANOVA with group as an independent factor and state as a repeated measure. Post-hoc tests were performed as noted, using Bonferroni correction. Withdrawal measures were compared using simple t-tests. Non-parametric tests were used when data sets did not meet the homogeneity of variance assumption for ANOVA.

Results

Experiment 1a

Delay Discounting

There were no significant differences between baseline discounting curves for rats that were to be made dependent rats and naive controls (group effect and interaction, NS), indicating that subjects in both groups were trained to a similar level of performance (Figure 2a). The preference for the larger reward at zero delay remained similar for drug experienced rats and controls at baseline (Figure 2a), after daily morphine dependence was induced (Figure 2b). After abstinence, formerly dependent rats appear to show an increased zero delay preference for the large reward (Figure 2c) but this effect was not significant (group effect and interaction, NS). Morphine dependent animals showed accelerated discounting compared to their morphine-naïve controls with the same amount of experience (group by delay interaction, $F(4,24)=9.70$, $P< .001$) (Figure 2b). This difference disappeared after they were withdrawn from morphine (group effect and interaction, NS) (Figure 2c). In addition, rats in the dependent and naïve groups had a equal number of omissions during baseline and abstinence DD testing (post hoc, NS), whereas

dependent rats showed a higher rate of omissions during dependence compared to controls (post hoc t=5.345, df=6, $p < .01$ (Bonferroni corrected). There were no group differences in response latency throughout testing.

AUC analysis confirmed the differences between groups and conditions in DD (group by time interaction, $F(1,6)=7.942$, $P< .05$). As can be seen in Fig 3, dependent rats were more impulsive than naïve controls during the dependence period (post hoc t=4.16, df=6, $p < .02$ (Bonferroni corrected)) but the groups did not differ in impulsivity after a 14-day abstinence period (post hoc t-test, NS).

During the baseline period the dependent and morphine-naïve groups did not differ in their consumption of sucrose solution during the 1-h free consumption tests (Median test, NS) (Figure 4). However during the period of morphine dependence the sucrose consumption of dependent rats was significantly lower than naïve rats ($p = .029$; Median test). After withdrawal from morphine, sucrose consumption in both groups was similar to that in the baseline period (Median test, NS).

Morphine Dependence Tests

After receiving 5 daily doses of 30mg/kg morphine, dependent rats showed reduced analgesia after 10mg/kg morphine in the tail flick test compared to their first experience with 10m/kg morphine. ($t=3.76$, $df=3$, $p < .05$) (Table 1). Dependent rats also exhibited significantly more withdrawal signs than controls after a 1mg/kg dose of naloxone. Specifically, dependent rats exhibited more writhing, rearing and teeth chattering than morphine-naïve rats. Only dependent rats exhibited ptosis, piloerection and scream on touch (median tests, all $p < .05$). Wet dog shakes were similarly infrequent for both groups (median test, ns). One hour after 1mg/kg naloxone, dependent rats were hyperalgesic compared to controls (median test, $p < .05$). In addition, dependent rats lost significantly more weight over the 24-hr period after naloxone injection (median test, $p < .05$).

Experiment 1b

Rationale

The increase in impulsivity observed in dependent rats in Experiment 1a was associated with a decrease in consumption of the sucrose solution used as reinforcement in the DD task. This raises the possibility that the change in impulsivity might result from a decrease in motivation to acquire sucrose-reinforced responses. To test whether a shift in motivation would alter DD performance we examined discounting in morphine naïve rats when they were switched from a deprivation schedule to free feeding.

Delay Discounting

As expected, rats that were switched to free feeding after baseline testing consumed less sucrose than control rats that were maintained at 85% of their free feeding weight throughout testing ($t=4.06$, $df=8$, $p < .005$).

There were no significant differences between free fed and control rats in DD at baseline (group effect and interaction, NS) (Figure 5a) but during free feeding the slopes of discounting curves was significantly lower for the free-fed group (interaction $F(4,32)=4.14$, $p<.01$) (figure 5b). Post-hoc analysis revealed that free fed rats had a reduced preference for large reward at zero delay compared to control rats ($t=3.34$, $df=8$, $p<.01$). When evaluated by area under the curve free-fed and food-restricted controls both exhibited the same degree of impulsivity as they had under baseline conditions (post hoc, NS). The groups did not differ in omissions or response latency (group effect and interaction, NS) though omissions and latencies increased from the baseline period to the test period in both groups (time effect $F(1, 8) = 6.232$, $P<.05$; $F(1,8) = 8.315$, $P<.05$ respectively).

Discussion

The present study shows that making rats physically dependent on morphine is sufficient to increase impulsivity in a delay-discounting paradigm. This increase in impulsive choice relative to naïve controls was observed while rats were dependent but 22.5h removed from their daily morphine doses. The effect was restricted to the period of dependence. After withdrawal and a subsequent 14-d abstinence period, previously dependent rats exhibited no more impulsive choice than controls.

The effects of repeatedly experiencing opioids depend on the context and the mode and timing of administration. In addition to the direct pharmacological effects and neural adaptations induced by opioids, there are Pavlovian conditioning effects arising from associations between the drug and the context of administration, and effects due to associations between the drug and behaviors under the control of reinforcement produced by the drug or modified by the drug (Hand & Franklin, 1986; Jacobs, Smit, de Vries, & Schoffelmeer, 2003; Stewart, de Wit, & Eikelboom, 1984). When opioids are administered by the experimenter, in a context remote from the testing situation, conditioned associations between the drug and reinforced behaviors or the context would not be activated by the testing situation. Consequently direct pharmacological effects would predominate. In our experiment, a daily 30mg/kg dose of morphine experienced in the home cage produced physical dependence, as evidenced by tolerance to morphine analgesia and significantly greater signs of naloxone-precipitated withdrawal than naïve controls. Rats made dependent on morphine in this way displayed significantly accelerated DD when compared to morphine-naïve controls. This suggests that impulsivity was a pharmacological effect induced by the chronic exposure to morphine.

Previous research has shown acute doses of morphine can cause accelerated DD (Kieres et al., 2004). In our study, the increase in impulsive choice in dependent rats was observed 22.5h after daily morphine administration. Morphine elimination curves in the rat are exponential with a half-life of 30-60 min (South, Edwards, & Smith, 2009; Stain et al., 1995) though the terminal half-life may be as long a 6.8 h (Ekblom, Hammarlund-Udenaes, & Paalzow, 1993). Nevertheless, even after lethal doses less than 1% of the peak

morphine concentration is detectable in plasma by 7 hours after administration (Bhargava & Villar, 1992; Strandberg et al., 2006). Consequently, the impulsivity induced by morphine dependence cannot be attributed to a direct effect of morphine, since no acute effects should be observed 22.5h removed from dosing. Instead, the observed increase in impulsivity is likely maintained by the state of dependence and/or the daily withdrawal resulting from an intermittent morphine-dosing regimen. Interestingly, all rats in our dependent group showed increased impulsivity (positive values for ΔImpulsivity). This would suggest that our dosing regimen was sufficient to increase impulsivity independent of baseline DD rates. However, we did not have sufficient statistical power to correlate changes in impulsivity changes with baseline measurements, and thus, determine whether a relationship exists between the two measures.

The human literature also suggests that opioid withdrawal may play a significant role in impulsivity. Giordano and colleagues, (2002) found that opioid dependent individuals were significantly more impulsive prior to their daily maintenance dose than when tested 2h after opiate administration. Likewise Kirby & Petry (2004), found that abstinent heroin users exhibited accelerated DD compared to controls, but were less impulsive than currently dependent users. Rats in our experiment should be experiencing acute withdrawal; rats made dependent using a similar dosing regimen exhibit withdrawal signs at 24hr but not 12hr removed from their maintenance dose (Cooper, Truong, Shi, & Woods, 2008). Thus, our experiment suggests that pharmacological effects of morphine play a large role in the increase in impulsivity observed during withdrawal.

It should be noted that the increase in impulsivity was transient; dependent rats retested after naloxone-precipitated withdrawal and a 14-day abstinence period, showed equivalent DD to controls. This suggests that the underlying mechanism responsible for the change in impulsive choice may not be long lasting. The lack of an extended effect of morphine dependence on DD also supports the inference that either the state of dependence and/or some symptom/s of withdrawal are causing impulsivity. Rats return to baseline levels of impulsivity when they are no longer physically dependent and do not exhibit symptoms of withdrawal. However, further experiments are needed to disentangle the relative contribution of dependence and withdrawal to opiate induced impulsivity. Additionally, our results are not inconsistent with Harty et al., (2011) who did not find an

effect of 2 mg/kg heroin on impulsivity. If morphine dependence and/or withdrawal cause a short-term increase in impulsivity, then a 2 mg/kg dose should not result in a change in impulsivity since it would not be expected to produce dependence and withdrawal (Tjon, et al., 1995).

Human cocaine users also show increased impulsivity compared to controls; however, this effect appears not to dissipate with abstinence (Kirby & Petry, 2004). This finding is consistent with animal experiments, which have found that increased impulsivity in rats chronically exposed to cocaine persists up to 3 months after cessation of the dosing regimen (Mendez et al., 2010; Simon, et al., 2007). Some studies report that cocaine has no protracted effects on DD, albeit with lower doses and after a shorter abstinence period (Paine, et al., 2003). It has been proposed that chronic impulsivity due to prolonged drug exposure may require a long "incubation period" (Setlow, Mendez, Mitchell, & Simon, 2009). A similar requirement for an incubation period to produce long-term behavioral sensitization has been shown previously for cocaine craving (Grimm, Hope, Wise, & Shaham, 2001). If the mechanism that underlies increased impulsivity following drug exposure is conserved between opioids and cocaine, then it is possible that we would observe accelerated DD in our dependent group after a longer abstinence period. Alternately, it is possible that chronic cocaine and opioids produce increased impulsivity through different mechanisms.

Finally, we observed that changes in free sucrose consumption in dependent rats mirror changes in impulsivity. Dependent rats show both increased impulsivity and decreased sucrose consumption during dependence, but not after abstinence. Thus, we conducted Experiment 1b to determine if a decrease in motivation to acquire the reward used in our DD task was sufficient to produce accelerated DD. We found that rats that were switched to a free feeding diet after baseline testing reduced their free sucrose consumption from baseline. This manipulation significantly altered delay discounting curves but did not significantly change AUC. The seemingly inconsistent findings can be reconciled when the decreased preference for the large reward at zero delay exhibited by the free fed rats is taken into account. The decrease in zero delay preference implies that free feeding reduced the relative subjective value of the large reward in comparison to the smaller reward as would be expected (Herrnstein, 1970). However, impulsivity in the

context of DD is the *rate* at which the subjective value of a reward decreases as a function of its time to delivery, that is, the *change* in relative preference for the large reward from zero delay to subsequent delays. When zero delay preference is equivalent between groups, this rate of change can be easily garnered from raw DD data by comparing preference for the large reward at each delay. However, between group differences in zero delay preference render this method of comparison hard to interpret since the y-intercept of DD curves are not equivalent between groups. Differences in zero delay preference are normalized in AUC analysis, which allows extraction of the rate of discounting regardless of y-intercept (Myerson, et al., 2001). Thus our data suggest that a change in motivation may affect the subjective value of the reward but does not affect the rate of delay discounting.

Theoretical models of DD posit that effects of differences in the relative subjective value of rewards are dissociable from the effects on rate of discounting (Ho, Mobini, Chiang, Bradshaw, & Szabadi, 1999; Myerson, et al., 2001). Likewise, previous research suggests that the neural systems that contribute to the processes of subjective value and delay discounting are divergent (Bezzina, Body, & Cheung, 2008; Adam C. Mar, Walker, Theobald, Eagle, & Robbins, 2011). Previous animal research addressing the effect of motivational state on DD has produced conflicting results. For example, in a t-maze DD paradigm, Eisenberger, Masterson, & Lowman, (1982) found that rats who had not been fed for 22hr were more impulsive than partially satiated rats. Conversely, in a lever task similar to ours, but with a much smaller ratio of free choice to forced choice trials, Bradshaw & Szabadi (1992) found that rats maintained at 90% of free feeding weight exhibited accelerated DD compared to rats maintained at 80% of free feeding weight. The former result suggests that increased motivation to obtain reward results in decreased impulsivity the latter result suggests the opposite. However, in our experiment, free feeding rats resulted in a decrease in the subjected value of the reinforcer but did not appear to a have a significant effect on the rate of DD. Moreover, on the basis of the raw discounting curves, the effect of reducing sucrose consumption by free feeding was towards reducing slope whereas decreased sucrose consumption associated with morphine dependence resulted in an increased slope. This shows that the decreased motivation to acquire sucrose reward in dependent rats cannot be the cause of the observed increase in impulsivity. Thus, our experiment provides strong evidence that morphine dependence can induce impulsivity in rats.

Figures

Measure	Units	Dependent± SEM	Naive± SEM	Sig.
Morphine Tolerance				
Tail-Flick (10mg/kg Mor – dep vs initial)	%MPE	14.95±15.99	83.33 ±10.93	**P< .05**
Precipitated Withdrawal Signs (1mg/kg Nal)				
Tail-Flick (1hr post-1mg/kg Nal)	%Baseline	-15.56±10.2	50.48±17.2	**P< .05**
Teeth-Chattering	Count	4.88±2.35	0±0	**P< .05**
Wet-Dog Shake	Count	.25±.25	0±0	NS
Writhing	Count	5±1.10	0±0	**P< .05**
Rearing	Count	13±2.33	30.25±3.3	**P< .05**
Ptosis	%present	75	0	N/A
Scream on Touch	%present	50	0	N/A
Piloerection	%present	75	0	N/A
Weight Change (10-min post-1mg/kg Nal)	mg	-3.5±1.71	0.5±0.29	NS
Weight Change (24-h post-1mg/kg Nal)	mg	-15.75±3.45	0±1.68	**P< .05**

Table 1 Indicators of morphine tolerance and withdrawal. The results of tests of tolerance to morphine analgesia and naloxone precipitated withdrawal in their appropriate units are presented ± SEM. Significance tests were not performed for %present measures.

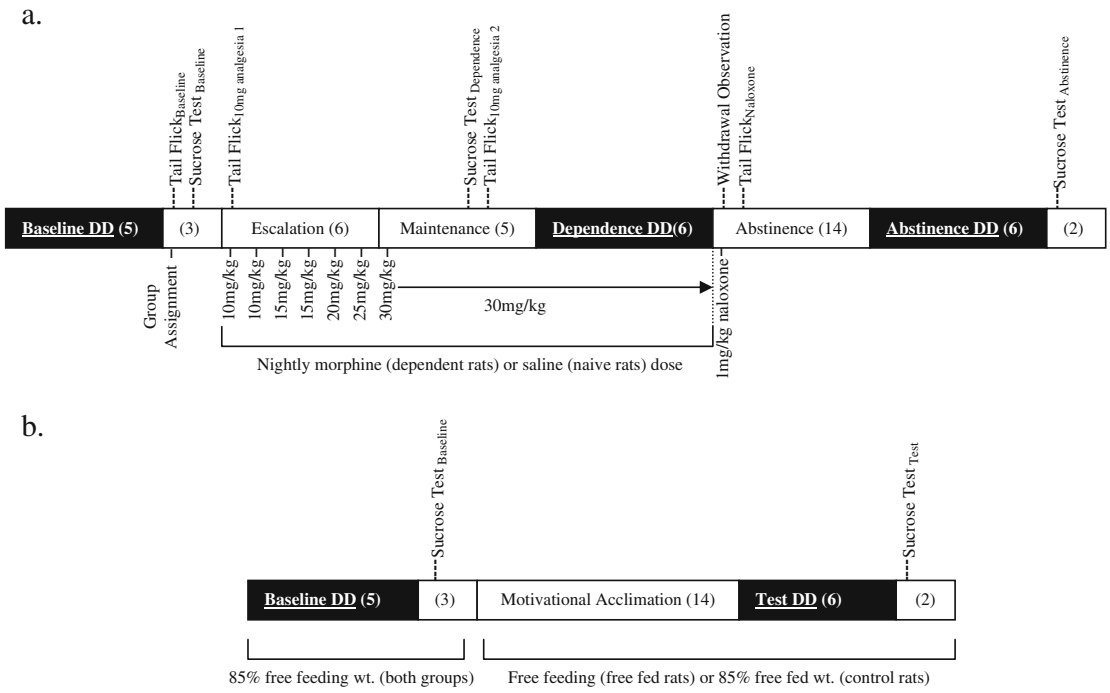

Figure 1 Schematic showing the timeline (a) Experiment 1a and (b) Experiment 1b. Filled boxes represent days in which rats were tested on the delay discounting task, unfilled boxes represent days they were not. The number of days spent at each interval is shown timeline in brackets. Auxiliary behavioral tests are represented above the timeline and dosing/feeding regimen is represented below the timeline.

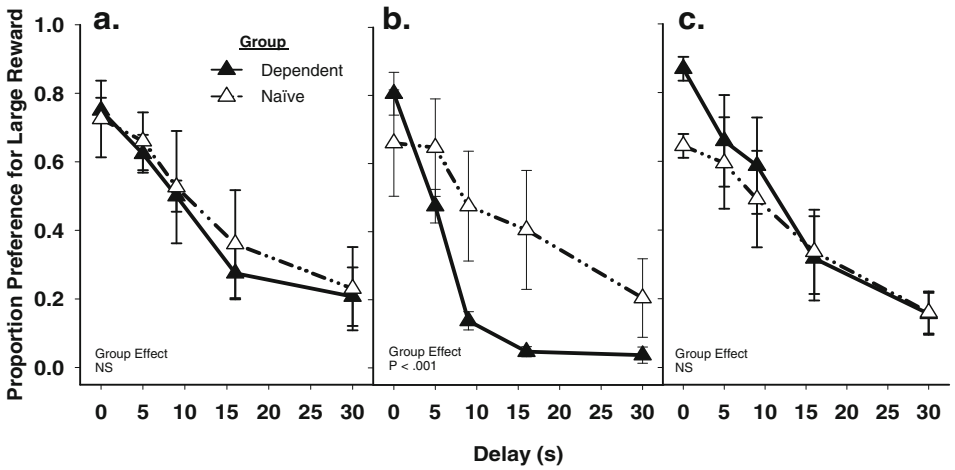

Figure 2 Delay discounting curves for dependent (filled triangles) and naïve rats (open triangles) at (a) baseline, (b) during the period of dependence and (c) after a 14-day abstinence period. The significance of the differences between groups at each stage (simple effect) is also shown.

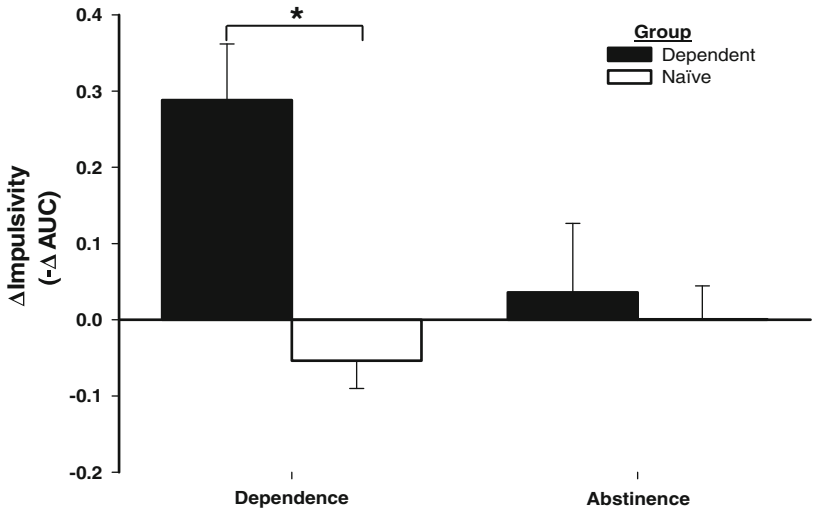

Figure 3 Changes in impulsivity from baseline for rats in the dependent (filled bars) and naïve groups (open bars) during the period of dependence (left) and after a 14-day abstinence period (right). Asterisks represent significance of t-test to a probability of $p<.02$ (Bonferoni corrected).

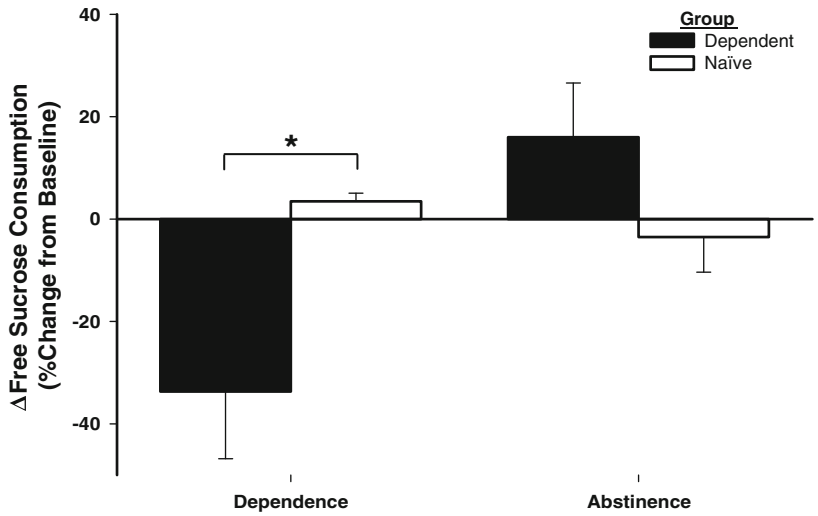

Figure 4 Change in free sucrose consumption for rats in the dependent (filled bars) and naïve groups (open bars) during the period of dependence (left) and after a 14-day abstinence period (right). Asterisks represent significance of median test to a probability of $p<.05$.

