The Effect of Morphine Dependence and Withdrawal on Morphine Reward Efficacy as Evaluated by the Intra-Cranial Self-Stimulation Rate-Frequency Function

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Abstract

An important issue in the drug dependence literature is the extent to which dependence and withdrawal contribute to the motivational forces driving drug taking. One theory asserts that dependent individuals re-administer opiates primarily to remove the negative effects of withdrawal; a second theory predicts that administration of increasing doses is due to motivational desensitization to the acute rewarding effects of opiates. Studies of drug reward utilizing self-administration rates are hard to interpret because of the complex effects of tolerance and drug kinetics on response rate. Since rewards summate, the efficacy of drug rewards can be assessed by their effects on the rewarding effect of electrical brain stimulation. We examined the influence of morphine dependence on the function relating response rate to the pulse frequency of brain stimulation. The M50 index of this function assesses changes in reward efficacy independent of a drugs effect on performance. Rats were randomly assigned to one of 3 groups. A dependent (D) group that received a nightly subcutaneous dose of morphine of 30 mg/kg, a non-dependent (ND) group that received a nightly saline injection or a food deprivation (FD) group that also received nightly saline but food consumption was controlled to match the loss of body weight in the D group. Rats were tested to determine the M50 1-h and 3-h after doses of morphine, and 18+ hr after nightly injections – a time point during which dependent animals are in withdrawal. Doses tested in ascending order were 0mg/kg, 1 mg/kg, 3 mg/kg, 10 mg/kg and 30 mg/kg morphine s.c. administered once a day for three successive days. Morphine decreased the M50 to a dose ceiling after which higher doses caused less facilitation. Morphine 1 mg/kg and 3 mg/kg caused equivalent decrease in

M50 in D, ND and FD rats but for D rats the ceiling was shifted up to 10mg/kg. The morphine dose-response curve was otherwise the same in D, ND and FD rats. Additionally, withdrawal had no effect on M50. Our result support neither changes in reward efficacy due to withdrawal nor desensitization of reward due to tolerance. Rather the results suggest that high doses of morphine are more effective rewards to dependent rats because tolerance removes a reward or performance depressing effect.

Resume

Un important point dans l'étude de la dépendance aux drogues dures est l'ampleur avec laquelle le syndrome de manque contribue aux forces motivationnelles qui incitent a la consommation de drogues. Une théorie suggère que le consommateur accro aux drogues continue la consommation d'opiacées principalement pour alléger les effets de manque ; une deuxième théorie prédit que l'augmentation de doses d'opiacées administrées est un effet de dé-sensitizations au effet benthiques aigue de ces dites drogues. Les études examinant l'effet récompensant des drogues en utilisant l'autostimulation intracrânienne sont difficiles a interpréter due aux a la complexité des effets qu'a tolérance et la cinétique chimique des drogues sur la cadence de réponse. La propriété sommatrice des récompenses fait en sorte que l'efficacité des drogues peut être évaluée grâce a leurs effets sur l'autostimulation intracrânienne. Nous avons examine l'influence de la dépendance a la morphine sur la fonction reliant la cadence de réponse au pulse de fréquence durant la stimulation intracrânienne. L'indexe M50 de cette fonction jauge les changements de l'efficacité de la récompense indépendamment de l'effet qu'a la drogue sur la performance de l'animale. Les rats ont été assigne a un des 3 groupes aléatoirement. Un groupe dependant (D) qui a reçu une dose de morphine de 30mg/Kg sous-cutanée chaque nuit, a groupe Non-Dependant (ND) qui a reçu une dose de solution saline chaque nuit ou un groupe qui a ete prive de nourriture (food deprivation –FD) qui a aussi reçu une dose de solution saline chaque nuit, cependant la consommation de nourriture a été contrôlée de façon a coïncider avec la perte de poids subite par le group D. Les rats on ete teste afin de déterminer le point M50 1 heure et 3 heures après l'injection de morphine, ainsi que 18+ heures après l'injection nocturne – qui est un

moment ou les rats dépendants sont en phase de manque. Les doses testées en ordre croissant sont 0mg/Kg, 1mg/Kg, 10 mg/Kg et 30 mg/Kg de morphine injectée sous-cutanée une fois par jour. La morphine réduit le point M50 à une dose plus haute suite a quoi une dose plus haute a un moindre effet de facilitation. Les doses de morphine de 1mg/Kg et 3 mg/Kg on réduit le point M50 de façon similaire pour les groupes D, ND et FD cependant dans le cas du group D, l'effet plafond est déplace au niveau de 10 mg/Kg. La courbe de dose-dependence de morphine est similaire pour les groupes D, ND et FD. De plus, le phénomène de manque n'a eu aucun effet sur le point M50. Nos résultats ne suggèrent pas de changement d 'efficacité de récompense relie au phénomène de manque ni de desensitization due a la tolérance. Les résultats suggèrent qu'une forte dose de morphine est une récompense plus efficace pour les rats dépendants et ce parce que la tolérance soustrait la récompense même ou l'effet de réduction de performance.