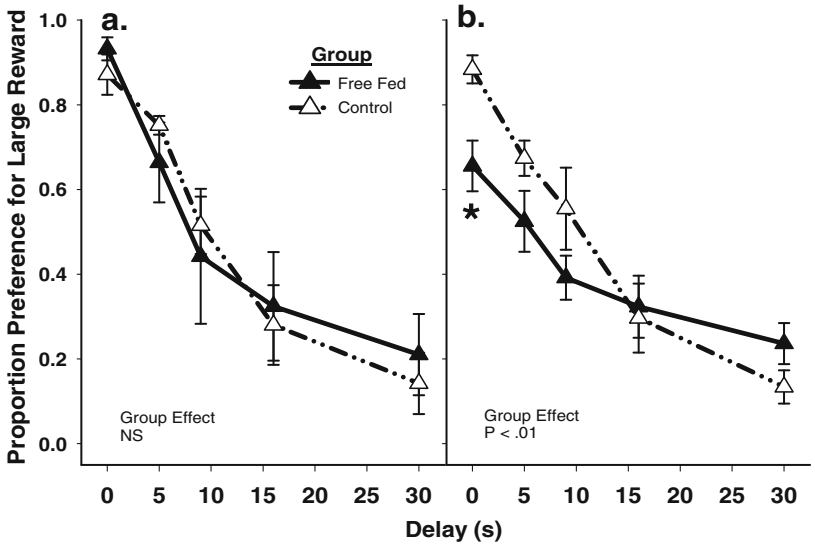

Figure 5 Delay discounting curves for rats is the free-fed (filled triangles) and control groups (open triangles) (a) at baseline, and (b) after free fed rats were switched to an free feeding diet and control rats were maintained at 85% free feeding weight. The significance of the differences between groups at each stage (simple effect) is also shown. Asterisks represent a significant difference ($p<.01$, t-test) in the preference for the larger reward at zero delay.

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Chapter 3: Reward-specific impulsivity in dependent rats: the effect of morphine rewards on delay discounting.

3.1 Transition to Manuscript 2

Manuscript one served two purposes. Firstly, it is the first published study to show that opiate dependence can cause increased impulsivity in rats. Secondly, it supports previous literature that suggests that motivational factors can cause changes in impulsivity in delay discounting paradigms. In the human literature, the effect of motivational factors on delay discounting in the opiate dependent population is most apparent when delay discounting of drug and non-drug rewards are compared; opiate dependent individuals show increased motivation to acquire drug rewards and significantly more impulsive choice thereof when compared to non-drug rewards (Cooper et al., 2010; G. C. Harris & Aston-Jones, 2002; Kirby et al., 1999; Kirby & Petry, 2004; Rouibi & Contarino, 2011) . Due to the aforementioned constraints of the human literature, it is unknown if this observed reward-specific impulsivity is restricted to the dependent population, and whether or not it is specifically caused by dependence. Manuscript two (Experiment 2a and 2b) aim to answer the question: Are dependent animals more impulsive towards drug rewards than non-drug rewards? In order to answer this question a novel method of operant oral self-administration of morphine had to be designed and validated. The results of this validation are contained in the first sub-experiment (Experiment 2a) of manuscript 2. The second sub-experiment (Experiment 2b) of manuscript two directly addresses the question of how drug and non-drug reward are discounted in both dependent and non-dependent animals. The specificity of the impact of drug rewards on impulsivity in dependent animals is

address through the use of a non-dependent control group and control non-drug solutions. In turn the Experiment 2b also serves to generalize the initial finding of increased impulsivity towards sucrose rewards to other types of rewards, specifically grapefruit juice rewards and morphine cocktail rewards.

3.2 Manuscript 2: Delay Discounting of Oral Morphine and Sweetened Juice Rewards in Dependent and Non-Dependent Rats.

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Abstract

Rationale: Opioid dependent humans are reported to show accelerated delay discounting of opioid rewards when compared to monetary rewards. It has been suggested that this may reflect a difference in discounting of consumable and non-consumable goods not specific to dependent individuals. Here, we evaluate the discounting of similar morphine and non-morphine oral rewards in dependent and non-dependent rats

Methods: We first tested the analgesic and rewarding effects of our morphine solution. In a second experiment, we assigned rats randomly to either dependent or non-dependent groups that, 30 minutes after daily testing, received 30mg/kg subcutaneous dose of morphine, or saline respectively. Delay discounting of drug-free reward was examined prior to initiation of the dosing regimen. We tested discounting of the morphine reward in half the rats and retested the discounting of the drug-free reward in the other half. All tests were run 22.5 h after the daily maintenance dose.

Results: Rats preferred the morphine cocktail to the drug free solution and consumed enough to induce significant analgesia. The control quinine solution did not produce these effects. Dependent rats discounted morphine rewards more rapidly than before dependence and when compared to discounting drug-free rewards. In non-dependent rats both reward types were discounted similarly.

Conclusions: These results show that morphine dependence increases impulsiveness specifically towards a drug reward while morphine experience without dependence does not.

Introduction

Impulsive choice or impulsivity is defined here as an exaggerated preference for small immediate rewards over larger delayed ones. Opioid dependent individuals are reported to be more impulsive than non-dependent and abstinent individuals, as evaluated by delay discounting tasks (DD) tasks (de Wit, 2009; Madden, Bickel, & Jacobs, 1999; Pau, Lee, & Chan, 2002; Perry & Carroll, 2008). However, in such tasks the level of observed impulsivity depends on the nature of the reward being discounted. Drug dependent individuals discount drug rewards at a faster rate than monetary rewards (Bjork, Hommer, Grant, & Danube, 2004; Coffey, Gudleski, Saladin, & Brady, 2003; Kirby & Petry, 2004; Madden, Petry, Badger, & Bickel, 1997). These findings suggest that when drugs are involved, dependent individuals make even more impulsive decisions than usual. Drug rehabilitation requires non-impulsive, long-term goal-oriented decision-making and thus, enhanced impulsivity towards drug rewards may make relapse more likely (Bickel & Marsch, 2001).

The literature on humans does not fully elucidate the nature of the relationship between impulsivity, dependence and drug rewards. In particular, the difference in modality of the rewards of interest, drugs and money, appears to affect discounting rate independent of other factors. The tendency of the general population to discount consumable goods far more quickly than money is well established (Estle, Green, Myerson, & Holt, 2007; Raineri, 1993). This effect appears to generalize to the drug literature; heavy cigarette users (10+ cigarettes a day) discount food and cigarettes similarly but more steeply than money (Odum & Baumann, 2007). Furthermore, the difference in discounting rates of drug and monetary rewards may not be specific to the dependent population; non-dependent individuals discount alcohol more quickly than monetary rewards but at the same rate as food (Estle et al., 2007). Whether discounting of illicit drugs follows a similar trend in non-dependent users is unknown. Thus, it is possible that the reported difference in discounting rates for opioid and monetary rewards in the dependent population may reflect a more general tendency towards accelerated discounting of consumable goods.

One problematic feature of studies that have reported similar discounting rates of drugs and consumable rewards is that the rewards being discounted are of different types.

Often these rewards are hypothetical (Estle et al., 2007), and/or they differ in their route of administration (e.g. cigarettes versus food Odum & Baumann, 2007). When both rewards are delivered orally, such as alcohol versus food, they differ in their non-alcoholic components (Estle et al., 2007). These experiments do not evaluate, for comparative purposes, the discounting of the most suitable control: the alcohol-free vehicle. As a result these studies do not clarify whether the drug content of a reward within itself contributes to observed impulsivity in dependent individuals.

Similarly, a few studies have examined discounting of cocaine (Woolverton, Myerson, & Green, 2007), PCP (Newman, Perry, & Carroll, 2008) and remifentanil (Maguire, Gerak, & France, 2013) in monkeys, and cocaine in rats (Perry, Nelson, Anderson, Morgan, & Carroll, 2007). These studies demonstrate that animals show delay discounting of drug rewards. However, because these studies do not directly compare discounting of drug and non-drug rewards it is hard to determine the extent to which discounting is affected by the type of reward. We have, therefore, examined the relative discounting of similar morphine and morphine-free rewards in dependent and non-dependent animals. We selected an opioid as the drug reward for several reasons. Firstly, opioid dependence manifests physiologically, and can be assayed by the physical withdrawal after discontinuation of a dosing regimen (Koob, 2006). Secondly, the daily dosage needed to induce dependence in rats is high (usually at least 10mg/kg for several days), and thus rats can have repeated exposure to morphine without becoming severely dependent (Frumkin, 1974). Like recreational drug users, rats can thus self-administer morphine rewards and remain as non-dependent – albeit morphine-experienced – controls (Mucha, Kalant, & Linseman, 1979; Nicholson, White, & Duncan, 1998). Finally, morphine can be self-administered by rats orally, usually through incorporation into a sweetened buffer solution (Hadaway, Alexander, Coambs, & Beyerstein, 1979). This route of self-administration models a large portion of the human dependent population since prescribed oral-opioids are the most frequently reported abused opioids (Mendelson, Flower, Pletcher, & Galloway, 2008). Once tampering is taken in account, these drugs are still frequently (estimated between 30% and 76% depending on the opioid) orally administered in the dependent population (Butler et al., 2013). Oral administration is particularly useful in the context of this experiment. There are no known non-drug intravenous reinforcers and comparing food rewards to opioids

administered by the more commonly used intravenous method results in a mismatched between rewards for route of administration and type. Using an oral morphine reward facilitates the use of sweetened buffer sans morphine as a non-drug reward matched with the morphine reward for type, vehicle and route of administration.

The low gastrointestinal (GI) bioavailability of morphine and its bitter taste has discouraged the use of oral morphine rewards in DD tasks in rats. The required volumes of low concentration morphine solution are too large to be ingested within the short time course of the typical operant task. Using grapefruit juice as a vehicle can circumvent the low GI bioavailability. Grapefruit juice contains quinidine, a P-glycoprotein inhibitor that increases the absorption of morphine in the GI tract thereby more than doubling the bioavailability of morphine (Okura, Morita, Ito, Kagawa, & Yamada, 2009; Okura et al., 2008).

Our first experiment validated the use of sweetened grapefruit morphine solution as a pharmacological active morphine reward in a two lever operant paradigm by testing: (1) Rat choice of this solution over a similar drug-free sweetened grapefruit solution (2) The antinociceptive effect of its ingestion. To verify that results were not solely attributable to the bitterness of the morphine solution a control group received bitter quinine in solution in lieu of morphine. The second experiment evaluated DD of these morphine and drug-free solutions in dependent and non-dependent rats.

Methods

Subjects

This research was reviewed by the Animal Ethics Committee of McGill University and carried out in accordance with the guidelines of the Canadian Council on Animal Care.

Subjects were 57 male Long Evans rats received at weights between 150g and 175g (Charles Rivers Laboratories, Montreal, Que, Canada) and began training at ages between 7 and 9 weeks. 14 rats were used in Experiment 2a, and a different group of 41 experimentally naïve rats were used in Experiment 2b. Rats were individually housed in a

colony maintained on a 12h light: 12h dark light cycle (light phase: 7am to 7pm). After 7 days of acclimatization/ handling, food access was restricted to maintain rats' weight at 85% of free-feeding values, adjusted over the duration of the experiment to reflect their natural growth curve.

Operant Conditioning Apparatus

Testing for both experiments took place in clear Plexiglas boxes (29.5cm wide, by 28cm deep, by 27.5cm high). One wall of the box had metal siding with two metal levers (Coulbourn Instruments, Allentown, PA) mounted 3cm apart, 6.5cm above the metal rod floor. Beneath each lever was a small metal sipper cup with an overflow capacity of approximately 200 µl. Liquid solutions were supplied to the sipper cup at a flow rate of 100µl per second using an automated syringe pump (Model 22, Harvard Apparatus, St. Laurent, Que., Canada). Above each lever was a metal panel containing 3 small LED stimulus lights (Coulbourn Instruments, Allentown, PA). Each operant conditioning box was housed in a sound- and light- attenuating chamber (65cm by 50cm by 52cm) that contained a small house light 41cm above the floor (Coulbourn Instruments, Allentown, PA). Programming of events and recording of data was controlled by a personal computer.

Nociceptive Testing Apparatus

An Isotemp 3016 (Fischer Scientific, Ottawa, Ont., Canada) circulation bath was used to heat water to a temperature of 54 +/- 0.1˚C. Latencies were timed using a foot-pedal operated timer (Model 54519-A, Lafayette Instruments, Lafayette, IN)

Operant Response Training

Operant task training and reward size discrimination

Prior to the experimental testing, rats in both experiments underwent operant training and reward size discrimination; the procedure for which were identical to those previously

reported by Harvey-Lewis (2012). In brief, animals were first trained to drink 150 µl of 20% sucrose solution presented in sipper cups. Following this rats were trained to press each of two levers that resulted in delivery of this solution to sipper cups beneath, on continuous reinforcement schedule. Then, in reward size discrimination, rats were trained to discriminate between pressing a lever that resulted in delivery of a small reward (50µl 20% sucrose) and one that resulted in delivery of a large reward (150µl 20% sucrose). Training continued until rats selected the large reward lever on at least 80% of free choice trials on 2 successive days – for one of which the large reward was associated with the left lever, and for the other the large reward was associated with the right lever. Rats (n=2) that did not reach this criterion after 16 sessions were excluded from further testing.

Operant Task Structure

Daily sessions in both experiments consisted of 5 blocks of 14, 55s trials. Each block began with 4 forced trials (2 trials on each lever in random order) in which each lever was programmed to deliver a solution of a particular size. The active lever was signaled by illumination of the corresponding cue light. The 4 forced trials were followed by 10 free choice trials wherein both levers were active and both cue lights were illuminated. A response on an active lever caused the cue lights to be extinguished and the associated reward to be delivered in the sipper cup under the selected lever after a programmed delay. The house lights automatically extinguished 35s after trial initiation for a time out period of 20 s., thus all trials lasted exactly 55s. If the rat had not responded in the 35s choice period then the cue lights extinguished simultaneously with the house lights and the trial was scored as an omission. Immediately after the time out period, the house lights and the appropriate stimulus lights were illuminated and the following trial began.

Determination of Dependence State

The effectiveness of morphine self-administration was tested by morphine's analgesic effect. The extent to which self- or experimenter-administered morphine resulted in dependence was verified by assaying signs of withdrawal. Analgesia and hyperalgesia were

measured using a modified tail withdrawal test (Franklin & Abbott, 1989). Rats were acclimatized to the testing room for 30-min prior to testing. The distal 5cm of the rat's tail was immersed into a 54^{o}C warm water bath. Latency from immersion to withdrawal was measured to the nearest 0.1s. Infrequently (< 5% of total trials), rats exhibited aggressive squirming for > 1s without exhibiting tail-flick. In such instances their tail was removed from the water bath and this latency was recorded. The upper limit of withdrawal latency was 10s, after which the rat's tail was removed from the water bath by the experimenter. Each testing session consisted of 3 test trials at 5-min intervals. The first trial served as a warm-up trial and was not analyzed. The final 2 trials were averaged to determine mean withdrawal latency. For Experiment 2a, the analgesic effect of drug consumed was tested 30min after completion of each of the test days during the initiation period. The initial test day of the initiation period (0mg/mL quinine/morphine) served as the baseline measure for the task. For Experiment 2b, drug-free baseline latencies were determined 1 day before initiation of the morphine-dosing regimen. Additionally, morphine analgesia was determined 60 min after the initial 10mg/kg dose, and 60 minutes after 10mg/kg morphine as part of the 5th 30mg/kg maintenance dose (the remaining 20mg/kg was given immediately following the pain test). Data were represented as percentage of maximal possible effect (%MPE) using the equation:

(1)%MPE = (upper limit – test latency) *100 / (upper Limit – baseline latency)

30 minutes after completion of the final testing session in Experiment 2a, and one day after test DD was completed in Experiment 2b withdrawal signs (including hyperalgesia) were assayed using a procedure derived from (Bläsig, Herz, Reinhold, & Zieglg nsberger, 1973). Fifty minutes after SA testing was completed (Experiment 2a) or the final (30mg/kg) maintenance dose (Experiment 2b), rats were placed in a clear Plexiglas container (dimensions 24cm wide, by 46cm deep, by 20cm high) for 10 minutes to acclimatize. Rats then received a 1mg/kg naloxone subcutaneously. Over the 10-minute period following injection, the number of occurrences of the following signs was scored for each rat; rearing (a complete transition from standing on all fours to standing on only the hind paws and back), wet dog shakes, teeth chattering and writhing. At the 10-minute

mark, rats were tested for presence or absence of; scream on touch, piloerection and ptosis. Scoring was done by an experimenter who was blind to the group designation of each rat using operational definitions provided in (Bläsig et al., 1973) or noted above. One hour after naloxone, the tail withdrawal test of nociception was administered. Hyperalgesia was presented as percentage of baseline latency (%baseline). The weight change 10-min and 24-h after naloxone injection was also recorded.

Procedure

Morphine Oral Self-Administration – Experiment 2a

After criterion was achieved in size discrimination training, rats were randomly assigned to receive morphine in 20% sucrose/10% grapefruit juice vehicle as a reward or quinine in 20% sucrose/10% grapefruit juice vehicle as their drug reward. Rats first received an acclimatization session where they pressed levers to obtain 150µl of the novel 20% sucrose + 10% grapefruit juice solution at both levers. Thereafter, Experiment 2a consisted of 3 stages (Figure 1). Stage 1 consisted of 7 sessions where rats chose between pressing a lever that resulted in delivery of 150µl of drug reward and pressing a lever that resulted in no programmed consequences. A fading procedure was selected so to expose rats to increasing bitter solutions in a fashion to maintain operant responding and ensure continued consumption of solutions. The drug concentrations were kept constant during each session but were systematically increased between sessions over days 1-7 as follows: Morphine: 0, 0.5, 0.5, 0.75, 1.0, 1.0, 1.0 mg/mL or Quinine: 0, 0.05, 0.05, 0.75, 0.1, 0.1, 0.1 mg/mL. The final quinine concentration was selected to match morphine bitterness based on pilot testing of free consumption rates of morphine/quinine + grapefruit juice + sucrose solutions in naïve rats. The spatial location of the active lever was kept constant for each rat but counterbalanced between rats.

On the day following completion of the initiation period rats began 9 sessions during which they could choose between the drug lever and the non-drug lever (stage 2), followed immediately by 11 sessions in which the location of drug and non-drug levers was

reversed (stage 3). During these test sessions the rat could press a lever that resulted in delivery of 150µl of drug solution (20% sucrose, 10% grapefruit juice vehicle + either 1 mg/mL morphine or 0.1 mg/mL quinine), or a lever that resulted in delivery of the non-drug solution (vehicle only). In stage 2 the spatial location of the drug-reward lever was the same as in the initiation period with the formerly inactive lever now serving as the non-drug-reward lever. For stage 3, these locations were reversed. Self-administration graphs were calculated by averaging the proportion of choices of the drug reward (total drug reward choices in completed choice trials/ total completed choice trials) for each session.

To confirm that rats experience of morphine did not induce dependence, 30 minutes after completion of the final testing session withdrawal signs (including hyperalgesia) were assayed using a procedure derived from (Bläsig et al., 1973).

Delay Discounting - Experiment 2b

After size discrimination criteria were achieved, rats in Experiment 2b were given 2 additional discrimination sessions where they chose between 50µl or 150µl of the non-drug solution containing 20% sucrose + 10% grapefruit juice. The location of the larger reward alternated on successive days and was counterbalanced between groups. This was immediately followed by 14 days of DD training (see Figure 2). In DD sessions pressing one lever resulted in the delivery of an immediate small reward (50 µl of ascribed solution), and the other lever resulted in the delivery of a large reward (150 µl of ascribed solution) after a specified delay. Delays remained constant within a block but increased between blocks in ascending order (0s, 5s, 9s, 16s, 30s). For DD training and baseline testing both large and small reward solutions was the non-drug vehicle. The location of the immediate and the delayed lever remained constant for all DD sessions for each rat but were counterbalanced between rats. In order to assure that rats included in our sample were competent in the DD task, rats (n=6) that exhibited no change in large reward choice with increasing delay were excluded from further testing. It must be noted that this sample could contain rats that are competent in the task but extremely delay insensitive. However, if no discounting is evident at the 30 sec delay these rats would be extreme outliers for the rat population.

After DD training, rats were given 5 days of baseline DD testing using the same procedure. Individual baseline curves were calculated by averaging the proportion choice of the large reward (total large reward choices in choice trials/ total completed choice trials) at each delay for this 5-day period. Rats were then randomly assigned to dependent or non-dependent group which was subdivided into drug and no drug groups (corresponding with the solution they would discount during the test period), for a total of 4 independent groups.

After baseline DD curves were determined, all animals received 9 days of self-administration training. The first 5 days thereof were identical to the first 5 days of initiation in Experiment 2a, except that lever location (left versus right) alternated each day for each rat. In the final 4 days of training, rats in Experiment 2b chose between either pressing a lever that resulted in delivery of 150µl of drug solution immediately, or pressing a lever that resulted in delivery of 50µl of the same solution. Lever location also alternated day-to-day for this period. Morphine maintenance began during, and overlapped fully with this period. Testing took place approximately 22.5hr after daily morphine/saline maintenance dose. Rats were then given an additional 4 days of DD training using the group specific solution (drug or non-drug) for both large and small rewards. This was followed by 5 days of DD testing using the same solution. Each rat's test DD curve for drug reward or retest DD curve for non-drug rewards was determined during this 5-day period. One day after test DD was completed, withdrawal signs (including hyperalgesia) were assayed .

Drugs and dosing regimens

Drug solutions for oral self-administration were made by dissolving either morphine sulphate (Sigma-Aldrich, St. Louis, MO) or quinine hydrochlorid dihydrate (Sigma-Aldrich, St. Louis, MO) in a solution of 20% (by weight) sucrose and 10% GFJ (Oasis) at concentrations noted in text. For Experiment 2b, injectable morphine solutions were made by dissolving morphine sulphate sulphate (Sigma-Aldrich, St. Louis, MO) in 0.9% saline. Injections were administered subcutaneously by the experimenter in a volume of 1mL/kg.

To induce dependence the maintenance dose was escalated over 7 days (10, 10, 15, 15, 20, 25, 30mg/kg Morphine/Saline). Thereafter, dependent rats were maintained on 30mg/kg morphine. The initial dose coincided with the first day of oral morphine self-administration. Delay-discounting testing occurred in a drug-free state, approximately 22.5-h after the previous daily maintenance dose. Approximately 30-min after the daily test session, morphine-dependent rats received a maintenance injection of morphine, while non-dependent animals received an injection of saline.

Data Analysis

For Experiment 2b, Mean DD curves were derived for all groups at baseline and during the test period. In addition, area under the curve (AUC) was used as a summary measure of discounting adjusted for any differences in y-intercept. AUC is a theoretically neutral method used to measure rate of discounting. It is similar to the commonly used discounting rate parameter (k), except that it requires no assumptions about the mathematical form of DD function(Myerson, Green, & Warusawitharana, 2001). Impulsivity in the context of DD is measured by the *rate* at which the subjective value of a reward decreases as a function of its time to delivery, that is, the *change* in relative frequency of choosing the large reward from zero delay to subsequent delays. When choice at zero delay is equivalent between groups, this rate of change can be easily garnered from raw DD data by comparing preference for the large reward at each delay. However, between group differences in the choice of large reward at zero delay can render this method of comparison hard to interpret since the y-intercept of DD curves are not equivalent between groups. AUC specifically addresses this shortcoming by normalizing differences in zero delay choice.

The method used to calculate AUC was derived from (Myerson et al., 2001), and detailed in full in Harvey-Lewis et al., (2012). In brief, each data point was expressed as a proportion of the maximal delay and proportion choice was represented as a proportion difference from the zero delay proportion choice using these equations:

(2) Normalized Delay = (Maximum delay – delay)/ (maximum delay)

(3) Normalized Proportion choice =

(proportion choice for large reward$_{delay=0}$ – proportion choice for large reward$_{delay=n}$)/ zero delay proportion choice)

The area underneath this normalized curve was calculated using the equation:

(4) Area = $[(x_2-x_1)[(y_1+y_2)/2]$

Where x_2 and x_1 are successive delays and y_1 and y_2 are the proportion choice values associated with these delays. Test areas were subtracted from baseline area to calculate Δarea. By convention we refer to the negative of Δarea (hereafter referred to as ΔAUC) to represent the change in impulsivity from baseline to test:

(5) ΔAUC = -1 * (Area$_{test}$ – Area$_{baseline}$)

Increases from baseline in the frequency of choosing the smaller-immediate reward are displayed as postive ΔAUC scores and represented above the x-axis whereas decreases are negative ΔAUC scores and represented below the x-axis.

Statistics

Statistics were calculated using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA) and Sigmaplot 11.0 (Systat Software Inc., Point Richmond, CA, USA). For Experiment 2a, self-administration and tail-flick test data were analyzed with a 2-way mixed model ANOVA with group (morphine v quinine) as a between groups factor and test session (for all test days) as repeated measure. In addition one-sample t-tests (one-tailed) were used to compare proportion choice of drug lever to chance (population mean = .50) for each group for each test day. Post-hoc testing (Tukey HSD corrected) was then used to compare the groups at each test day. For Experiment 2b, a 4-way mixed model ANOVA was performed to analyze raw DD data with state (dependent v non-dependent) and reward type (drug v drug-free) as a between groups factor and delay (0, 5, 9, 16 or 30) and test point (baseline v

test) as repeated measures. When significant 2 and 3-way interactions were observed, simple main effect analysis was used to compare the effects of delay (repeated measure) and reward type (between groups) at each level of state (dependent and non-dependent) separately for baseline and test sessions. Each groups' baseline AUC was compared to their own test AUC using the Wilcoxon sign-rank test, as this data did not meet the equal variance assumption for ANOVA. ΔAUC was compared between reward types within state using the Mann-Whitney test for the same reason. Withdrawal measures for both experiments were compared using non-parametric tests, as a subset of these data sets did not meet the homogeneity of variance assumption for ANOVA.

Results

Experiment 2a

Self-Administration

On the first day of the initiation period, rats whose drug solution was to contain morphine and those whose drug solution was to contain quinine did not differ (Tukey HSD, q = .642, p = .65) in the proportion of trials in which they pressed the lever that resulted in delivery of the novel vehicle solution (Figure 3a, session 1). Over the next 6 days, as the concentration of drug in solution increased from 0mg/mL to 1mg/mL in the morphine group and from 0mg/kg to 0.1mg/mL in the quinine group, both groups equally selected the drug reward lever over the inactive lever (Tukey HSD, q's = 1.345, 0.754, 0.0, 0.064, 0.032, 0.0 respectively, all p NS) (Figure 3a). For the duration of initiation training both groups selected the drug lever more frequently than expected by chance (T-test (df=6) all P< .05). When compared to rats receiving quinine solution, morphine consuming rats had longer tail flick latencies in post-sessions tail-flick tests (main effect of group, F (1,12) = 11.40, p < .01, group by session interaction and main effect of session, ns) (Figure 3b). Including forced choice trials; a mean daily volume of 8.9 and 7.2 ml of drug reward solution was

delivered to quinine and morphine rats respectively over the duration of the initiation period.

When rats were initially given a choice between the drug-free vehicle and a solution containing either 1mg/mL morphine or 0.1mg/mL quinine, there was no difference in the frequency with which rats selected their respective drug solution (Tukey HSD, $q = .546$, $p = .70$) (Figure 3c, session 1). As the free choice period continued, rats in the morphine group selected the drug reward in a greater proportion of trials than did rats in the quinine group (group by session interaction, $F(26,312)=1.858$, $P<.01$) (Figure 3c). Specifically, during sessions 5(Tukey HSD, $q = 2.761$, $p = .05$), 6 (Tukey HSD, $q = 4.205$ $p < .01$), 7 (Tukey HSD, $q = 3.628$, $p = .01$) and 9 (Tukey HSD, $q = 3.85$, $p < .01$) rats in the morphine group selected the drug reward significantly more often than rats in the quinine group. Moreover, with the exception of session 8 ($t(6)= .89$, NS), rats in the morphine group selected the morphine solution significantly more often than they selected the non-drug solution ($t(6) = 17.5, 9.7, 2.5, 4.3, 5.9, 6.6, 4.8, 5.7$, all $P < .05$.) In the first two days of stage 2, Rats in the quinine group selected the drug reward significantly more frequently than the drug-free reward on the first 2 days ($t(6) = 8.1, 6.4$, $P < .05$) but thereafter, did not show a preference for either lever (T-test (df=6) all $P> .05$). Including forced choice trials; a mean daily volume of 4.74 and 7.83 ml of drug reward solution was delivered to quinine and morphine rats respectively over the duration of the drug lever versus non-drug lever choice period.

The preference for the drug reward in the morphine group was re-established quickly after lever locations were reversed (Figure 3d). During the first 5 days of the reversal period, the morphine group's choice of the drug reward lever steadily trended upwards but did not differ significantly from the quinine group's choice for the drug reward lever during these days (Tukey HSD, $q = .995, .706, .305, .385, 1.504$, all p's NS). Thereafter, rats in the morphine group selected the morphine reward significantly more often than the quinine group on days 6 (Tukey HSD, $q = 3.146$, $p = .03$), 8 (Tukey HSD, $q = 2.81$, $p = .05$), 9 (Tukey HSD, $q = 4.94$, $p < .01$), 10 (Tukey HSD, $q = 3.93$, $p < .01$) and 11 (Tukey HSD, $q = 3.76$, $p < .01$) but not on day 7 (Tukey HSD, $q = 2.41$, $p = .09$). The morphine reward lever was selected significantly more frequently than the drug-free vehicle lever during sessions 7 through 11 ($t (6) = 2.3, 2.3, 3.2, 2.0, 3.7$, all $P < .05$). In contrast the quinine group chose vehicle reward in more than 50% of completed trials

from day 5 onward, though no significant preference for either solution was established (T-test (df=6) all P< .05). Including forced choice trials; a mean daily volume of 5.6 and 7.1 ml of drug reward solution was delivered to quinine and morphine rats respectively over the duration of the free choice spatial location reversal

Morphine Dependence Tests

In rats that had been receiving morphine, naloxone elicited significantly more writhing and teeth chattering and significantly less rearing than the quinine group (table 1;. MW Rank Sum, all $p < .05$). The groups did not differ in occurrences of burying and wet dog shakes and both groups had similar weight loss 10 minutes and 24hrs after injection (MW Rank Sum, all p's NS). No significant differences were seen in the scream on touch, piloerection and ptosis scores (Fischer Exact, all p NS); a single rat in the morphine group exhibited piloerection and there was no incidence of scream on touch or ptosis in either group. One hour after 1mg/kg naloxone, pain thresholds were similar in both groups (median test, p NS).

Experiment 2

Delay Discounting

There were no significant differences between the baseline discounting curves of the four groups, indicating that subjects in all groups were trained to a similar level of performance (main effect of group, reward type and interaction effects, NS). During the test period, reward type had a significantly different effect in dependent and non-dependent rats (reward type interaction, $F(4, 27) = 3.41$, $p = .01$) (Figure 4). Non-dependent rats discounted vehicle and the morphine solutions similarly (reward type by delay interaction, $F(4, 16) = .363$, $p = .83$) (Figure 4a) while dependent rats discounted the morphine solution more steeply than they discounted vehicle (reward by delay interaction, $F(4,14) = 4.955$, $p < .01$) (Figure 4b). Comparisons between dependent and non-dependent rats discounting of vehicle or drug reward during the test phase did not reveal significant differences, $F(4,30) = .957$ and 1.90 respectively, p NS). Of note, at zero delay we observed

a trend (Tukey HSD, q = 2.671, p = .07) for rats discounting vehicle to choose the large reward less frequently.

It can be seen in Figure 5 that the steeper discounting observed in the dependent drug group was also reflected in accelerated discounting from the pre-dependence baseline discounting of non-drug reward (timepoint by delay interaction, $F(4, 28) = 3.785$, $p = .02$). Dependent rats discounting vehicle and non-dependent rats discounting drug-solution both exhibited significantly flattened discounting from baseline (timepoint by delay interaction, $F(4, 28) = 7.191$, , $p < .01$ and $F(4, 32) = 4.728$, $p < .01$). The control non-dependent vehicle group did not exhibit any change in discounting from baseline values (timepoint by delay interaction, $F(4, 32 = 0.419$, p NS).

Change in area under the curve analysis confirmed that drug content of the reward had a significant effect in the dependent group (Mann-Whitney, $p = .03$) but not in the non-dependent group (Mann-Whitney, $p = .34$) (Figure 6). Dependent animals were significantly more impulsive toward morphine rewards, as evidenced by a significant decrease in AUC from their pre-dependence discounting of vehicle (Wilcoxon sign-rank test, $p= .05$). On the other hand, dependent animals discounted vehicle rewards similarly before and during dependence (Wilcoxon sign-rank test, $p= .31$). In non-dependent rats, AUC were unchanged from baseline whether discounting drug or drug-free rewards during the test period (Wilcoxon sign-rank test, both p NS).

The was a significant increase in omissions from 0.6% of trials to 4.0% of trials from baseline to test (main effect of timepoint, $F(1, 30) = 10.124$, $P < .01$) however omissions did not vary between groups at either time point (time point by group interaction, $F (3, 30) = 0.72$, P NS).

Morphine Dependence Tests

As evaluated by tail flick latencies, rats maintained on morphine showed decreased analgesic response to 10mg/kg morphine on the fifth day of their 30mg/kg/day dosing regiment compared to their first experience with 10m/kg morphine (Wilcoxon sign-rank test, $p< .01$) (Table 2). Dependent rats also exhibited significantly more withdrawal signs than non-dependent rats after a 1mg/kg dose of naloxone. Specifically, dependent rats

exhibited more writhing and tooth chattering, and less rearing (a negative sign of withdrawal) than non-dependent rats (Wilcoxon sign-rank test, all $p < .01$). Rats on morphine maintenance frequently exhibited ptosis and piloerection, and did so significantly more often than controls (Fischer exact test, all $p < .01$). Wet dog shakes, burying, and scream on touch did not significantly differ between morphine-maintained rats and non-dependent rats (all p NS). One hour after 1mg/kg naloxone, morphine-maintained rats were hyperalgesic compared to controls (median test, $p < .05$) and lost significantly more weight 10 minutes after naloxone injection (Wilcoxon sign-rank test, all $p < .01$).

Discussion

We found that rats will work to obtain a solution of 10% grapefruit juice, 20% sucrose and 1mg/mL morphine more frequently than a similar drug-free solution (10% grapefruit juice, 20% sucrose only) in an operant task. The volume of morphine solution consumed in this way was sufficient to cause significant analgesia, with only minor withdrawal signs which may indicate acute dependence (Harris and Gewirtz, 2005). When rats were administered nightly 30mg/kg morphine, they exhibited signs typical of a morphine withdrawal syndrome and showed significantly accelerated DD of this morphine solution reward compared to pre- and peri-dependence discounting of the drug-free solution. On the other hand, non-dependent rats did not show significant differences in discounting of the morphine and drug-free solution.

Home cage oral morphine consumption has previously been used to study the effects of environmental enrichment (Alexander, Coambs, & Hadaway, 1978), stress (Shaham, Alvares, Nespor, & Grunberg, 1992) and morphine dependence (Badawy, Evans, & Evans, 1982) but, the use of discrete oral morphine rewards in an operant task is a novel method of studying opoiod self-administration in rats. Experiment 2a validated the efficacy of a cocktail of 10% grapefruit juice, 20% sucrose and 1mg/mL morphine as a psychopharmacologically active morphine reward. Rats exhibited analgesia after consuming an average of 7.5ml of morphine solution/day. In 6 of 7 testing sessions that

occurred thirty minutes after SA, rats in the morphine group showed significant increases from baseline tail flick latency when compared to rats in the quinine group. The morphine solution still produced analgesia on the seventh day of consumption, showing that pharmacological effects were maintained after repeated exposure. Total volume of solution consumed was similar in both groups, showing that morphine, and not the other constituents shared by both solutions, is responsible for the observed analgesia. The increases in tail flick latencies was within the range (37-65% MPE) to be expected from a 20-30mg/kg p.o. dose of morphine buffered by grapefruit juice(Okura et al., 2008).

To establish the rewarding properties of the morphine solution we examined operant choice behavior in rats in 3 stages. During the first initiation stage, we allowed rats to choose between an active and an inactive lever and showed that rats would self-administer the oral morphine solution. Rats also orally self-administer a similarly bitter 10% grapefruit juice, 20% sucrose and 0.1mg/mL quinine solution. This suggests that the sucrose – used to mask the bitter-tasting morphine and acidic grapefruit juice combination – serves as a food reward to the weight-restricted rats, as suggested in previous literature (e.g. Hajnal, Smith, & Norgren, 2004).

In order to determine whether morphine contributes to the rewarding qualities of the bittersweet solution we gave rats a choice between equal quantities of morphine solution and similar drug-free solution. During this free choice stage of Experiment 2b, rats choose the drug solution significantly more often than the vehicle solution, and more often than rats given a choice between quinine and drug-free solutions. The preference for the morphine solution was established within 5 sessions, and was robust; rats chose the morphine 6.5 times more often than the drug-free solution at maximum. In contrast, increasing bitterness with quinine had little effect; rats were indifferent to the choice between quinine and quinine-free solution. This suggests that the morphine solution, despite its bitter taste, is more rewarding than the sweetened grapefruit juice vehicle.

In the third stage of Experiment 2a, we reversed the location of the levers associated with the drug and drug-free solution to show that rats were tracking the morphine solution. Rats quickly re-established their preference for the morphine solution at its new spatial location. Rats are known to use taste and associative spatial cues acquired during training to orient their choice behavior. Zellner and colleagues (1985) showed that oral

morphine consumption can result in rats associating the bitter taste of the solution with the positive hedonic effect, causing animals to prefer its taste over water. Thus, it is reasonable to conclude in this experiment, rats acquired a preference for the morphine solution's taste due to its positive pharmacological effects in spite of its bitterness.

The quantity of morphine consumed by rats over the course of Experiment 2a resulted in some minimal signs of physical dependence. When given a 1mg/kg dose of naloxone at the conclusion of self-administration training, rats in the morphine group showed increased incidence of writhing and wet-dog shakes albeit it at low frequency. However, rats in the quinine and morphine groups showed only a single occurrence of the 3 "presence/absence" signs which are particularly indicative of dependence (Mucha et al., 1979) and did not differ from morphine naïve animals in any of the other withdrawal signs. This limited profile of positive signs of dependency is consistence with evidence that even single exposures to morphine may induce an acute dependence state (Meyer & Sparber, 1977) (A. C. Harris & Gewirtz, 2005); but is not comparable to the physical withdrawal signs following chronic morphine dependence. Likewise, in Experiment 2b, rats that had only self-administration experience showed much weaker signs of dependence than rats that were maintained on 30mg/kg morphine daily. Indeed, we would not expect rats in Experiment 2a or the non-dependent group in Experiment 2b to exhibit strong physical dependence since they received daily doses between 20-30mg/kg p.o., that have bioavailability similar to 3-4mg/kg s.c. doses . Daily doses of 10mg/kg s.c. and greater are traditionally used to establish dependence (Tjon et al., 1995). Taken as whole, Experiment 2a shows that a morphine solution buffered by a grapefruit juice and sucrose mixture can serve as pharmacological active opiate reward in an operant self-administration task.

In Experiment 2b, we found that chronic morphine dependence altered the delay discounting of morphine and drug-free solutions. Dependent rats that discounted morphine solution during the test period did so at a significantly faster rate than dependent rats discounting drug-free solution. As shown by AUC analysis, dependent rats showed significantly accelerated discounting of morphine rewards compared to their baseline-discounting rate of drug-free solution. The difference in impulsivity is attributable to the single difference between the two solutions, that is, the presence or absence of morphine. Thus, morphine-dependent animals are more impulsive towards morphine

rewards than non-morphine rewards. Dependence did not have a significant effect on discounting of the drug-free solution; rats showed less steep discounting of the vehicle during dependence, and a trend towards decrease in the proportion of choice for large reward at zero delay. This finding was surprising, since we previously found that drug dependence significantly accelerated discounting of a pure sucrose solution (Bymaster et al., 2002). However, it is hard to directly compare the findings since different solutions were being discounted in the two experiments.

Non-dependent rats discounted morphine rewards and non-morphine rewards at a similar rate. As evaluated by AUC, discounting rates for both morphine and drug-free solutions in non-dependent rats during the test period did not significantly deviate from their baseline discounting rates of the non-drug reward. This suggests that morphine content of a reward does not affect the rate of discounting in non-dependent rats. Furthermore, it appears that a history of oral morphine self-administration is not sufficient to induce an increase in impulsivity in rats. Similarly, Harty and colleagues (Harty, Whaley, Halperin, & Ranaldi, 2011) found that low-dose i.v. heroin did not have a lasting effect on DD in rats.

As previously mentioned, it has been hypothesized that the difference in discounting of drug rewards and monetary rewards in the dependent population may reflect a non-specific difference in discounting between consumable and non-consumable rewards (Odum & Baumann, 2007). In our experiment, where both rewards are consumable and differ only in their drug content, we still observe a difference in discounting of drug and non-drug rewards in dependent rats. Unlike discounting of alcohol and monetary rewards in humans, the effect does not generalize to non-dependent animals (Estle et al., 2007). Thus, when oral rewards are identical except for the presence or absence of morphine, dependent rats are more impulsive towards the reward that contains morphine.

We suggest that the underlying motivational changes that accompany dependence may drive the observed accelerated delay discounting of drug rewards. Morphine dependence can selectively alter motivation so that increases in the incentive salience of drug rewards -- that is, the extent to which they are 'wanted' or valued-- can occur simultaneously with decreases in the salience of some non-drug rewards (Cooper, Shi, & Woods, 2010; G. C. Harris & Aston-Jones, 2002; Rouibi & Contarino, 2011). Koffarnus &

Woods (2011) showed that increased demand for cocaine rewards was associated with accelerated delay discounting. We observe a similar result, wherein the dependent morphine reward group was the only group to show significantly accelerated discounting compared to their non-drug baselines in either raw data or AUC analysis.

Morphine dependence and withdrawal can cause decreased appetite and motivation to acquire food rewards (Sanger & McCarthy, 1980). In the current experiment, two findings suggest that deceased motivation to acquire the non-drug reward in dependent animals is also contributing to the difference in discounting between dependent groups: (1) the drug-free solution DD curve for dependent animals is flattened compared to their baseline, and (2) during dependence we observed a trend towards decreased proportion of choice of the large reward at zero delay in the non-drug group. The latter finding specifically suggests a decrease in subjective value of the non-drug reward according to Herrnstein's Matching law (Herrnstein, 1970), and both findings mirror the effects on DD of decreasing motivation for sucrose by switching rats from a restricted diet to an ad libitum diet (Harvey-Lewis et al., 2012). This might explain the failure to find a difference between dependent and non-dependent animals in test discounting of drug reward in the raw data analysis. However, when the difference between groups in choice at zero delay is accounted for with AUC analysis, which allows extraction of the rate of discounting regardless of y-intercept , we do resolve a significant difference in discounting of drug rewards in the dependent group. Taken as a whole, our results suggest that differential discounting of drug rewards in the dependent population may result from a combined effect of increased motivation to acquire drug reward, and decreased motivation to acquire the non-drug solution. This would lead to a significant divergence in the relative value of drug and non-drug rewards, which is reflected in relatively accelerated discounting of drug rewards.