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1. Introduction

1.1 Background

In 1954, James Olds and Peter Milner attempted to use brain stimulation of the reticular formation to produce conditional firing of the arousal system, which they theorized would facilitate learning in a T-maze paradigm (Milner, 1989). The planned paradigm proved to be largely unsuccessful. However, a single rat appeared to experience reward whenever given a bout of electrical stimulation. It was later determined by x-ray that the electrode had been serendipitously misplaced in the region of the septal area (Milner, 1989). Olds and Milner (1954) designed a set of experiments to explore the possibility that inter-cranial electrical stimulation would act as a reward. The results of these experiments showed that rats would perform an operant task of lever pressing to deliver a pulse of 60 Hz of alternating current to several areas of the brain. Olds and Milner postulated that this stimulation excited a system in the brain, the function of which was to reward behavior. Thus, they believed that this technique could be used to explore the physiological basis of reward. Five decades later, the original methodology of Olds and Milner has been pruned and honed to produce the contemporary methodology of intra-cranial self-stimulation (ICSS), a paradigm that allows us to effectively investigate the efficacy of rewards.

Soon after the original discovery of ICSS, researchers began investigating its properties and appropriateness as a reinforcer. According to the law of effect, if brain stimulation reward (BSR) was truly a reinforcer then a response that resulted in its

administration would in fact increase in probability (Leslie, 2006). To address its generalizability as a reinforcer, BSR was used to condition a wide-variety of responses including bar pressing (J. Olds & Milner, 1954), locomotion (J. Olds, Killam, & Bach-Y-Rita, 1956), licking (Gibson, Reid, Sakai, & Porter, 1965), and nose poke (Ross, 1973). Thus, while ICSS fit in the theoretic framework of reward with traditional methods of reinforcement, such as water and food, some properties of ICSS differed significantly from traditional methods of rewarding behavior. Firstly, ICSS-trained responses seemed to have a rapid extinction; trained responses decayed to almost a tenth of average rate during extinction following maximal responding trials (Deutsch, 1963). Additionally, when responses were rewarded at a threshold level they extinguished completely within 30 responses (Deutsch, 1963). Secondly, responding at large response-reward ratios was hard to achieve; Deutsch (1963) reported that the uppermost limit of reinforcement for rats was a variable interval (VI) of sixteen seconds, or a fixed ratio (FR) of seven, levels much fewer than what could be achieved with food or water reinforcement. Thirdly, ICSS responding seemed to be insatiable, as responding would continue uninterrupted for as long as 24 hours (Trowill, Panksepp, & Gandelman, 1969). When given a two-lever paradigm, one lever for food and another for BSR, rats would often exclusively administer brain stimulation to the point of self-starvation (Routtenberg & Lindy, 1965). Lastly, rewarding properties of ICSS were non-transferable; secondary reinforcer responses extinguished with the removal of the brain stimulation (Mogenson, 1965). It must be noted that these particulars of ICSS were later shown to not be related to ICSS per se, but to the method of delivery, that is, rapid onset and offset (Gibson et al., 1965). Nevertheless, the overall conclusion was that BSR was rewarding. However, The

question of whether the peculiarities of ICSS would be helpful or a setback in the studying of reward was yet to be addressed.

In traditional conditioning experiments rats were often food deprived under the empirically supported rationale that rewards were more salient if a subject was in a state of deprivation. Thus, many traditional rewards were hypothesized to fit into a drive-reducing hypothesis; their reinforcing nature was based on eliminating an organismic state of need (Milner, 1975). If ICSS truly tapped into the drive system responsible for rewarding behavior, then inducing a state (such as hunger) that would cause an increase in drive to obtain a natural reward (in this case food) should cause a parallel shift in the drive to obtain BSR. Studies found that hunger increased rates of ICSS (Brady, Boren, Conrad, & Sidman, 1957) and delayed the time to extinction (M. E. Olds & Christenson, 1970), but only when electrodes were placed in areas of the brain associated with feeding, specifically the hypothalamus (Goldstein, Hill, & Templer, 1970, Olds, 1958 #48). Similar results were found for water deprivation (Brady et al., 1957) and an increase in male androgen hormone to mimic sex drive (J. Olds, 1958).