That the incentive salience of drugs and drug-related cues increases, and the incentive salience of non-drug rewards decreases during dependence is well documented in the human dependent population (for review see "Incentive-sensitization and addiction - Robinson - 2002 - Addiction - Wiley Online Library," 2001). This increased motivation to acquire drug rewards is also seen in addicts purchasing behavior; heroin addicts have been found to spend on average, 81% of their monthly income on drugs (Roddy & Greenwald,

2009). The increased impulsivity towards drug rewards observed in this population may result from changes in motivation rather than changes in judgment or other non-specific effects of reward type. Such an explanation aligns with the current finding that, after controlling for route of administration, reward content and morphine experience, morphine dependence causes a selective increase in impulsivity towards morphine rewards in rats.

Figures

Measure	Units	Morphine±SEM	Quinine± SEM	Sig.
Teeth-Chattering	Count	1.71 ±0.42	0.58 ±0.30	P = .71
Wet-Dog Shake	Count	1.0±0.54	0.5±0.30	**P < .05**
Rearing	Count	15.29±5.68	30.14±5.7	**P<.01**
Burying	Count	1.29±1.11	2.43±0.30	P = .07
Writhing	Count	3.14±0.77	0.0±0.0	**P<.01**
Pstosis	%present	0	0	N/A
Scream on Touch	%present	0	0	N/A
Piloerection	%present	14	0	N/A
Weight Change (10-min post Nal)	g	-5.9±0.52	-2.7±1.6	P = .21
Weight Change (24-h post Nal)	g	-10.4±5.18	-12.0±2.42	P = .71
Hyperalgesia (10-min post Nal)	%Baseline	125.7±16.2	110.5±9.0	P = .38

Table 1 Signs of morphine tolerance and withdrawal in Experiment 2a. The results of naloxone precipitated withdrawal in their appropriate units are presented alongside standard error of the mean. Fischer's exact significance of non-parametric independent samples median test was used to compare %present measures for morphine and quinine groups. For all other signs, a Wilcoxon rank-sum test was used to compare the groups. Significant tests are indicated in bold.

Measure	Units	Dependent ± SEM	Non-Dependent. ± SEM	Sig.
Morphine Tolerance				
Tail-Flick (10mg/kg Mor - Initial)	%MPE	72.89±9.23	N/A	
Tail-Flick (10mg/kg Mor –Dep)	%MPE	-1.28±5.43	N/A	**P<.01**
Precipitated Withdrawal Signs				
Teeth-Chattering	Count	3.38±0.53	0.39±0.24	**P<.01**
Wet-Dog Shake	Count	1.06±.41	0.56±0.15	P=.69
Rearing	Count	16.0±1.53	30.8±1.63	**P<.01**
Burying	Count	3.25±0.96	1.11±0.31	P=.22
Writhing	Count	5.31±1.37	0.61±0.50	**P<.01**
Pstosis	%present	87.5	11.11	**P<.01**
Scream on Touch	%present	31.25	11.11	P=.21
Piloerection	%present	81.25	16.66	**P<.01**
Weight Change (10-min post- Nal)	g	-3.50±0.48	±0.29	**P<.01**
Weight Change (24-h post- Nal)	g	8.81±2.58	13.50±2.93	P=.30
Tail-Flick (1hr post- Nal)	%Baseline	78.8±5.91	104.6±4.31	**P<.05**

Table 2 Signs of morphine tolerance and withdrawal in Experiment 2b. The results of tests of tolerance to morphine analgesia and naloxone precipitated withdrawal in their appropriate units are presented alongside standard error of the mean. Fischer's exact significance of non-parametric independent samples median test was used to compare %present measures. For all other signs a Wilcoxon rank-sum test was used to compare dependent to non-dependent groups or baseline to retest in the dependent group only (morphine tolerance). Significant tests are indicated in bold.

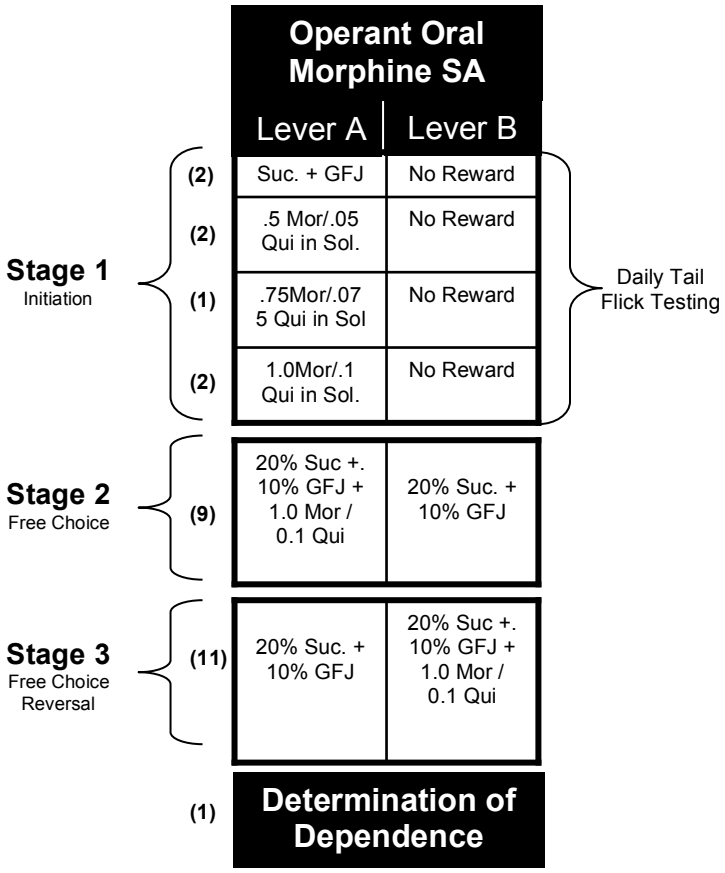


Figure 1 Schematic showing the time line of Experiment 2a. Number of daily session for each segment presented on the left in brackets. Experimental conditions that are group specific are both listed, separated by a forward slash. Solutions are delivered in 150 ul quantities unless otherwise specified. Concentrations of morphine/ quinine are listed in mg/mL Grapefruit juice is abbreviated as GFJ.

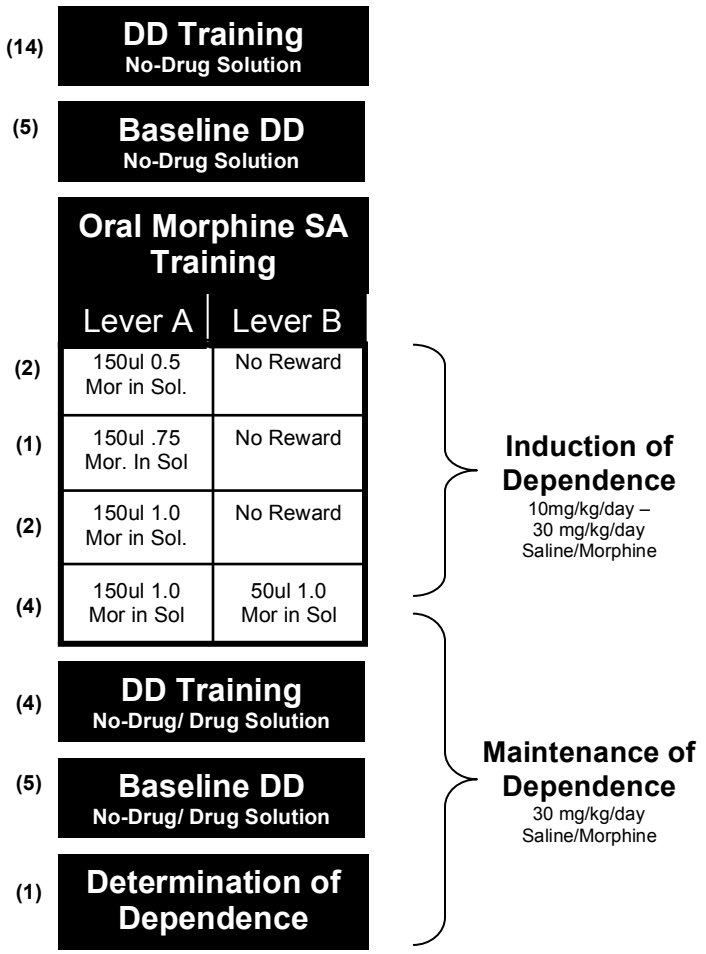
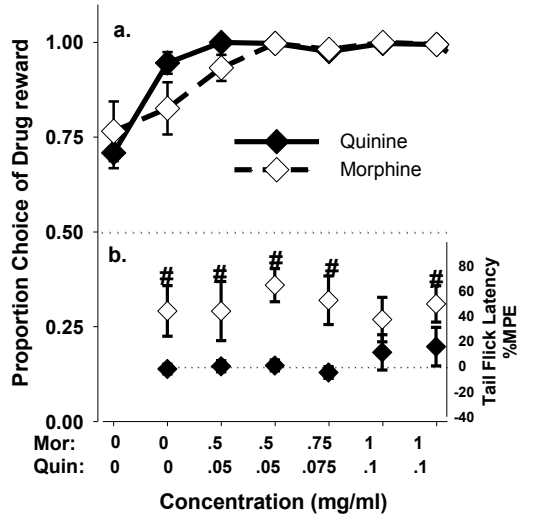
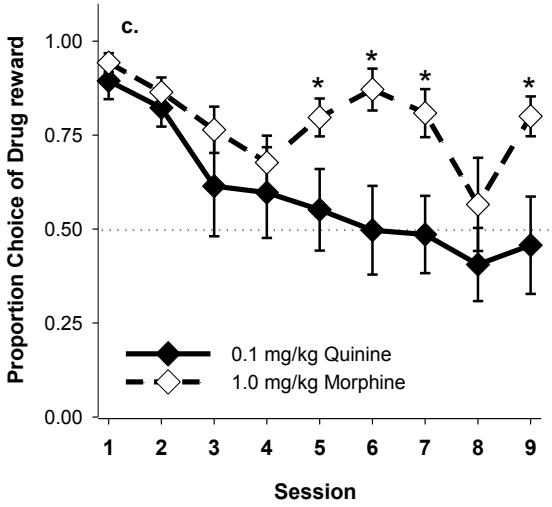


Figure 2 Schematic showing the time line of Experiment 2b. Number of daily session for each segment presented on the left in brackets. Experimental conditions that are group specific are both listed, separated by a forward slash. Solutions are delivered in 150 ul quantities unless otherwise specified. Concentrations of morphine/ quinine are listed in mg/mL. 'Sol.' And 'No Drug Solution" refers to a solution of 20% sucrose, 10% grapefruit juice. Drug solution refers to 1mg/mL morphine in 20% sucrose, 10% grapefruit juice.


a.
1.00
0.75
0.50
0.25
0.00
Proportion Choice of Drug reward
Quinine
Morphine
b.
#
80
60
40
20
0
-20
-40
Tail Flick Latency
%MPE
Mor: 0 0 .5 .5 .75 1 1
Quin: 0 0 .05 .05 .075 .1 .1
Concentration (mg/ml)


c.
1.00
0.75
0.50
0.25
0.00
Proportion Choice of Drug reward
*
0.1 mg/kg Quinine
1.0 mg/kg Morphine
1 2 3 4 5 6 7 8 9
Session

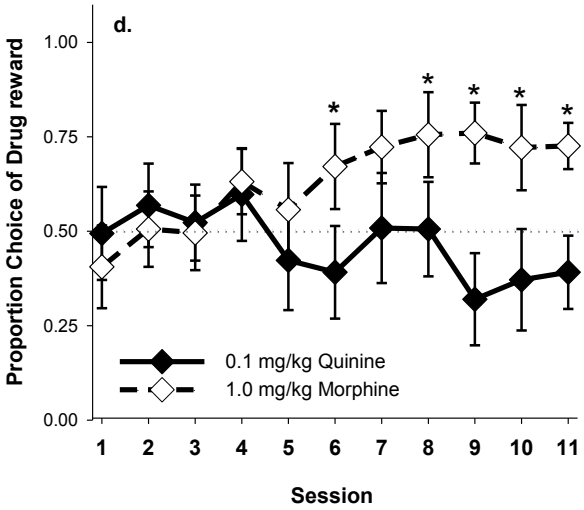

d.
1.00
0.75
0.50
0.25
0.00
Proportion Choice of Drug reward
*
0.1 mg/kg Quinine
1.0 mg/kg Morphine
1 2 3 4 5 6 7 8 9 10 11
Session

Figure 3 Time Line of Morphine (n=7, white diamonds) and Quinine (n=7, black diamonds) Oral Self-Administration during initiation (a), free choice (c) and free choice – reversal (d) portions of Experiment 2a. The antinociceptive effects (b) of self-administration during initiation is also presented. *p < .05, significance of Tukey HSD post-hoc tests, #p < .05, median test.

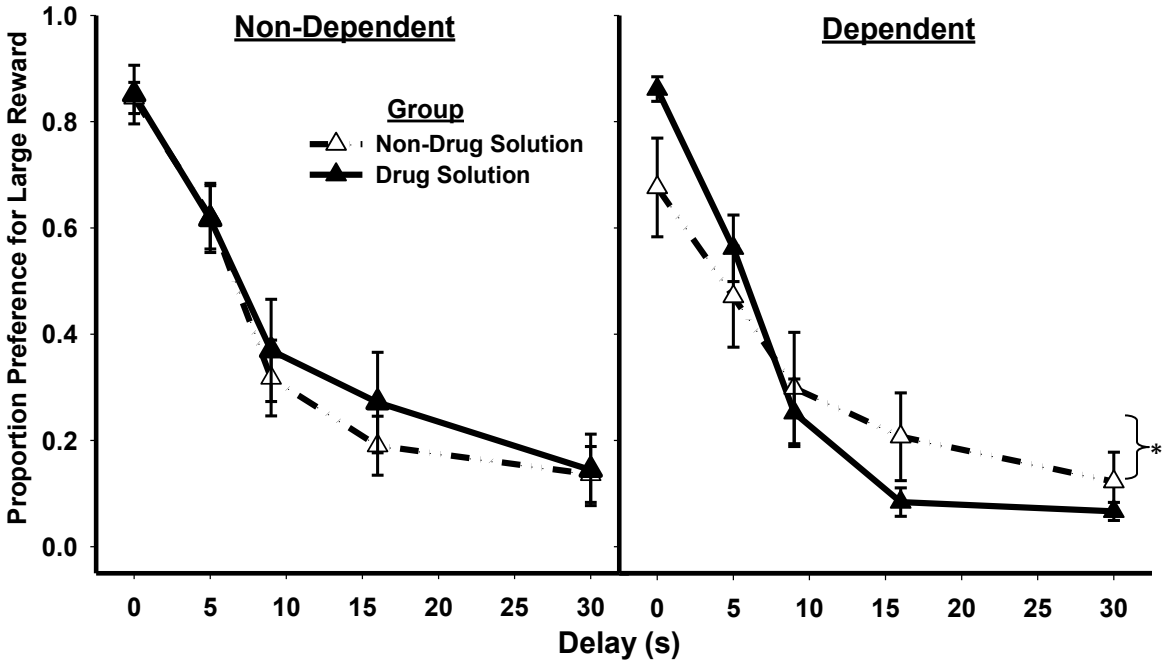


Figure 4 Test Delay discounting curves for non-dependent (n=9, left) and dependent (n=8, right) rats receiving either a morphine solution (white) or non-drug solution (black) as a reward. *p < .05, significance of Tukey HSD post-hoc tests, #p < .05, median test.

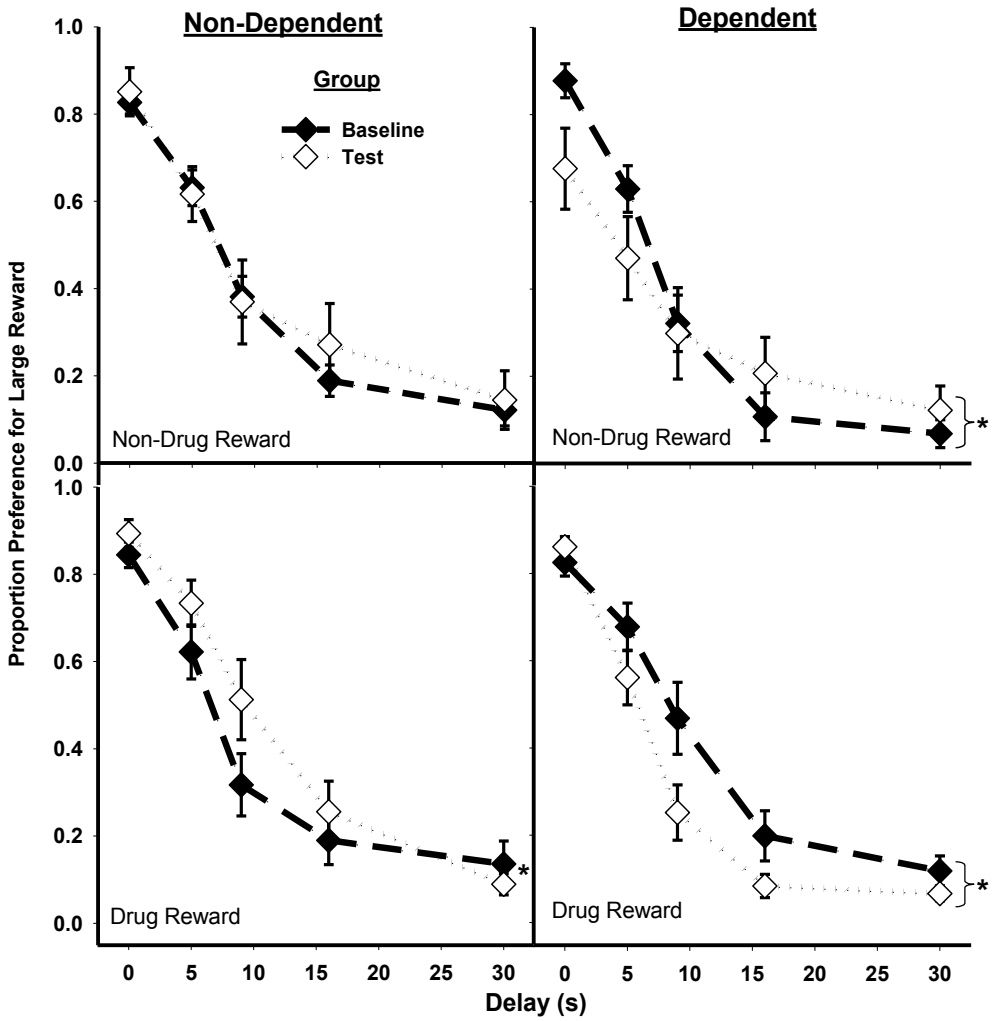


Figure 5 Group baseline and test delay discounting curves for: (a) non-dependent rats receiving non-drug solution at test (n=9, top left) (b) non-dependent rats receiving non-drug solution at test (n=9, bottom left) (c) dependent rats receiving non-drug solution at test (n=8, top right) (d) dependent rats receiving non-drug solution at test (n=8, bottom left) *p < .05, significance time point by delay interaction, simple main effects

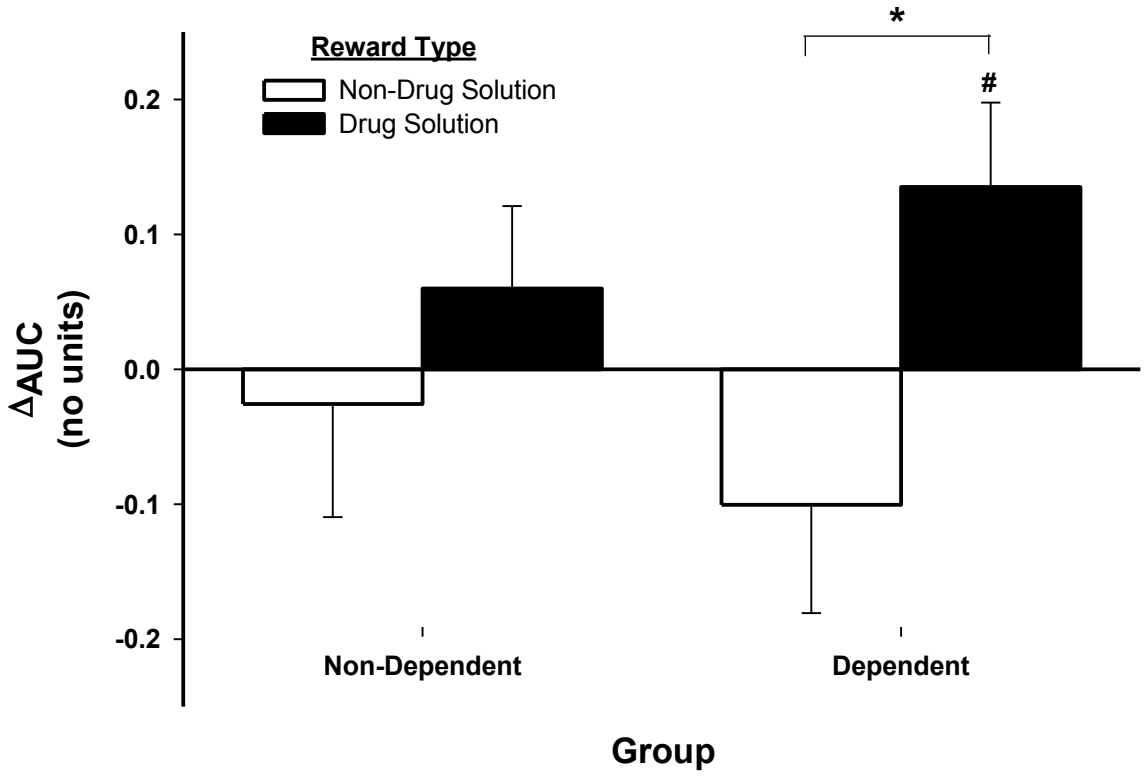


Figure 6 Change in area under the curve from baseline to test. # $p < .05$ significance of Wilcoxon sign-rank test within group, * $p < .05$ significance of Wilcoxon sign-rank test between group.

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Chapter 4: Tolerance to the acute effects of morphine in dependent rats.

4.1 Transition to Manuscript 3

One major contribution of manuscript two was the validation of a novel method of operant oral self-administration of morphine rewards. Using this method Experiment 2b served to confirm the finding from the human literature that morphine dependent individuals are more impulsive towards drug rewards than non-drug rewards. Like experiment 1, Experiment 2b also showed that morphine dependence can cause increased impulsivity, in this case towards drug rewards. In the context of this thesis, it is important to note that morphine dependent animals in Experiment 2b do not show increased impulsivity towards non-morphine rewards when compared to their pre-dependence levels of discounting. The bittersweet grapefruit juice rewards used in this experiment are less palatable than the pure sucrose rewards used in experiment 1. The failure to observe increased impulsivity towards grapefruit juice could indicate that only rewards with high incentive salience are discounted at an accelerated rate in dependent rats. It is also possible that experience with self-administration in the same operant context used for impulsivity testing, can cause associative and/or conditioning effects that affect the level of observed impulsivity when animals are retested on discounting. The acute effects of the drug rewards received by dependent rats should not have much effect on the rate of discounting in this experimental design. Testing sessions took one hour but oral morphine takes between 90-120 minutes to reach peak pharmacological activity (Okura, Morita, Ito, Kagawa, & Yamada, 2009; Okura et al., 2008). However, this gives an auxiliary reason to

examine the effects of acute morphine on delay discounting in both dependent and non-dependent rats.

The main goal of experiment three presented in manuscript three was to investigate the question: does dependence cause tolerance or sensitization to the acute effects of morphine on impulsivity? As will be discussed in the manuscript, this question not only has clinical implication but also serves to add to the animal literature on opiate sensitization/tolerance

Additionally, as it pertains to this thesis, the experiment also serves to replicate the initial finding by examining whether opiate dependence can cause an increase in impulsivity towards sucrose rewards.

4.2 Manuscript 3: The Effect of Acute Morphine on Delay Discounting of Sucrose Rewards in Dependent and Non-Dependent Rats.

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Abstract

Rationale: Chronic opiate use is associated with increased impulsivity in both humans and animals, and previous studies suggest that acute morphine can increase impulsivity in non-dependent rats. However, the extent to which chronic opiate usage modulates the effect of acute morphine is unknown.

Methods: Rats were trained to delay discount 20% sucrose solution and then randomly assigned to either a dependent group that received a nightly 30 mg/kg subcutaneous dose of morphine, or a non-dependent group that received a nightly saline injection. Once dependence was established, rats were then assigned to 1 of 4 acute morphine doses (0mg/kg, 1.25 mg/kg, 2.5 mg/kg, 5 mg/kg). For 5 days delay discounting curves were determined 22.5hrs after maintenance doses and 1hr after their prescribed acute injections.

Results: In non-dependent rats, 2.5mg/kg and 5mg/kg doses of morphine caused decreased preference for the large reward at all delays. Acute morphine had no effect on discounting curves in dependent rats.

Conclusions: Morphine dependence can cause tolerance to the effects of acute morphine on delay discounting.

Introduction

Delay discounting is a paradigm used in both animals and humans to evaluate the rate at which rewards are discounted as a product of time until their delivery. Here, impulsive choice or impulsivity is used exclusively to refer to an exaggerated preference for small immediate rewards over larger delayed ones in delay discounting paradigms. In both the human and animal literature, decision-making and choice behavior are reported to be altered by both acute drug effects, that is, immediate pharmacological effects of intermittent exposure to smaller doses, and chronic drug effects, that is, long-term neurochemical changes due to extended exposure to larger doses of drugs. In the human literature, chronic drug use is strongly associated with increased impulsivity; individuals dependent on drugs including cocaine (Coffey, Gudleski, Saladin, & Brady, 2003), opiates (Madden, Petry, Badger, & Bickel, 1997), alcohol (Bjork, Hommer, Grant, & Danube, 2004), cigarettes (Heyman & Gibb, 2006) and methamphetamine (Hoffman et al., 2006) are consistently reported to be more impulsive than non-dependent controls. Likewise, in animals, chronic exposure to cocaine (Mendez et al., 2010; Roesch, Takahashi, Gugsa, Bissonette, & Schoenbaum, 2007), morphine (Harvey-Lewis, Perdrizet, & Franklin, 2012), heroin (Schippers, Binnekade, Schoffelmeer, Pattij, & Vries, 2011) and methamphetamine (Richards, Sabol, & de Wit, 1999) have been reported to increase impulsivity.

On the other hand, the relationship between acute drug use and impulsivity is not clear. In humans, acute doses of drugs have been reported to cause both increases (Reynolds, Richards, & de Wit, 2006) and decreases (de Wit, Enggasser, & Richards, 2002;

Ortner, 2003) in impulsivity. In rats, the effect of acute dosage of drugs depends on the methodology of a given study. In particular, the prior length and strength of drug exposure appears to influence the effect of acute drug use on impulsivity. For example, acute cocaine may produce impulsivity after long periods of exposure to high doses (Tanno et al. 2014; Dandy & Gatch, 2009), whereas no changes in impulsivity are observed in rats who have been exposed to low dose regimens (Tanno et al., 2014; Winstanley et al., 2007). Similar results have been found for acute nicotine during periods of chronic exposure (Kelsey & Niraula, 2013) (Kolokotroni, Rodgers, & Harrison, 2013) but not after termination of the dosing regimen (Kolokotroni et al., 2013). Thus, it appears that a dose of a drug can have effects in drug-dependent animals or individuals that are exposed to drugs at high concentration over long periods that differ from the effects in animals or humans that are naïve or sensitized to low doses. However, the influence of dependence on the effects of acute drug use on impulsivity has not been explored directly.

The distinction between chronic and acute drug use is particularly pertinent to opiates. Opiates are routinely prescribed as a method of pain management in non-dependent individuals as well as part of replacement/maintenance therapy in dependent individuals (Amato, Davoli, Perucci, & Ferri, 2005). In addition, prescription opiates are increasingly used recreationally in the general populations with 2012 usage rates estimated at 5% (Fischer, Keates, Bühringer, Reimer, & Rehm, 2013). Thus, the acute effects of opiates, including their effects on decision-making, are relevant to a large population. As it pertains to the dependent population, chronic opiate use is associated with strong physical dependence and withdrawal. Repeated exposure to opiates has been shown to cause both sensitization and tolerance to the effects of acute doses on a wide

variety of behavioral measures (Stewart & Badiani, 1993; Stewart, de Wit, & Eikelboom, 1984). Tolerance is more often observed than sensitization, especially with analgesic effects (Roerig, O'Brien, Fujimoto, & Wilcox, 1984), respiratory depression and sedation (Heyman & Gibb, 2006; LeBlanc & Cappell, 1974), and aversive stimulus properties (as evaluated by conditioned taste aversion e.g. Hunt, Spivak and Amit 1985) . However, a subset of processes that involve the mesolimbic dopaminergic system show sensitization to opiates, including excitatory locomotive effects (Bartoletti, Gaiardi, Gubellini, Bacchi, & Babbini, 1983; Hoffman et al., 2006) and conditioned reward processes (Zarrindast, Ebrahimi-Ghiri, Rostami, & Rezayof, 2007). Thus, it is possible that tolerance or sensitization may cause the effects of acute opiate doses on impulsivity to differ in dependent and non-dependent populations.

The few studies that have investigated the acute effects of opiates in humans report either decreased or unaltered impulsivity. Giordano et al. (2002) found that opioid dependent individuals were significantly less impulsive two hours after their daily maintenance dose than immediately prior thereto. Zacny and colleagues (2009) reported no effect of acute oxycodone on impulsivity in a non-dependent population. On the other hand, acute opiates are reported to either cause increased impulsivity in rats or to have no effect. For example, Kieres (2004) reported that acute morphine (.5mg/kg – 2.0 mg/kg) caused a dose-dependent increase in impulsivity. Tanno (2014) found that morphine produced a dose-dependent increased preference for the large reward at all delays and when immediately delivered, suggesting a decreased sensitivity to reward size and possibly an increase in impulsivity. On the other hand, Garcia-Lecumberri (2011) reported no effect of acute doses of morphine ranging from 0.25-2 mg/kg in two different rat strains.

Similarly, Pattij (2009) reported that large doses of morphine (6 mg/kg) decreased preference for the large reward at zero delay but did not affect the slope of delay discounting.

One possible explanation for the inconsistencies within and between literatures is the wide range of length and strength of exposure to opiates prior to testing. The aforementioned human studies that investigated effects of acute opiates used very dissimilar populations; that is, a largely naïve sample (Zacny & de Wit, 2009) and a sample of dependent individuals who had been repeatedly exposed to large doses of opiates (Giordano et al., 2002). Similarly, in the animal literature, the length of prior experience with opiates ranged from one exposure per dose (Tanno et al., 2014), to six weeks of total exposure (Kieres et al., 2004). All the aforementioned animal studies exposed rats to the full range of intra-experimental doses, but the maximum dose in each study ranged from a relatively low dose of 1.8 mg/kg (Kieres et al., 2004), to a dose bordering on one sufficient to induce physical dependence (Pattij et al., 2009). Thus, the literature suggests that the effects of acute doses of opiates vary with prior history and the resulting tolerance or sensitization that exposure might produce. This may make interpretations of dose-response curves within experiments problematic, since tolerance or sensitization effects may disproportionately affect later doses in the testing sequences.

We have, therefore, tested the effects of acute doses of morphine in samples of dependent rats and morphine-experienced but non-dependent rats. In order to investigate a range of doses while keeping the duration of exposure to the drug limited -- to avoid developing dependence in our non-dependent sample -- each does was tested in a different group of animals. With this methodology we find that acute morphine induces a dose-

dependent shift in preference towards smaller immediate rewards, and morphine dependence induces tolerance to this effect.

Methods

Subjects

This research was reviewed by the Animal Ethics Committee of McGill University and carried out in accordance with the guidelines of the Canadian Council on Animal Care.

Subjects were 90 male Long Evans rats received at weights between 150g and 175g (Charles Rivers Laboratories, Montreal, Que, Canada). Rats were individually housed in a colony maintained on a 12h light: 12h dark light cycle (light phase: 7am to 7pm). For the duration of the experiment, access to food was restricted to maintain rats' weight at 85% of free-feeding values.

Operant Conditioning Task Apparatus

Training and testing for operant conditioning tasks took place in clear Plexiglas boxes (29.5cm wide, by 28cm deep, by 27.5cm high). Two metal levers (Coulbourn Instruments, Allentown, PA) were mounted 3cm apart, 6.5cm above the metal rod floor on the metal side of the box. Above each lever was a metal panel containing 3 small LED stimulus lights (Coulbourn Instruments, Allentown, PA). Beneath each lever was a small metal sipper cup (overflow capacity: approximately 200 μl). Reward solutions were supplied to the sipper cup at a flow rate of 100μl per second using an automated syringe pump (Model 22, Harvard Apparatus, St. Laurent, Que., Canada). Each testing box was housed in a sound- and light-attenuating chamber (65cm by 50cm by 52cm) illuminated by a small house light on the left side of the operant chamber 41cm above the floor (Coulbourn Instruments, Allentown, PA). A personal computer controlled the programming of events and recording of data.

Nociceptive Testing Apparatus

An Isotemp 3016 (Fischer Scientific, Ottawa, Ont., Canada) circulation bath was used to heat water to a temperature of 54 +/- 0.1˚C. Latencies were timed using a foot-pedal operated timer (Model 54519-A, Lafayette Instruments, Lafayette, IN)

Operant Response Training

Operant conditioning task training and reward size discrimination

Prior to experimental testing, rats underwent operant conditioning task training and reward size discrimination using the methods previously reported in full detail by Harvey-Lewis (Harvey-Lewis et al., 2012). In brief, animals were first trained to drink 150 µl of 20% sucrose solution freely presented in sipper cups. Following this, rats were trained to press each of two levers to activate delivery of this reward to sipper cups beneath on a continuous reinforcement schedule during daily (for 6 days) sessions of 50 trials that lasted until the appropriate lever was pressed with an intertribal interval of 20s. The active lever was altered randomly between trials. Then, in reward size discrimination, rats were trained to discriminate between pressing a lever that resulted in delivery of a small reward (50µl 20% sucrose) and one that resulted in delivery of a large reward (150µl 20% sucrose). Daily session consisted of 5 blocks of 14, 55s second trials. The first 4 trials of a block were forced choice trials (where only one lever was active) and the following 10 trials were free choice trials where both levers were active. Reward discrimination training continued until rats selected the large reward lever on at least 80% of free choice trials on 2 successive days -- for one of which the large reward was associated with the left lever, and for the other the large reward was associated with the right lever. Rats (n=7) that did not reach this criterion after 16 sessions were excluded from further testing.

Delay Discounting Task

Daily delay discounting sessions consisted of 5 blocks of 14 trials. Each trial consisted of a 35s choice period followed by a 20s time out period. During these sessions, pressing on one lever resulted in the delivery of an immediate small reward (50µl of 20% sucrose solution), and pressing the other lever resulted in the delivery of a large reward (150µl of 20% sucrose solution) after a specified delay. Delays remained constant within a block, but increased between blocks in ascending order (0s, 5s, 9s, 16s, 30s). Each block began with 4 forced trials (2 trials on each lever in random order) in which only one lever was active as signaled by illumination of the corresponding cue light. The 4 forced trials were followed by 10 free choice trials wherein both levers were active as signaled by illumination of both cue lights. A response on an active lever caused the cue lights to be extinguished and the associated reward to be delivered in the sipper cup under the selected lever after the appropriate delay. The house lights were extinguished 35s after trial initiation (irrespective of large reward delay) and reactivated after 20s with the beginning of the new trial. If the rat had not responded in the 35s choice period then the cue lights extinguished simultaneously with the house lights and the trial was scored as an omission. Immediately after the time-out period, the appropriate stimulus lights were illuminated (along with the house lights) and the following trial began. The location of the immediate and the delayed lever remained constant for all delay discounting sessions for each rat but were counterbalanced between rats. Rats (n=5) that exhibited no change in preference with increasing delay were excluded from further testing.

Procedure

The structure of testing is depicted in Figure 1. Rats were trained on the delay discounting task, once daily, for 14 days. This was immediately followed by a 5-d baseline delay discounting period (baseline discounting prior to initiation of the dosing regimen) using the same procedure. Individual baseline delay discounting curves were calculated by averaging the proportion choice of the large reward at each delay for this 5-day period using the equation:

(1) Proportion choice = total large reward choices in choice trials/ total completed choice trials.

After baseline delay discounting, pain testing and sucrose consumption was complete, rats were then randomly assigned to either a dependent group that received nightly 30mg/kg morphine (s.c.) or a non-dependent group that received nightly saline. Groups were further subdivided into 4 acute dose groups (corresponding with the morphine dose received 1-h prior to testing): 0mg/kg, 1.25mg/kg, 2.5mg/kg or 5mg/kg, for a total of 8 independent groups.

Once assigned, the 12-day escalation/maintenance period began. Delay discounting testing did not occur during the escalation and sensitization periods. First, the morphine maintenance dose for dependent rats was escalated over the first 8 days (see dosing regimen) while non-dependent rats received saline. Then during the following five days, rats were sensitized to their ascribed acute dose of morphine with a daily injection. All rats were given their ascribed acute dose (0mg/kg, 1.25mg/kg, 2.5mg/kg or 5mg/kg) 1-h prior to the time-period during which their regular delay discounting training period would take place. Approximately 2.5-h after acute sensitization injections, dependent rats received the remainder of their 30 mg/kg dose of morphine, while non-dependent rats received saline. Thus, all animals received 5days of sensitization to acute dose prior to delay discounting testing in addition to their regular maintenance injections.

Following sensitization, a 6-d test delay discounting testing period took place, that is, animals delay discounted sucrose rewards after receiving acute doses of morphine while currently receiving maintenance doses of morphine or saline. Approximately 21.5-h after the daily morphine/saline maintenance dose, rats were given their prescribed acute dose. One hour later, rats were tested on delay discounting (Figure 1, above timeline). Approximately, 30-min after completion of the delay discounting protocol dependent rats received the remainder of their 30mg/kg dose of morphine, while non-dependent rats received saline. The first day of this testing period was used as a re-acclimatization day and the results were not analyzed. Individual test curves were calculated by averaging the proportion of times the large reward was selected over the small reward at each delay for the last 5-days of the testing period. As a summary measure of delay discounting we

performed non-linear regression to fit hyperbolic curves to both individual and group discounting data using the methods presented in Kayir, Semenova, and Markoua (2014), adopted from Mazur (1987) with the equation:

$$(2)\quad V = A / (1 + kD)$$

Where V is the preference for the large reward after a delay of D in seconds, A is the preference for the large reward at D = 0 s and the free parameter k describes how rapidly V declines with increasing delay. Goodness of fit (R^2) for each curve was also calculated. Large k values indicated increased slope of discounting and thus greater impulsivity whereas small k values indicate the opposite. During both baseline and test periods, the average lever press latency (time from trial initiation to initial contact with a lever) and average omissions per session were recorded.

Free sucrose consumption task

On the 3 days prior to initiation of the morphine-dosing regime (baseline), and on the days following the 3rd and 4th 30mg/kg maintenance dose (test), rats were injected with saline (baseline testing) or their prescribed acute dose (test period) in the colony. Then, rats were transported in their home cages to the testing room and allowed to habituate while their water bottle was made unavailable. After 1hr, a sipper bottle containing 20% sucrose solution was placed in the home cage. Rats were allowed to freely drink from this bottle for one hour and five minutes at the time that delay discounting testing would usually occur. The total volume of sucrose solution consumed during this period was determined for each rat. The baseline period consisted of one training session followed by two consecutive testing days. The test period consisted only of two consecutive testing days. The mean sucrose consumption was determined by averaging the latter two testing days for each session. Change in sucrose consumption was calculated by subtracting mean test sucrose consumption from baseline sucrose consumption.

Determination of Dependence State

The production of dependence via experimenter-administered morphine was assayed by evaluating tolerance to morphine-analgesia and recording signs of withdrawal following a 1mg/kg dose of naloxone.

Baseline pain thresholds were evaluated using a modified tail withdrawal test (Franklin & Abbott, 1989) on the day following the final baseline delay discounting session. For all tail-flick sessions, rats were first habituated to the testing room for 30-min. During testing, the distal 5cm of the rat's tail was immersed into a 54^{o}C warm water bath. The latency from immersion to withdrawal (or sufficient squirming to indicate intention to withdraw the tail) was measured to the nearest 0.1s. The upper limit of withdrawal latency was 10s, after which the rat's tail was removed from the water bath by the experimenter. Each testing session consisted of three test trials at 5-min intervals. The first trial served as a warm-up trial and was not analyzed. Drug-free baseline latencies were determined one day before initiation of the morphine-dosing regimen. Morphine analgesia was determined 60-min after the initial 10mg/kg dose, and 60-min after 10mg/kg morphine as part of the fifth 30mg/kg maintenance dose (the remaining 20mg/kg was given immediately following the pain test). Data were represented as percentage of maximal possible effect (%MPE) using the equation:

(3) %MPE = (upper limit – test latency) *100 / (upper Limit – baseline latency)

One day after the final delay discounting testing session, and 50-min after a final 30mg/kg maintenance dose, rats were placed in a clear 24cm wide, by 46cm deep, by 20cm high Plexiglas container for 10-min to habituate. Rats then received 1mg/kg naloxone subcutaneously. Over the 10-minute period following injection, the number of occurrences of the following signs was scored for each rat; rearing, digging, wet dog shakes, teeth chattering and writhing. At the 10-min mark, rats were tested for presence or absence of; scream on touch, piloerection and ptosis. One hour after later hyperalgesia (represented as % change from baseline) was measured using the tail withdrawal test. Weight change 10-min and 24-h after naloxone injection was also recorded.

Drugs and Dosing Regimens

Injectable morphine solutions were made by dissolving morphine sulphate (Sigma-Aldrich, St. Louis, MO) in 0.9% saline. Injections were administered subcutaneously by the experimenter in a volume of 1mL/kg.

To induce dependence the maintenance dose was escalated over 7 days (10, 10, 15, 15, 20, 25, 30mg/kg Morphine). Thereafter, dependent rats were maintained on 30mg/kg morphine given approximately 30-min after the daily test session or the time during which test would occur, Non-dependent rats were maintained on saline.

Data Analysis

Statistics were calculated using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA) and Sigmaplot 11.0 (Systat Software Inc., Point Richmond, CA, USA). A 4-way mixed model ANOVA was performed to analyze proportion preference data with state (dependent v non-dependent) and acute morphine dose (0, 1.25, 2.5 and 5 mg/kg) as between group factor and delay (0,5,9,16 or 30s) and test point (baseline v test) as repeated measures. When significant 2 and 3-way interactions were observed, simple main effect analysis was used to compare the effects of delay (repeated measure) and acute morphine dose (between groups) at each level of state (dependent and non-dependent) separately for baseline and test sessions. Post-Hoc tests were performed as noted in text, corrected for multiple comparisons using Tukey HSD. 3-way mixed model ANOVAs were performed to analyze k values, R^2 values, omissions and tail flick latency with state (dependent v non-dependent) and acute morphine dose (0, 1.25, 2.5 and 5 mg/kg) as between groups factors and test point (baseline v test) as repeated measures. Withdrawal measures were first compared using a 2 way ANNOVA (state v acute dosage). When no significant main effect of acute dosage or dose by state interaction was resolved, dependent and non-dependent groups were analyzed as homogenous groups ignoring acute dosage. Since a subset of these data sets did not meet the homogeneity of variance assumption for t-tests, the Mann-Whitney rank sum test was used to compare continuous measures, and the Fischer exact test was

used to compare categorical measures. For the same reason, free sucrose consumption was compared using the Mann-Whitney rank sum test.