On the other hand, there was a competing theory of incentive motivation, that is, external contingencies pull the organism to execute a response through processes such as increasing arousal or priming (Crespi, 1942). In the purest form an incentive motivation is "a response energized by anticipation of a stimulus and reinforced by its realization" (Trowill et al., 1969 p. 271). Viewed under the umbrella of this theory, a train of brain stimulation is inherently rewarding and cannot be assumed to energize solely by reducing a drive. This theory could explain the persistence of ICSS responding without the presence of any apparent drive. Incentive motivation theory predicts that each train of

brain stimulation acts as both a reward for the instigating response and a prime for a subsequent response. Reid, Hunsicker, Kent, Lindsay, & Gallistel (1973) showed that when rats had to first transverse a runway and then bar press to receive successive bouts of ICSS, increasing the length of the runway decreased both the speed of the rat and the subsequent bar pressing rate. Thus as the priming effect decayed so did the response rate. Additionally, Koob (1977) found that incrementally increasing the current of the brain stimulation – presumably increasing the magnitude of both the reward and the prime – caused a significant increase in response rate when compared to incrementally decreasing or randomly changing the current. This provides evidence that a decrease in the magnitude of the prime decreases the associated response rate. In turn, the quick decay of the priming effect helps to explain the apparent quick extinction of ICSS responding. Though there is support for the incentive theory, it does not subtract from, or disprove, drive theory. Unfortunately, drive and incentive cannot be truly dissociated since drive can inherently increase incentive of a reward (Trowill et al., 1969).

Taken together, the findings on drive and incentive motivation, coupled with the unique properties of the procedure, suggested that ICSS response parameters might be affected by the intrinsic motivational state of the organism at the neurological level. This notion played its part in the subsequent usage of ICSS to evaluate the salience of "unnatural" rewards and, in particular, to investigate the motivational properties that influence the addictive properties of narcotics.

1.2 Using Drugs of Abuse to study ICSS

The first published combination of a euphorigenic drug and ICSS was morphine. J. Olds & Travis (1960) investigated the effect of morphine and four other anti-psychotics on the lever response rate for ICSS. The underlying theory behind this experiment was that drugs that suppress psychotic behavior might actually operate through suppressing the same positive reinforcement system that brain stimulation taps into. Conversely, the authors found that morphine at low doses augmented the response rate for brain stimulation of the tegmental area. However, the authors did not associate the results with the positive reinforcing potential of morphine. Instead, J. Olds & Travis (1960) suggested that tegmental stimulation often produced an escape response, and the increased response rate simply represented moderation between opposing forces of motivation and escape. This argument was supplemented with evidence that morphine caused a depression of response rates with septal electrodes. Subsequent papers reported increased ICSS-related response rates after injections of amphetamine (Stein, 1964) and cocaine (Benesova, 1968).

During the groundwork period of drug-ICSS interaction the narcotics were primarily used to investigate the properties of ICSS rather than visa versa. However, in 1972, Adams, Lorens, & Mitchell found that a 10 mg/kg dose of morphine caused an increase in response rates at 4 hours after initial dosing. Subsequent doses in the same rat increased response rate at more proximal time points. This was the first suggestion of using ICSS to investigate euphoric properties of morphine.

One of the reasons ICSS was not originally investigated as a methodology to study drugs of abuse was because drug self-administration (SA) was already an established gold standard for evaluating the motivational and addictive potential of drugs (Kornetsky & Esposito, 1979). It was thought that SA served as the most face valid measure of addictive potential. However, it has been shown that SA methodology has its own set of limitations. One of the problems with SA is that it cannot separate motivational qualities that contribute to readministration (Kornetsky, Esposito, McLean, & Jacobson, 1979). In particular, with physical dependence inducing drugs, it is impossible to determine if SA results from the animals attempting avoidance of withdrawal effects or from a desire to experience the euphorigenic effects of the drug. In addition, desensitization and sensitization of the reward system can have parallel effects with SA. A rat may work harder for a more salient reward (sensitization) or may work harder for a less salient reward (desensitization) to summate rewards to a supra-threshold level. These limitations of SA imply that an alternate measure is necessary to understand the motivational properties of drug administration. In the late 1970's, research began to evaluate the possibility of using ICSS to remedy the shortcomings of SA.