Results

Delay Discounting

During baseline testing there were no significant differences in the discounting curves of the eight groups, indicating that subjects in all groups were trained to a similar level of performance (main effect of state, acute dosage and interaction effects, NS). During the test period, acute morphine had significantly different effects in dependent and non-dependent rats (state by dose by delay interaction ($F(12, 280) = 2.35$, $p < .01$) (Figure 2). In non-dependent rats there was a significant dose-dependent effect of morphine on delay discounting (dose by delay interaction ($F(12, 144) = 5.67$, $p < .01$)) (Figure 2). Specifically, rats that received 2.5 or 5.0 mg/kg morphine 1-h prior to testing, showed significantly decreased preference for the large reward at all delays when compared to either rats receiving vehicle or 1.25mg/kg morphine (Tukey HSD, all $P< .01$). There were no significant differences in discounting between rats receiving 2.5mg/kg and rats receiving 5.0mg or between rats receiving vehicle and rats receiving 1.25mg/kg (Tukey HSD, all P NS) In dependent rats, there was no significant effect of acute morphine dose on discounting ($F(12,132) = 1.146$, $P = .33$).

Logistic regression hyperbolic curve fitting to discounting curves confirmed the raw data analysis (Table 1). There were significantly different effects of acute morphine on the k coefficient of delay discounting in dependent and non-dependent rats (state by dose interaction ($F(3,70) = 3.67$, $p < .05$)). In non-dependent rats 5mg/kg morphine significantly increased the slope of discounting when compared to both the 0mg/kg and 1.25 mg/kg dose (Tukey HSD, all $p<.05$). There was a trend towards increased slope of discounting in non-dependent rats receiving 2.5mg/kg when compared to the 1.25mg/kg dose (Tukey HSD, $q=3.43$, $p=.08$). Acute morphine had no significant effects on the slope of discounting

in dependent rats at any dose (Tukey HSD, all p>.49). There were no significant differences in R^2 value between groups.

There was an increase in response latency from baseline to test in all groups (main effect of test period, F (1, 70) = 18.6, p < .01), this effect was significantly exaggerated in the dependent group (simple main effects, effect of state, F(1,70) = 6.23, P < .01) (Table 2). There was no significant effect of acute dose on response latency (main effect of acute dose and interactions, F(3, 70) = 1.08, NS). On average, during the test period dependent rats made significantly more errors of omission than non-dependent rats (main effect of state, F (1,70) = 7.64, p < 0.01) irrespective of acute dose (main effect of dose, state by dose interaction (F(3, 70), (3,78) = 1.23, 1.38 all p NS) (Table 2).

Sucrose Consumption

During the morphine dosing regimen, dependent rats exhibited a significant decreases in sucrose consumption from baseline when compared to non-dependent rats (main effect of state F(1,7) = 50.03, p < .01) (Table 3). This effect was not modulated by the acute morphine dose received prior to testing (main effect of dose, dose by state interaction F(3,7), (3,70) = .15, 1.27, all P NS).

Morphine Dependence Tests

It can be seen in Table 3 that with the exception of a drop in weight 24-hr after withdrawal testing (main effect of dose, F(3,7) = 3.33, p = .02, dose by state interaction F(3,70) = .56, P NS), there were no effects of acute morphine dose on measures of withdrawal (main effects of dose all F(3,7) < 2.34, all P NS, dose by state interaction all F(3,70) < 1.47, all P NS).

As evaluated by tail flick latencies, rats maintained on morphine showed tolerance to 10mg/kg morphine after their fifth 30mg/kg dose when compared to their first experience with 10m/kg morphine (Wilcoxon sign-rank test, p< .01) (Table 4). Dependent rats also exhibited significantly more withdrawal signs than non-dependent rats after a 1mg/kg dose of naloxone. Specifically, dependent rats exhibited more writhing, burying,

teeth-chattering, and less rearing (a negative sign of withdrawal) than non-dependent rats (Mann Whitney rank sum test, all $p < .01$). Rats maintained on morphine frequently exhibited ptosis, piloerection and scream on touch and did so significantly more often than controls (Fischer exact test, all $p < .01$). Incidence of wet dog shakes did not differ significantly between groups (Mann Whitney sign-rank test p NS). One hour after 1mg/kg naloxone, morphine-maintained rats showed lower pain thresholds than non-dependent controls (Mann Whitney rank sum test, $p < .05$) and lost significantly more weight 10 minutes and 24hrs after naloxone injection (Wilcoxon sign-rank test, all $p < .05$).

Discussion

To date, this is the first study in the animal literature to examine the interaction between acute doses of opiates, opiate dependence and impulsivity. We found that the effect of acute morphine on delay discounting of liquid sucrose rewards varied with the extent of prior morphine experience. At all delays, including the no delay condition, moderate acute doses of morphine caused an increase in preference for smaller immediate rewards in morphine-experienced rats that did not exhibit signs of dependency. On the other hand, rats made dependent with experimenter administered nightly 30mg/kg morphine showed similar rates of delay discounting at all acute doses. These results suggest that morphine dependence can induce tolerance to the effects of acute morphine on delay discounting exhibited in non-dependent rats.

In non-dependent rats, a low dose of morphine had no effect on impulsivity in our delay discounting task. Our low dose (1.25mg/kg) was similar to doses previously reported to produce no effect on delay discounting (García-Lecumberri et al., 2011; Kieres et al., 2004). On the other hand, we found that moderate dose morphine caused increased preference for the small reward whether delivered immediately or after a delay. Firstly, the finding that rats have decreased preference for the large reward when both rewards are delivered without delay is unsurprising; previous studies have reported this effect in delay discounting tasks (Pattij et al., 2009; Tanno et al., 2014) and reward discrimination tasks (Castellano, 1975). One possible explanation for this effect is that acute morphine caused a

decrease in motivation to acquire sucrose reward, which, similar to satiation, might cause a decreased preference for the large reward. However, we also found that doses that altered zero delay preference did not significantly affect free sucrose consumption or omissions in our delay discounting paradigm, two measure that should asses the motivation to acquire sucrose reward. Since rewards are delivered differently in the free sucrose consumption task, it is possible that this measure is less sensitive to the effects of motivation than operant response tasks. However, previous studies have suggested that, if anything, acute opiates increase motivational drive to acquire food rewards in operant response tasks (Solinas and Goldberg 2005; Cooper and Kirkham 1990). Morphine has been shown to impair discrimination in 2-lever visual discrimination tasks which procedurally resemble the delay discounting task (Andrews and Holtzman 1988; Koek and Slangen 1984) hence, it is more likely that the change in zero delay preference is a reward- size discrimination deficit or some more general disturbance in operant task responding.

Secondly, since our non-dependent rats, regardless of acute dose, had limited exposure to morphine and showed no signs of dependence the effects can be attributed to pharmacological effects of the drug and not latent effects of state. We found that these doses of morphine caused an increased preference for the smaller, immediate reward when delivered after a delay, and rats reached an asymptote of discounting at a shorter delay. Similarly, moderate doses of morphine caused increased rate of discounting in non-dependent rats as determined by non-linear regression. Thus, both measures suggest that acute morphine accelerated delay discounting which suggests an increase in impulsivity in non-dependent rats. Previous research suggests that the neural systems that contribute to the processes of lever choice preference and delay discounting rate are divergent (Bezzina et al. 2008; Mar et al. 2011; Marshall et al. 2014). But, the extent to which the effects of morphine on reward-size discrimination and delay discounting are independent cannot be ascertained in our experiment. Thus, we cannot exclude the possibility that acute morphine causes a downward shift in the dose-response curve on delay discounting without specifically altering impulsivity, or that morphine simply causes a general disruption of behavior unrelated to impulsivity. Nevertheless, our results are similar to Kieres (2004) and Tanno (2014) in suggesting that moderate to high doses of morphine cause decreased preference for larger delayed rewards.

In contrast, there was no effect of acute morphine on delay discounting in dependent rats. Rats that received nightly 30mg/kg morphine and subsequently showed significant signs of withdrawal after receiving 1mg/kg naloxone, discounted sucrose rewards at similar rates irrespective of the acute dose of morphine. Similarly, acute morphine had no significant effects on reward size discrimination as evidenced by a similar proportion preference for large reward at zero delay in all dependent groups. These results suggest that repeated exposure to large doses of morphine induces tolerance to the effects of acute morphine on delay discounting, including the reduced sensitivity to magnitude of rewards. It is possible then, that previous studies reporting no effects of acute morphine on impulsivity may be explained by tolerance from exposure to extended and/or large doses of morphine prior to testing. Similarly in the human literature, the observation that acute doses of opiates decrease impulsivity might reflect a combination of unchanged impulsivity due to the acute dose of drug and reduced impulsivity-inducing withdrawal (Giordano et al., 2002). Alternatively, large acute doses closer to a dependent individual's or animal's maintenance dose (30mg/kg in our experiment) might be necessary to produce decreased impulsivity.

The fact that we observe tolerance to effects of morphine on delay discounting may provide insight into the neurochemical underpinning of changes to impulsivity caused by morphine. Previous studies have implicated many areas of the brain in the decision making processes of delay discounting, including various orbitofrontal areas, the hippocampus and the limbic system (Abela & Chudasama, 2013) (Pothuizen, Jongen-Rêlo, Feldon, & Yee, 2005) (Jo, Kim, Lee, & Jung, 2013). In humans, recent studies have suggested that these area might play different roles in component processes of delay discounting, for example the mesolimbic dopaminergic system may primarily encode future reward magnitude whereas prefrontal areas may encode delay (Ballard & Knutson, 2009; Day, Jones, Wightman, & Carelli, 2010). The underlying mechanisms that lead to sensitization and tolerance are complicated. However, most effects of morphine showing sensitization, including reward processes and locomotion, are theorized to result from increased dopaminergic release from mesolimbic dopamine neurons (Stewart et al., 1984). The fact that we observe tolerance and not sensitization in our dependent rats, suggests that morphine induced changes in delay discounting are due to alterations in sensitivity to

delay rather than future reward magnitude. Consistent with this notion, morphine has been shown to affect timing mechanisms including circadian rhythms (Glaser, Reyes-Vázquez, Prieto-Gómez, Burau, & Dafny, 2012) and perception of time (Ward & Odum, 2005).

As it pertains to humans, our findings have two implications. Firstly, they suggest that accelerated delay discounting may be a side effect of receiving opiates during pain management, and this should be taken into consideration when patients are making decisions that require weighting immediate and delayed rewards. Secondly, our findings suggest that opiate replacement therapy should not increase impulsive decision making in opiate dependent individuals. Over the last few years opiate substitution using buprenorphine, methadone, heroin or morphine has been increasingly common as a method of treating opiate dependence (Amato et al., 2005; Ferri, Minozzi, Bo, & Amato, 2013). However, if acute opiates caused increased impulsivity in the dependent population replacement therapy might indirectly and counterproductively promote relapse since accelerated discounting has specifically be shown to be a negative predictor of abstinence from opiates (Passetti, Clark, Mehta, Joyce, & King, 2008), (for review see Stevens et al., 2014). However, our results that dependence leads to tolerance to the effects of an opioid on impulsivity suggests opiate replacement therapy would not increase the likelihood of relapse via impulsivity.

Figures

Group (**State** - *Acute Dose*)	**N**	**k coefficient** ± SEM (Individual)	**R^2** ± SEM (Individual)	**k coefficient** (Group)	**R^2** ± SEM (Group)
Dependent					
0 mg/kg	9	0.289 ± 0.09	0.93 ± 0.04	0.280	0.99
1.25 mg/kg	10	0.432 ± 0.11	0.92 ± 0.02	0.329	0.96
2.5 mg/kg	9	0.260 ± 0.05	0.88 ± 0.03	0.180	0.94
5.0 mg/kg	10	0.349 ± 0.10	0.72 ± 0.09	0.211	0.97
Non-Dependent					
0 mg/kg	9	0.239 ± 0.07	0.85 ± 0.03	0.143	0.93
1.25 mg/kg	9	0.149 ± 0.05	0.85 ± 0.03	0.105	0.90
2.5 mg/kg	11	0.468 ± 0.09	0.90 ± 0.07	0.369	0.98
5.0 mg/kg	11	0.645 ± 0.14	0.84 ± 0.06	0.199	0.98

Table 1 Delay discounting k coefficient and R^2 values. Number of animals per group, mean k coefficient and R^2 values of non-parametric regression of individual rat delay discounting curves (individual) and SEM are presented with k coefficient and R^2 values of non-parametric regression of mean group delay discounting curves (group).

Group (**State** - *Acute Dose*)	**Latency(s)** ± SEM (Baseline)	**Latency(s)** ± SEM (Test)	**Omissions** ± SEM (Baseline)	**Omissions** ± SEM (Test)
Dependent				
0 mg/kg	2.54 ± 0.18	3.45 ± 0.39	0.44 ± 0.12	6.96 ± 2.65
1.25 mg/kg	3.45 ± 0.26	4.09 ± 0.39	0.87 ± 0.15	2.38 ± 0.48
2.5 mg/kg	2.84 ± 0.49	3.80 ± 0.45	0.78 ± 0.32	2.24 ± 0.49
5.0 mg/kg	3.30 ± 0.56	3.90 ± 0.50	0.74 ± 0.39	6.24 ± 3.32
Non-Dependent				
0 mg/kg	2.80 ± 0.34	2.52 ± 0.26	0.21 ± 0.15	0.93 ± 0.42
1.25 mg/kg	2.40 ± 0.30	2.72 ± 0.35	0.32 ± 0.10	0.87 ± 0.35
2.5 mg/kg	2.62 ± 0.26	2.92 ± 0.38	0.36 ± 0.16	1.87 ± 1.12
5.0 mg/kg	2.59 ± 0.41	3.95 ± 0.52	0.32 ± 0.08	1.78 ± 0.83

Table 2 Delay discounting response latency and omissions. Mean response latency in seconds and mean number of errors of omissions for both baseline and test delay discounting period presented by groups alongside the standard error of mean.

Group (**State** - *Acute Morphine Dose*)	**Sucrose Consumption** (%change ± SEM)
Dependent	
0 mg/kg	-35.7 ± 8.78
1.25 mg/kg	-39.5 ± 6.61
2.5 mg/kg	-28.9 ± 4.30
5.0 mg/kg	-27.4 ± 2.71
Non-Dependent	
0 mg/kg	0.1 ± 7.14
1.25 mg/kg	4.8 ± 6.34
2.5 mg/kg	-7.4 ± 5.90
5.0 mg/kg	-1.49 ± 5.89

Table 3 Free Sucrose consumption in the sucrose bottle test. Mean percentage change in sucrose consumption from baseline to test for each group is presented alongside standard error of the mean.

Measure	Units	Dependent ± SEM	Non-Dependent. ± SEM	Sig.
Morphine Tolerance				
Tail-Flick (10mg/kg Mor - Initial)	%MPE	81.61±4.73	N/A	
Tail-Flick (10mg/kg Mor - Dep)	%MPE	4.60±5.34	N/A	**P<.01**
Precipitated Withdrawal Signs				
Teeth-Chattering	Count	3.55±0.63	0.51±0.62	**P<.01**
Wet-Dog Shake	Count	1.00±.22	0.73±0.21	P=.48
Rearing	Count	15.42±1.08	29.39±1.05	**P<.01**
Burying	Count	2.28±0.34	1.52±0.33	**P<.01**
Writhing	Count	4.90±0.4	0.10±0.39	**P<.01**
Pstosis	%present	55.2	0	**P<.01**
Scream on Touch	%present	58.0	2.5	**P<.01**
Piloerection	%present	84.2	10.0	**P<.01**
Weight Change (10-min post- Nal)	g	-4.43±0.34	-0.60±0.33	**P<.01**
Weight Change (24-h post- Nal)	g	-3.78±0.66	-1.56±0.65	**P=.04**
Tail-Flick (1hr post- Nal)	%Baseline	92.9±6.54	107.5±6.40	**P=.03**

Table 4 Signs of morphine tolerance and withdrawal. The results of tests of tolerance to morphine analgesia and naloxone precipitated withdrawal in their appropriate units are presented alongside standard error of the mean. Fischer's exact significance of non-parametric independent samples median test was used to compare %present measures. For all other signs a Wilcoxon rank-sum test was used to compare dependent to non-dependent groups or median test to compare baseline to retest in the dependent group only (morphine tolerance). Significant tests are indicated in bold.

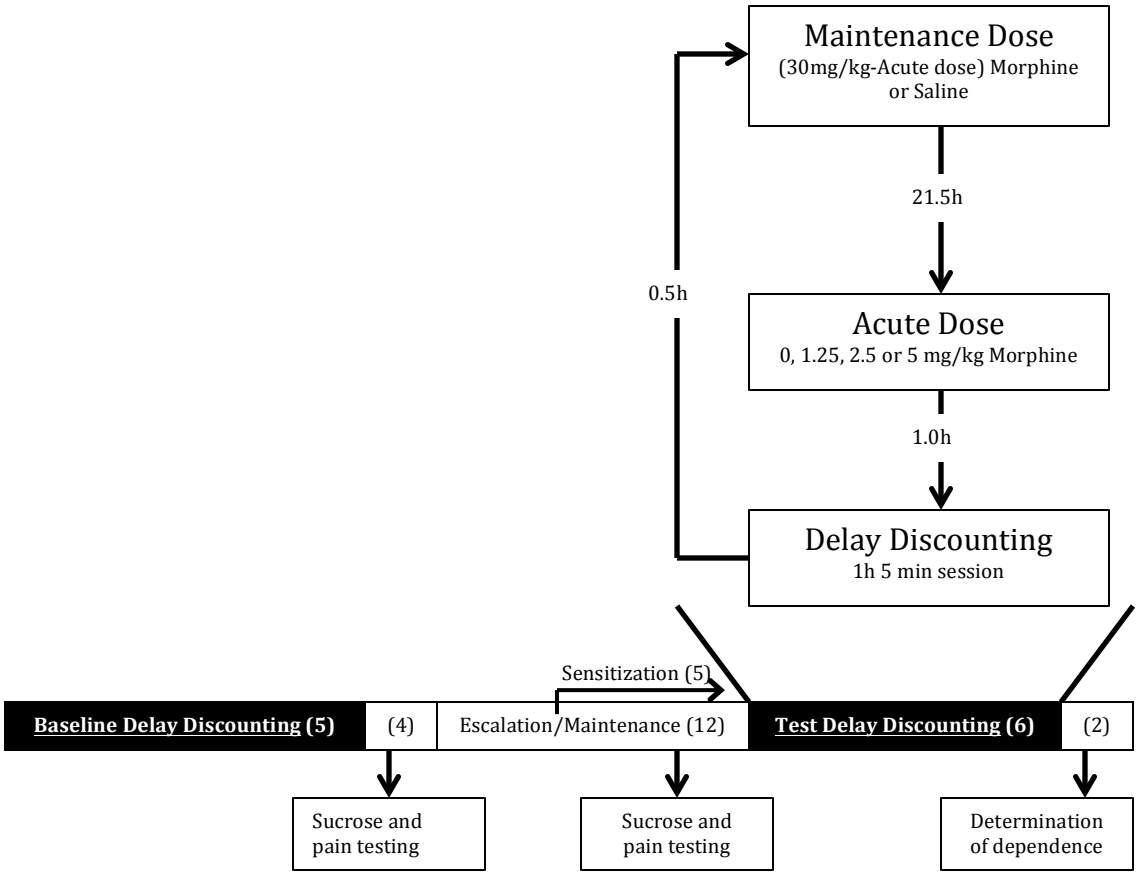

Figure 1 Schematic showing the full experimental structure (horizontal timeline), daily structure during the test period (above timeline) and auxiliary behavioral tests (below timeline). Filled boxes on the horizontal time line represent days in which rats were tested on the delay discounting task (baseline or test as indicated), unfilled boxes represent days they were not. The number of days spent at each interval is shown on the timeline in brackets.

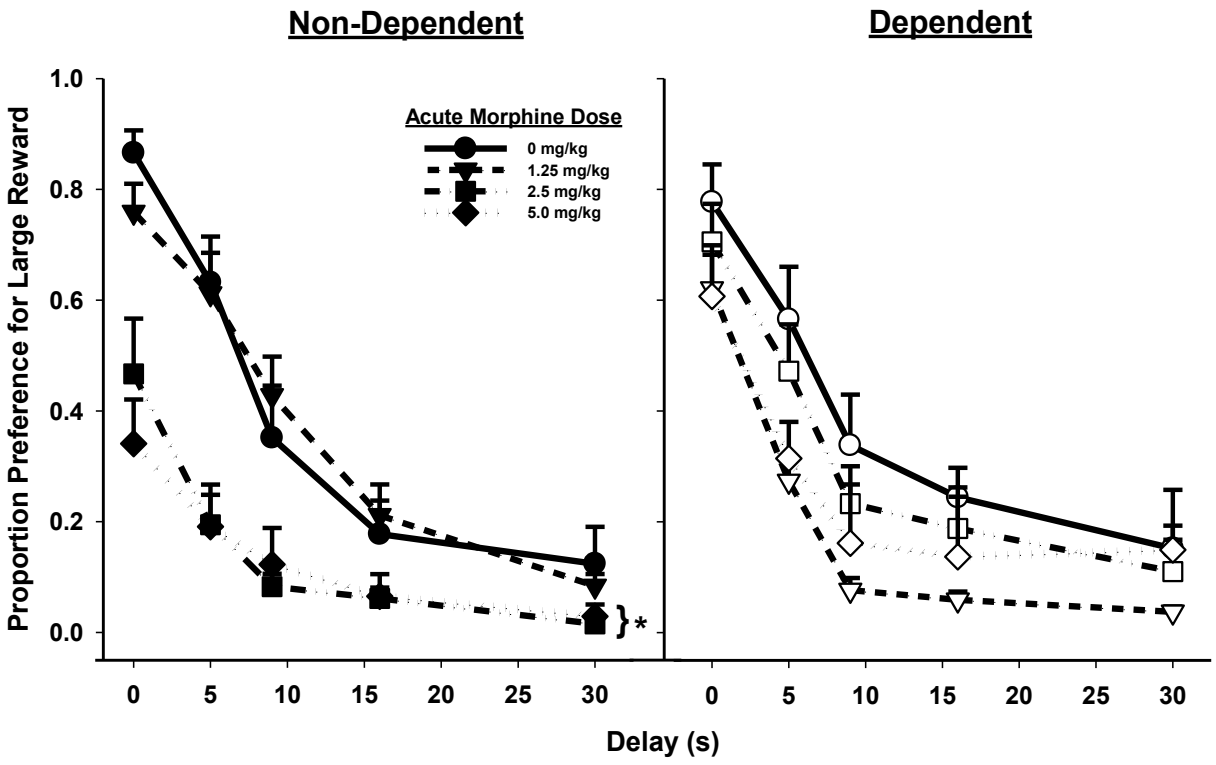

Figure 2 Delay Discounting. Delay discounting curves for non-dependent (left, filled symbols) and dependent rats (right, unfilled symbols) receiving either 0m/kg (circles, straight lines), 1.25mg/kg (inverted triangle, dashed lines), 2.5 mg/kg (squares, dot dashed lines) or 5.0mg (diamonds, dotted lines) prior to testing. N = 9-11 per group. * Significance of Tukey HSD $p<.01$.

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Chapter 5: The effect of withdrawal on impulsivity

5.1 Transition to Manuscript 4

In addition to the results reported in the third manuscript, experiment three also served as a replication of the original finding from experiment one, that morphine dependence can cause an increase in impulsivity in rats. In experiment three, dependent rats that received acute morphine during the dependence test period show significantly accelerated discounting compared to their baseline rates of discounting (2-way repeated measures ANNOVA, main effect of test period $F(1,9) = 9.204$, $p = .01$) (Supplemental Figure 1, appendix). However, the effect size of the increase in impulsivity was much smaller than that observed in experiment 1. Although animals in experiment three showed significant signs of naloxone-precipitated withdrawal compared to non-dependent rats, the extent to which they exhibited these signs were less extensive than observed in experiment one. This suggests that either some of the rats in the dependent group did not exhibit signs of withdrawal or that the group as a whole showed less intense withdrawal than expected. In the human literature, opiate dependent individuals are more impulsive when in withdrawal (Giordano et al., 2002). It follows that increased impulsivity in the period of dependence could have a dose-response relationship with withdrawal intensity. Thus, experiment four as presented in manuscript four was designed to both account for the discrepancies in effect size between experiment one and three and also to test the final proposed question of the thesis: Does withdrawal specifically contribute to impulsivity in dependent animals?

5.2 Manuscript 4: Naloxone-precipitated withdrawal causes an increase in impulsivity in morphine-dependent rats.

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Abstract

Rationale: Opiate dependence is associated with increased impulsivity in both humans and animals. Although the state of withdrawal appears to contribute to this effect, a causal relationship has not been shown. Here, we test whether precipitating withdrawal in morphine dependent rats via naloxone can cause increased impulsivity.

Methods: Rats were trained on a delay discounting task and then randomly assigned to either a dependent group that received a nightly 30 mg/kg subcutaneous dose of morphine, or a naïve group that received nightly saline. Once dependence was established, two-day test delay discounting curves were determined 1-h after 3 doses of naloxone (0mg/kg, 0.25 mg/kg, 0.5 mg/kg).

Results: In dependent rats both doses of naloxone caused increased preference for the small reward at short delays. Naloxone had no effect on delay discounting in naïve rats

Conclusions: Precipitating withdrawal in dependent rats can cause increased impulsivity.

Keywords: Delay Discounting, Impulsivity, Morphine, Naloxone, Opiate Dependence, Withdrawal, Decision Making.

Introduction

Humans and animals depreciate the subjective value of rewards as a function of the delay to their delivery. Impulsivity is an exaggerated preference for small immediate rewards over larger delayed rewards, and can be measured systematically using delay discounting. In such paradigms, drug dependent individuals (Bjork, Hommer, Grant, &

Danube, 2004; Madden, Petry, Badger, & Bickel, 1997; Heyman & Gibb, 2006; Hoffman et al., 2006) and chronically drug-exposed rats (Roesch, Takahashi, Gugsa, Bissonette, & Schoenbaum, 2007; Mendez et al., 2010; Richards, Sabol, & de Wit, 1999) show impulsivity compared to controls. This finding holds true in opiate-dependent individuals (de Wit, 2009; Madden, Bickel, & Jacobs, 1999; Pau, Lee, & Chan, 2002) and chronically opiate-exposed rats (Harvey-Lewis, Perdrizet, & Franklin, 2012; 2014; Schippers, Binnekade, Schoffelmeer, Pattij, & Vries, 2011).

In addition to chronic impulsivity, opiate dependent individuals may exhibit state-dependent increases in impulsivity. Opiate-dependent individuals are more impulsive when acutely-deprived -- just prior to their daily maintenance dose -- than one hour thereafter (Giordano et al., 2002). However, when compared to their respective opiate-dependent controls, abstinent individuals show decreased impulsivity (Kirby & Petry, 2004). Similarly, chronically opiate-treated rats show increased impulsivity during spontaneous withdrawal, but not after an extended period of abstinence (Harvey-Lewis et al., 2012; Schippers et al., 2011). Taken together, these results suggest that acute withdrawal may directly contribute to increased impulsivity in opiate dependent individuals.

Mu-opioid antagonists such as naloxone precipitate withdrawal in dependent rats, but neither cause withdrawal (Bläsig, Herz, Reinhold, & Zieglg nsberger, 1973; Frumkin, 1974) nor alter impulsivity in morphine-naïve rats (Kieres et al., 2004; Pattij, Schetters, Janssen, Wiskerke, & Schoffelmeer, 2009). Their effects in dependent rats are unknown. Here, by examining the effect of naloxone in morphine-dependent and morphine-naïve rats, we evaluate the effects of withdrawal on impulsivity.

Methods

Subjects

This research was reviewed by the Animal Ethics Committee of McGill University and carried out in accordance with the guidelines of the Canadian Council on Animal Care. 30 male Long Evans rats received between 150g and 175g (Charles Rivers Laboratories, Montreal, Que, Canada) were individually housed and maintained on a 12h light (7am to 7pm): 12h dark cycle. Food was restricted to maintain weight at 85% of age-adjusted values.

Testing Apparatus

Operant training and testing took place in two-lever (Coulbourn Instruments, Allentown, PA) plexiglas boxes, described in full by Harvey-Lewis (2012). Reward solutions were supplied to sipper cups below each lever by syringe pump (Model 22, Harvard Apparatus, St. Laurent, Que., Canada). An Isotemp 3016 (Fischer Scientific, Ottawa, Ont., Canada) was used to heat water to 54 +/- 0.1˚C for nociceptive testing.

Operant Response Training

Rats first underwent training using the methods in Harvey-Lewis (2012). Animals were trained to drink 20% sucrose solution from the sipper cups, and then trained to press each of the levers to receive 150 µl of this solution (FR-1). Next, rats learned to discriminate between pressing a lever that resulted in delivery of a small reward (50µl 20% sucrose) and one that resulted in delivery of a large reward (150µl 20% sucrose). Rats (n=4) not achieving criterion (80% large reward lever choice on 2 successive days, once per large reward location) after 16 sessions were excluded from further testing.

Delay Discounting Task

Daily sessions consisted of 5 blocks of 14, 55s trials. Each block began with 4 forced trials (2 per lever, randomized order) where only one lever was active, followed by 10 free choice trials wherein both levers were active. Pressing one lever produced an immediate small reward (50µl, 20% sucrose solution), and the other produced a large reward (150µl, 20% sucrose solution) after a delay. Delay increased in ascending order (0s, 5s, 9s, 16s, 30s) between blocks. The location of the two levers was counterbalanced between rats. A response on an active lever -- indicated by illumination of the corresponding cue light above -- caused cue lights to extinguish and the appropriate reward to be delivered after the programmed delay. If no response occurred within 35s the trial was scored as an omission and all lights were extinguished. A new trial began after 20s indicated by illumination of house and stimulus lights. Rats (n=2) that exhibited no change in preference with increasing delay were excluded from further testing.

Procedure

Fourteen days of delay discounting training immediately preceded the 2-d baseline period. Thereafter, rats were randomly assigned to receive nightly 30mg/kg morphine (dependent group) or saline (naïve group). 22-h after the 12th escalation dose (see dosing regimen), all rats were given a 1mL/kg dose of saline. 1-hr later they were tested on delay discounting as their initial 0mg/kg Naloxone test. Each test session was followed by 5 test-free days; no maintenance injections were given for 48-h, then 3 maintenance doses were given over the next 72-h. On the 6^{th} day animals received naloxone subcutaneously 1-h after the maintenance dose, and delay discounting evaluated 1-h later. This 6-d period was repeated so that 2 tests occurred per naloxone dose. To counterbalance order effects, rats received their naloxone doses, flanked by 0mg/kg tests, in either ascending order or descending order. Delay discounting curves were calculated by averaging the 2-d proportion preference at each delay for baseline and each dose of naloxone using the equation:

(1)Proportion preference = total large reward choices / total completed trials

Determination of Dependence

Dependence was assayed by tolerance to morphine-analgesia and signs of withdrawal precipitated by 1mg/kg naloxone. The average latency of withdrawal of a rat's tail from a 54^{o}C water bath was measured (10s upper limit) and used to calculate percentage of maximal possible effect (%MPE) with the equation:

(2)%MPE = (limit – test latency) *100 / (limit – baseline latency)

Morphine analgesia (compared to baseline) was determined 60-min after the initial 10mg/kg dose, and on the 12th day of morphine escalation. Following a 1mg/kg dose of naloxone, signs of withdrawal were scored; incidence of rearing, wet dog shakes, teeth chattering, writhing; presence or absence of scream on touch, piloerection and ptosis; weight loss; and 1-h hyperalgesia.

Drugs and dosing regimens

Morphine sulphate (Sigma-Aldrich, St. Louis, MO) was dissolved in 0.9% saline to volumes of 1mL/kg. Experimenter-administered maintenance doses were escalated over 12 days (10, 10, 15, 15, 20, 25, 30, 30, 30, 30 mg/kg Morphine). Naïve rats received saline injections as a control.

Data Analysis

Statistics were calculated using SPSS version 19.0 (SPSS Inc., Chicago, IL, USA) and Sigmaplot 11.0 (Systat Software Inc., Point Richmond, CA, USA). A 3-way mixed model ANOVA was performed to analyze proportion preference with group as a between groups factor and delay and dose as repeated measures. When significant interactions were observed, simple main effect analysis was used to compare the effects of delay and dose

(repeated measures) for each group. Post-hoc tests were corrected for multiple comparisons using Student-Newman-Kuels test. Withdrawal data was compared using the Mann-Whitney rank sum test for continuous measures and Fischer exact test for categorical measures.

Results

There were no significant differences in the baseline discounting curves of the two groups, suggesting subjects were trained to a similar level of performance (main effect of state, acute dosage and interaction effects, NS). During the test period, acute naloxone had significantly different effects in dependent and non-dependent rats (dose by group interaction ($F(2, 21) = 3.95$, $p = .035$)) (Fig. 1). Naloxone had no effect on delay discounting in non-dependent rats (simple main effects: dose and dose by delay interaction ($F(2, 24)$, $(8, 88) = 0.37, 0.61$, all p NS)) (Fig. 1). In dependent rats, there was a significant effect of naloxone dose on discounting (simple main effects: dose and dose by delay interaction ($F(2, 20)$, $(8, 80) = 6.08, 3.03$, all $p < .01$)) (Fig. 1). Specifically, when compared to saline, 0.25mg/kg naloxone caused an increased preference for the small reward at both 5 and 9s (SNK $q=6.316, 5.261$, p's $< .01$,), and 0.50mg/kg naloxone had similar effect at 9s delay (SNK $q=3.136$, $p = .03$). At the termination of the experiment, a 1mg/kg dose of naloxone induced significantly more withdrawal signs in dependent rats (table 1).

Discussion

To date, this is the first study in animals to examine the effects of precipitated withdrawal on impulsivity. In rats made dependent with nightly 30mg/kg morphine we found that naloxone produced accelerated discounting of sucrose rewards delivered after short delays. On the other hand, similar doses had no effect in naïve rats. This confirms that mu-opiate antagonist do not independently produce impulsivity (Kieres et al., 2004; Pattij et al., 2009). Instead the state of withdrawal, induced by mu-opiate antagonism in dependent rats, can cause increased impulsivity.

Previous studies have shown that drug-free but chronically opiate-experienced rats show increased impulsivity during periods of spontaneous withdrawal (Harvey-Lewis et al., 2012; 2014; Schippers et al., 2011). However in such studies, it is not clear whether withdrawal causes, or simply coincides with increased impulsivity in dependent rats. Here, by showing that inducing actue withdrawal with naloxone can directly cause increased impulsivity, we suggest that withdrawal itself is a contributing factor to the increased impulsivity observed in morphine dependence. These results confirm the parallel findings that withdrawal is specifically associated with increased impulsivity in opiate-dependent humans (Giordano et al., 2002).

Figures

Measure	Units	Dependent ± SEM	Naïve ± SEM	Sig.
Morphine Tolerance				
Tail-Flick (10mg/kg Mor - Initial)	%MPE	72.14±10.8	N/A	
Tail-Flick (10mg/kg Mor –Dep)	%MPE	13.68±9.81	N/A	**P<.01**
Precipitated Withdrawal Signs				
Teeth-Chattering	Count	5.55±1.55	0.15±0.15	**P<.01**
Wet-Dog Shake	Count	1.82±.70	0.54±0.31	P=.09
Rearing	Count	11.64±1.04	32.46±2.13	**P<.01**
Burying	Count	3.18±0.60	1.62±0.50	P=.06
Writhing	Count	5.0±1.36	0±0	**P<.01**
Pstosis	%Present	63.63	0	**P<.01**
Scream on Touch	%Present	72.72	0	P=.21
Piloerection	%Present	100	0	**P<.01**
Weight Change (10-min post- Nal)	g	-5.0±0.66	-1.0±0.41	**P<.01**
Weight Change (24-h post- Nal)	g	-4.73±1.54	-0.23±0.72	**P<.05**
Tail-Flick (1hr post- Nal)	%Baseline	-23.93±22.7	-13.04±8.96	P=.64

Table 1 Signs of morphine tolerance and withdrawal. Group means for tests of tolerance to morphine analgesia, and naloxone-precipitated withdrawal in their corresponding units are presented alongside standard error of the mean and significance of statistical tests (Significant tests are indicated in bold).

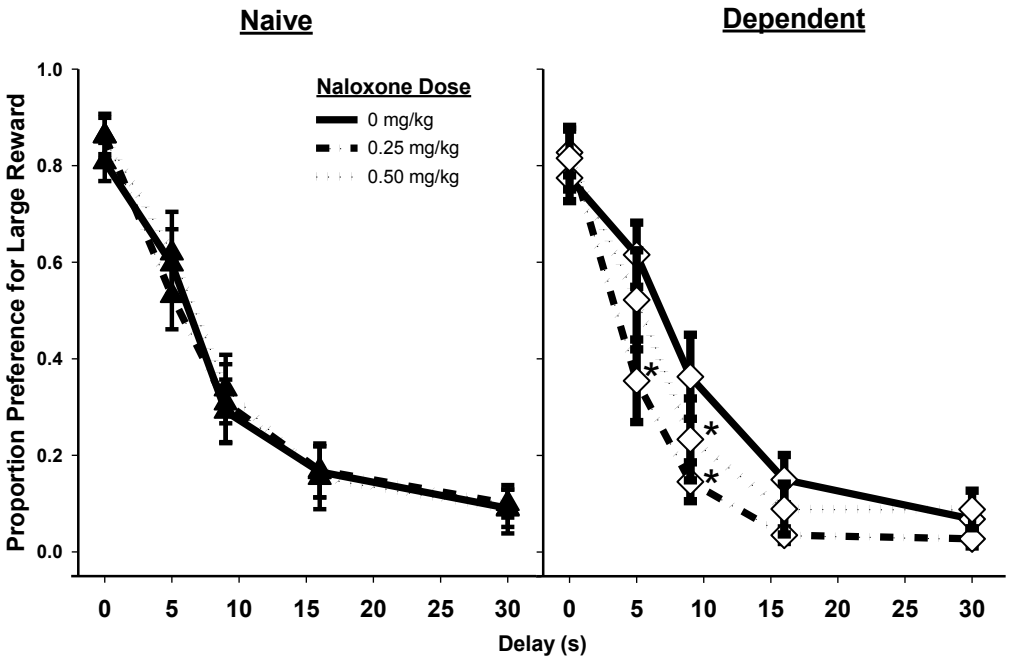

Figure 1 Delay discounting curves for naïve (left – filled triangles) and dependent rats (right – empty diamonds) receiving either 0m/kg (solid lines), .25 mg/kg (dashed lines) or 0.5mg (dotted lines) prior to testing. * Significance of SNK $p<.05$.

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Chapter 6: General Discussion

6.1 Summary

The experiments presented in this thesis are the first to extensively investigate the effects of opiate dependence on impulsivity in animals. Their results suggest that morphine dependence causes a transient increase in impulsivity restricted to the period of dependence. The increased impulsivity cannot be accounted for by a decrease in motivation to acquire sucrose rewards. The level of impulsivity observed is exaggerated when withdrawal is precipitated using a μ-opiate antagonist and when animals are made to discount morphine rewards. Additionally, dependence causes tolerance to the effects of acute morphine on delay discounting.

6.2 Extended Discussion of Results

First and foremost, our results support the hypothesis that the increased impulsivity observed in the human opiate dependent population is at least partially a consequence of repeated exposure to large doses of opiates. In experiment one we observe that inducing morphine dependence can cause accelerated delay discounting in rats during the period of dependence when compared to matched controls and pre-dependence baselines. Similarly, in experiment two, dependent rats show accelerated delay discounting of morphine rewards compared to pre-dependence baseline and matched non-dependent controls. We

also directly replicate our original finding in experiment three, where dependent rats that received a saline injection prior to testing showed accelerated discounting of sucrose rewards compared to their pre-dependence baselines. However, experiment three suggests that morphine dependence may have a more subtle effect on impulsivity than observed in experiment one.

The results of the experiments presented herein also suggest that the extent to which morphine dependence causes accelerated delay discounting in rats is modulated by at least two other factors. Firstly, preexisting/baseline levels of impulsivity modulate the magnitude of change in impulsivity conferred by morphine dependence. Across all experiments a significant correlation was found between low baseline impulsivity and greater magnitude of increased impulsivity caused by morphine dependence (supplemental figure 2, appendix). There was no significant relationship between preexisting impulsivity and change in impulsivity in non-dependent animals (supplemental figure 3, appendix). This suggests that the less impulsive an animal is prior to initiation of dependence, the greater the increase in impulsivity that will be exhibited during dependence. This could suggest that low impulsivity is a vulnerability trait for dependence-induced increased impulsivity. However, previous studies with inter-strain differences in impulsivity have had mixed results. Lewis rats, a more impulsive rat strain, have been shown to have greater increases in impulsivity conferred by chronic amphetamine administration than the less impulsive Fischer strain (Huskinson, Krebs, & Anderson, 2012). There were no differences in changes in impulsivity between these strains following chronic nicotine treatment (Anderson & Diller, 2010). It is possible that the two results differ due to use of different drugs, and this relationship is drug specific. Thus, these

previous studies suggest that high innate impulsivity due to genetics is a vulnerability trait for increased impulsivity as a result of chronic exposure to some drugs, but not others.

To date, the only study that has investigated this relationship within strain, found that low impulsivity animals show greater increases in impulsivity as a result of chronic nicotine exposure (Kayir, Semenova, & Markou, 2014). This result, similar to the findings presented here, may suggest that the tendency of low impulsive animals to exhibit larger effects of chronic drug exposure is an experimental artifact, specifically a ceiling effect. The delay discounting procedure has a maximal level of impulsivity it can measure, and due to this fact, high impulsivity animals simply have less room to show an effect. Low impulsivity animals are further from impulsivity ceiling and thus would have more room for change in impulsivity. The highest level of impulsivity detectable would vary between methodologies; those procedures that employ short initial delays or have a high large: small reward ratio are more sensitive to highly impulsive animals and would have a higher ceiling. This could also explain why the effect of chronic drug exposure on impulsivity varies within the literature, as ceiling effects will have varying impact with different procedures. Thus, preexisting levels of impulsivity -- acting either as vulnerability factor or through a floor effect -- could reasonably explain the discrepancy in effect size between the large increase in impulsivity conferred by dependence observed in experiment one and the relatively small effect in the replication groups in experiment three.

Secondly, our results suggest that withdrawal specifically contributes to the increased impulsivity exhibited by opiate dependent individuals. In experiment four, μ-opioid antagonism caused increased impulsivity in morphine-dependent rats, but not in morphine-naïve rats. This would suggest μ-opioid antagonists only cause impulsivity when

their actions precipitate withdrawal. The hypothesis that withdrawal specifically contributes to impulsivity is also supported by the increase in impulsivity (compared to pre-dependence baselines) observed during the period of dependence in experiments one and three. Specifically, the effect was observed at a time-point when animals were drug-free and experiencing spontaneous withdrawal. Likewise, animals in experiment one, tested after a period of abstinence far exceeding the duration of withdrawal did not exhibit increased impulsivity. Since withdrawal requires concurrent dependence it follows that increased impulsivity would only be observed during the period of dependence when withdrawal is present. These results mirror the finding of Giordano and colleagues (2002) that opiate dependent individuals are more impulsive during withdrawal than when satiated. Similarly, these results support both human and animal findings that increased impulsivity in opiate dependent individuals is restricted to the period of dependence (Kirby & Petry, 2004; Schippers et al., 2011).

Withdrawal severity and intensity may also be an explanation for the reduced effect of dependence on impulsivity observed in experiment three. When withdrawal was precipitated with 1mg/kg naloxone in experiment three, fewer and less extensive signs of withdrawal where observed in dependent rats than in experiment one. This would suggest that the dependent group in experiment three experienced a relatively weaker withdrawal syndrome than dependent animals in experiment one. Alternatively, a portion of the dependent animals in experiment three could have been withdrawal insensitive. In either case, given a relationship between withdrawal intensity and accelerated delay discounting, we would expect less effect of morphine dependence on discounting in experiment three. Although animals in both experiments had similar dosing regimens, many other factors

could possibility influence withdrawal severity including seasonality (Shoham-Moshonov & Weinstock, 1977; Williams & Spratto, 1978). All animals in experiment one were testing during winter, whereas animals in experiment three were evenly distributed throughout the year.

The neurochemical underpinnings of withdrawal also provide a plausible mechanism for the increased impulsivity observed in dependent animals. Mu-opioid antagonism in non-dependent animals has no effect on striatal dopaminergic release (Daudet, Leviel, Kerny, & Guibert, 1983) or dopamine neuron activity (Diana, Muntoni, Pistis, Melis, & Gessa, 1999). However, decreases in dopamine release in the nucleus accumbens are observed during naloxone-precipitated withdrawal (Pothos et al., 1991) and spontaneous withdrawal in dependent animals (Acquas & Chiara, 1992). Single-cell recordings show that withdrawal-induced depression of dopamine neurons persist for at least seven days but returns to baseline after fourteen days (Diana et al., 1999). Interestingly, lesions and disconnections of the whole nucleus accumbens or its core are associated with accelerated rates of delay discounting (Bezzina, Body, & Cheung, 2008) (Cardinal & Howes, 2005; da Costa Araújo, Body, & Hampson, 2009). Similarly, dopaminergic antagonism also causes accelerated delay discounting (van Gaalen, van Koten, Schoffelmeer, & Vanderschuren, 2006). It follows that decreased dopaminergic release in the nucleus accumbens during withdrawal could result in increased impulsivity in opiate dependent animals. This would also account for the lack of extending effect observed in experiment one; after an extended period of abstinence dopaminergic release returns to baseline causing a return to baseline levels of discounting.

The effects of acute morphine on impulsivity are also modulated by morphine dependence. Firstly, as shown in experiment two, morphine dependent animals made to discount a morphine solution do so at quicker rate than dependent animals made to discount a non-morphine solution. This would suggest that dependent animals are more impulsive towards morphine rewards than non-morphine rewards. This finding supports the similar finding in the human literature that opiate-dependent individuals are more impulsive towards opiate rewards (Kirby et al., 1999; Madden et al., 1997). Unlike human experiments, experiment two controls for reward type and further shows that this effect is specific to the dependent population; non-dependent animals show similar delay discounting of morphine and non-morphine rewards.

Motivational factors probably underlie the accelerated discounting of drug rewards in dependent animals. Morphine dependence can increase the motivational salience of drug rewards and simultaneously decrease motivational drive towards non-drug rewards (Cooper et al., 2010; G. C. Harris & Aston-Jones, 2002; Rouibi & Contarino, 2011). Previous studies have shown that increased motivation to acquire rewards causes accelerated discounting (Eisenberger et al., 1982). Here, dependent rats that have increased motivation to acquire drug rewards also show accelerating discounting thereof. In experiment one, decreasing motivation to acquire sucrose rewards by switching animals to an ad lib diet caused a trend towards flattening of delay discounting curves and decreased relative preference for large rewards over small ones. A similar change in discounting was observed when dependent rats were made to discount non-drug rewards in experiment two. Thus, changes in motivation in dependent rats, specifically the presence of acquirable

morphine rewards that are motivationally salient lead to their relative accelerated discounting when compared to less salient non-morphine rewards.

Secondly, morphine dependence also modulates the effects of acute morphine on delay discounting. In experiment three, small to moderate doses of morphine given one hour prior to testing, cause accelerated discounting of delayed rewards in non-dependent rats. Additionally, acute morphine caused a general shift towards preference for smaller rewards over larger ones when both are delivered without delay. On the other hand, similar doses of acute morphine resulted in neither of these effects in dependent rats; acute morphine has no significant effect on discounting in animals currently dependent on morphine. This would suggest that morphine dependence induces tolerance to the acute effects of morphine on delay discounting.

This result also provides a plausible explanation for the discrepancies in the previous literature pertaining to acute morphine and impulsivity. Previous studies have reported that acute morphine has no effect on delay discounting, or that it causes either accelerated discounting, decreased relative preference for large rewards, or both (Kieres et al., 2004; Pattij, Schetters, Janssen, Wiskerke, & Schoffelmeer, 2009; Tanno et al., 2014). Experiment three shows that in non-dependent rats low dose morphine has no effect. Moderate dose morphine can cause increased impulsivity and impaired reward size discrimination in non-dependent rats. The highest dose of morphine tested caused decreased reward size discrimination without increased impulsivity. Thus the full variety of results reported in previous studies reasonably fall within the dose-response curve tested here. However, Kieres (2004) find that morphine has no effect on discounting at moderate doses. It is possible that in this study repeated and/or prolonged exposure may

have inadvertently induced dependence; six weeks of exposure to even moderate doses of morphine is sufficient to cause tolerance to some acute effects of morphine (Tjon et al., 1995). Thus, the variation of the effect of acute morphine on delay discounting due to size of dose and previous exposure to morphine, sufficiently explains the discrepancy in previous literature.

The observation that morphine dependence causes tolerance towards the effects of acute morphine on delay discounting is particularly interesting as it suggests that the underlying mechanism could possibly be mediated through the system that usually produces sensitization. In dependent rats the rewarding effects of morphine are hypothesized to be produced via dopaminergic release in the nucleus accumbens, whereas these effects are modulated by a dopamine-independent process in the TPP in non-dependent rats (Ting-A-Kee & van der Kooy, 2012). Because the effects of acute morphine on delay discounting are exclusive to non-dependent animals, it is plausible that these effects are mediated through the TPP pathway. However, the involvement of the TPP in delay discounting has yet to be investigated. It follows that in dependent rats acute morphine could cause dopaminergic release in the nucleus accumbens (van Gaalen et al., 2006). As previously noted dopaminergic release in the nucleus accumbens can cause decreased impulsivity. In this way, the switch to dopamine-dependent effects of acute morphine in dependent rats could cause decreased impulsivity due to dopaminergic release in the nucleus accumbens. However, it is possible that decreased impulsivity due to dopaminergic release cancels some other effect of acute morphine that causes increased impulsivity as observed in non-dependent rats. The net effect would then be no changes in delay discounting from baseline when dependent rats receive acute morphine, as observed

in experiment three. This hypothesis can be tested by administering larger acute doses of morphine to dependent rats, which should increase dopaminergic release to such an extent to negate the impulsivity-inducing effects, resulting in a net decrease in impulsivity. It is also possible that the acute morphine-induced increased impulsivity observed in non-dependent rats and the lack thereof in dependent rats is not mediated through the morphine reward pathway. In this case, the tolerance observed in dependent rats would likely be due to either receptor desensitization or protein decoupling.

6.3 Future Directions and Implications

The methods used in experiment one through four, provide a scaffold to further investigate the nature of the mechanism underlying the effects of acute and chronic morphine on impulsivity. To date, no experiment has specifically investigated the areas of the brain that contribute to acute morphine's effect on both reward discrimination and impulsive choice. The extent to which the TPP pathway contributes to one or both of these effects could be explored through lidocane or optogenetic inactivation of the TPP (Ting-A-Kee et al., 2013) during delay discounting testing in non-dependent animals. Similarly, the hypothesis that tolerance to these effects is caused by the switch to the dopaminergic signaling in dependent rats can be systematically investigated. Specifically, this hypothesis can be tested by reverting the mechanism of opiate reward in dependent animals to the dopamine-independent mechanism using pharmacological manipulations such as: inactivating GABAa receptors using muscimol, inhibiting the carbonic anhydrase enzyme with Acetazolamide, or optogenetic inhibition of the BDNF trkB receptor. If the hypothesis

is correct, combining these manipulations with acute morphine administration in dependent rats should result in increased impulsivity and/or reward discrimination deficits similar to those seen in non-dependent rats.

Regardless of the underlying mechanism of action, experiment three is the first animal experiment to document dependence-induced tolerance to the effects of morphine on a decision-making task. This suggests that other higher order processes may be subject to tolerance and/or sensitization due to opiate dependence. Further experiments are needed in this field to examine how dependence alters the way in which opiates exert their influence on other decision-making processes. But, the results here suggest that tolerance and sensitization effects must be considered when evaluating the psychological mechanisms that promote continued reuse of opiates in the dependent population.

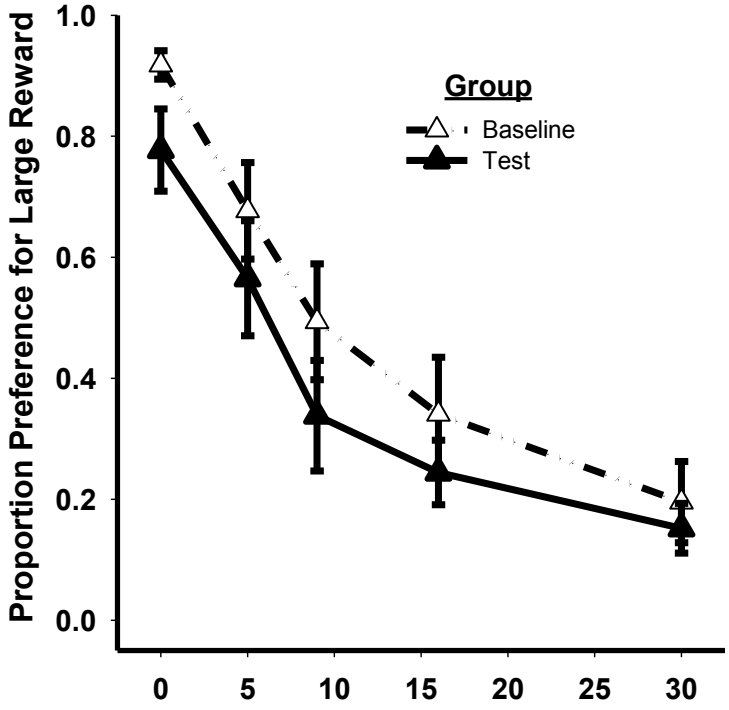
As it pertains to the human dependent literature, the findings presented in this thesis have particular relevance to opiate replacement therapy. Opiate replacement has become a frequently used method of treating dependence (Amato, Davoli, Perucci, & Ferri, 2005). But, one of the objections against its therapeutic use is that acute doses of opiates may have effects that are counterproductive to rehabilitation. For example, if acute doses of opiates can cause increased impulsive decision making then individuals might be more likely to relapse due to the increased salience of a immediate high that would be provided by drug use (Bickel & Marsch, 2001). The results presented here suggest that it is unlikely -- due to tolerance caused by dependence -- that individuals in opiate replacement therapy would become more impulsive as a result of their maintenance doses. This is also confirmed by the human literature that suggests that opiate dependent individuals are less impulsive after daily maintenance doses (Giordano et al., 2002). It will be instructive to

evaluate whether or not other relevant decision making processes, such as risk-taking, are affected by opiate maintenance therapy.

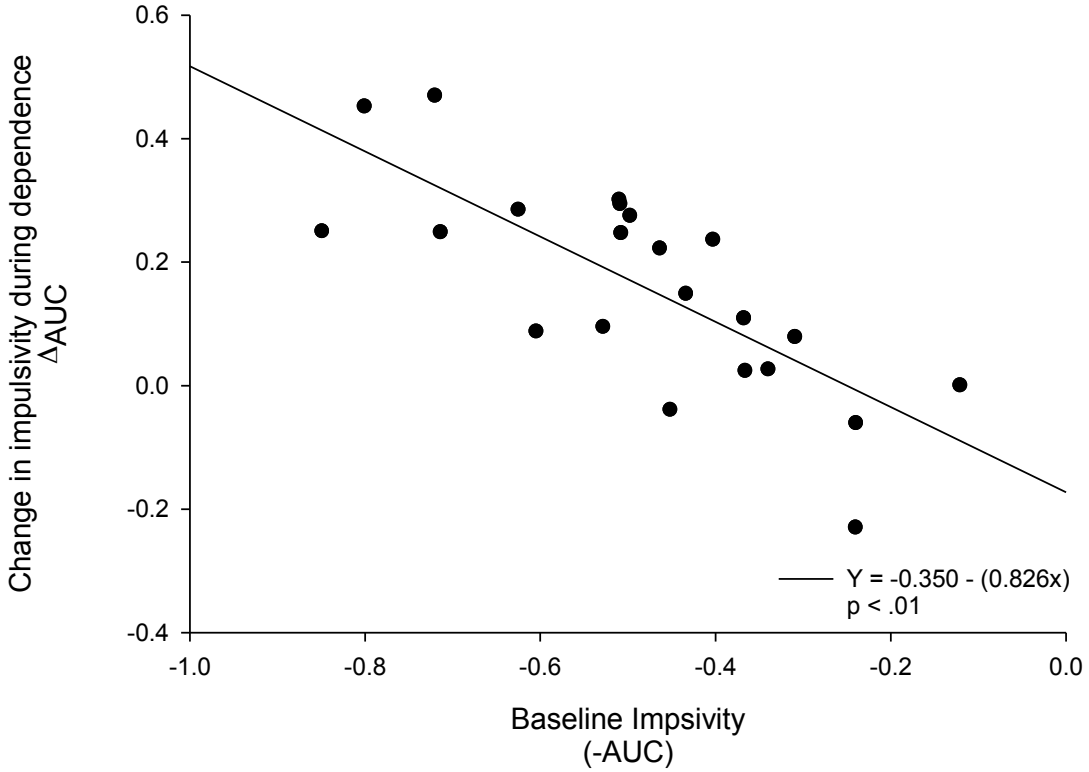
6.4 Conclusion

As stated in the thesis proposal the main questions addressed were: (1) Can morphine dependence cause impulsivity? (2) Are dependent animals more impulsive towards drug rewards than non-drug rewards? (3) Does dependence cause tolerance or sensitization to the effect of acute morphine on impulsivity? (4) Does withdrawal specifically contribute to impulsivity in dependent animals? In summary, the questions were successfully answered thusly: (1) Morphine dependence can cause an increases in impulsivity, (2) Dependent animals are more impulsive towards drug rewards than non-drug rewards, (3) Dependence causes tolerance to the effect of acute morphine on impulsivity and, (4) The state of withdrawal specifically contributes to impulsivity in dependent animals. The series of experiments presented here strongly support observations from the human literature that opiate dependence is associated with impulsivity. The results further add to that literature by specifically showing that increased impulsivity caused by opiate dependence is likely responsible for the association observed in the literature. Finally, this series of experiments also shows that opiate dependence can additionally cause state- and reward- specific changes in impulsivity including tolerance to the impulsivity-inducing effects of morphine.

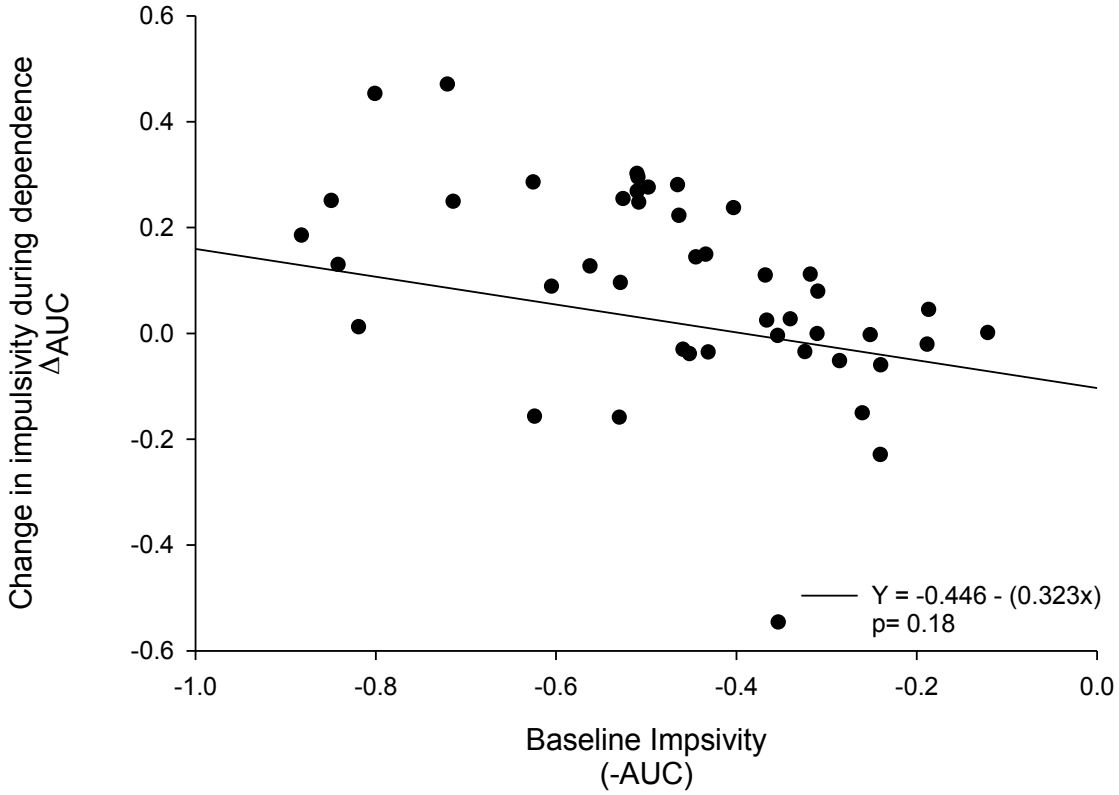
Appendix



Supplemental Figure 1 Replication of the effect of dependence on impulsivity in Experiment 3. Delay discounting curves for dependent rats either at baseline prior to initiation dependence (open triangles, dot-dashed lines) and at test while dependent and receiving saline injections prior to testing (filled triangles, solid lines).



Supplemental Figure 2 Linear regression of change in impulsivity caused by dependence against baseline impulsivity for dependent rats. This analysis incorporates all animals from replication groups in experiments 1-3. Equation of the regression and the significance of its slope is presented in the lower right corner.



Supplemental Figure 3 Linear regression of change in impulsivity caused by dependence against baseline impulsivity for non-dependent/naïve rats. This analysis incorporates all animals from replication groups in experiments 1-3. Equation of the regression and the significance of its slope is presented in the lower right corner

Group	**Latency(s)** ± SEM (Baseline)	**Latency(s)** ± SEM (Test)	**Omissions** ± SEM (Baseline)	**Omissions** ± SEM (Test)
Dependent	2.6 ± 0.31	4.0 ± 0.41	1.2 ± 0.32	10.8 ± 2.17
Naive	3.6 ± 0.22	3.0 ± 0.31	2.8 ± 1.12	1.4 ± 0.32

Supplemental Table 1: Delay discounting response latency and omissions for Experiment 1a. Mean response latency in seconds and mean number of errors of omissions for both baseline and test delay discounting period presented by groups alongside the standard error of mean.

Group (**State** – *Drug type*)	**Latency(s)** ± SEM (Baseline)	**Latency(s)** ± SEM (Test)	**Omissions** ± SEM (Baseline)	**Omissions** ± SEM (Test)
Dependent				
Drug	2.22 ± 0.19	4.10 ± 0.67	0.67 ± 0.12	0.73 ± 0.21
Non-Drug	2.98 ± 0.26	3.87 ± 0.41	3.44 ± 1.2	3.82 ± 1.3
Non-Dependent				
Drug	3.0 ± 0.34	2.52 ± 0.26	0.55 ± 0.15	6.27 ± 2.1
Non-Drug	2.40 ± 0.30	3.44 ± 1.2	0.70 ± .23	2.47 ± 3.5

Supplemental Table 2: Delay discounting response latency and omissions for Experiment 2b. Mean response latency in seconds and mean number of errors of omissions for both baseline and test delay discounting period presented by groups alongside the standard error of mean.

Measure	Units	Dependent ± SEM	Naïve ± SEM	Sig.
Delay Discounting - Omissions				
0mg/kg Naloxone	%Trials	4.32±0.62	2.19±0.40	
0.25mg/kg Naloxone	%Trials	3.77±0.82	2.54±0.36	
0.50mg/kg Naloxone	%Trials	2.86±0.54	2.50±0.40	
Delay Discounting - Latency				
0mg/kg Naloxone	s	4.09±0.21	3.07±0.25	
0.25mg/kg Naloxone	s	4.31±0.43	3.48±0.31	
0.50mg/kg Naloxone	s	3.93±0.32	3.38±0.25	

Supplemental Table 3: Delay discounting response latency and omissions for Experiment 4. Mean response latency in seconds and mean number of errors of omissions for both baseline and test delay discounting period presented by groups alongside the standard error of mean.

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