1.3. A Third Rationale

The first two rationales for using ICSS as a measure of the motivational properties of drugs of abuse are discussed above - (1) The unique ability of ICSS to tap into incentive motivation, and (2) The shortcomings of SA. It is pertinent to mention in brief a third rationale, the neurochemical overlap of ICSS and euphorigenic drugs, which does

not fit into the historical context of this paper. The literature supports the position that both ICSS and drugs of abuse derive their reinforcing properties through activation of the mesolimbic dopamine (DA) system (Wise, 1996). A current model for the neurological basis of ICSS is that brain stimulation activates long myelinated descending fibers that originate in the basal forebrain (Simmons, Ackermann, & Gallistel, 1998). This activation causes impulses to traverse their axons through the ventral tegmental area (VTA) and subsequently, indirectly excite dopaminergic neurons that release DA in the nucleus accumbens (Simmons, Ackermann, & Gallistel, 1998). Franklin (1978) showed that DA antagonists reduce rewarding properties of brain stimulation when controlling for performance effects. The rewarding properties of amphetamine, cocaine, opiates, and cannabis seem to be directly related to increased dopamine release in the forebrain, specifically the nucleus accumbens (Wise, 1996). Of particular interest is that morphine's reinforcing properties appear to be related to binding to opioid receptor on GABAnergic neurons in the VTA. This binding causes inhibition of GABAnergic cells that synapse on DA neurons. The net result is a disinhibition of DA neurons precipitating an increase in DA release in the nucleus accumbens (Wise, 1989). Furthermore, the levels of excitatory effects of DA on the tissue responsible for reward are believed to underlie the magnitude of reward produced by both drug use and ICSS (Edmonds, Stellar, & Gallistel, 1974), and thus the two processes should summate together. Since parameters of ICSS are believed to assess changes in the excitation in this tissue it follows that the level of reward produced by euphorigenic drugs can also be evaluated using this methodology. More specifically, the shift in the magnitude of reward produced by a drug-related change in the summation in the tissue can be quantified by the difference between ICSS parameters

with and without the presence of said drug. The specific parameters of ICSS that are needed to evaluate this interaction will be assessed for the remainder of this paper.

1.4. ICSS dependant measures: Bar pressing Rate.

Early studies using ICSS methodology to evaluate rewarding properties of drugs used bar pressing rate as the standard dependant measure. The response rate directly mirrored the dependant variable in the SA field. The rationale, using traditional behaviorist theory, was that response rate reflected the drive of the animal to obtain the reinforcer and thus the greater the rewarding properties of the reinforcer (in our case BSR), the greater amount of exertion to obtain it. This predicted that changes in ICSS-related response rate as a result of drug administration were directly related to drug-induced changes in reward efficacy (Liebman, 1983). The stimulus-reinforcement relationship can be fixed ratio (FR), variable interval (VI) or other manipulations.

Early reports showed a drug-induced increase in response-rate by administration of cocaine (Benesova, 1968) and amphetamine (Stein, 1964). This rate increase, assumed to indicate a drug-induced increase in reward efficacy of ICSS, correlated with cocaine and amphetamine's addictive properties in both the rat and human literature. However, this convergence did not hold true for other drugs. For example, the sedative ethanol caused a decrease in response rate (Carlson & Lydic, 1976). Additionally, morphine – a drug with strong abuse liability – had variable effects on response rate; depending on dose and time. Morphine caused both decreases and increases in response rates (J. Olds & Travis, 1960, Adams, 1972 #54). This discrepancy was often associated with the biphasic

activity of morphine, whereby it produced sedation at early time points and euphoria at later points. It was much more likely, however, that the discrepancies were a result of inherent flaws in using response rate as a dependant measure.

The first inherent problem with response rate was a readily apparent ceiling effect. Two manipulations (for example different drug doses) that result in the same response rate at a given frequency, current, and reinforcement schedule can have significant differences in rates when the parameters are altered (Valenstein, 1964; Wauquier, Niemegeers, & Geivers, 1972). This ceiling is due to a physical limit – the rate at which a rat can press a lever. Under moderate reinforcement schedules, an asymptote in response rate is readily achieved (Liebman, 1983). A related complication is that at higher current intensities certain steotaxic placements cause stimulation-related motor side effects, which may interfere with performance.

The second limitation of using response rate as a dependent measure is that changes in response rate may be due to non-reinforcing properties of the drug. There are motor side effects of narcotics that can be discernable from rewarding properties. For example, sedatives such as morphine can cause sedation-related depression of response rate independent of its reinforcing properties. Carey (1982) found that at low and medium current intensities there is a negative dose response relationship between apomorphine and response rate, but at high intensities there was no significant difference in response. This result was opposite to positive dose response stereotypy observed in the rats (Carey, 1982). The explanation is that the low response rate seen at low current intensities were readily suppressed by the sedative properties of apomorphine. Additionally, side effects of some drugs not directly related to an increase or decrease in

motor activity can alter the response rate. Anticonvulsants, which do not have abuse liability or rewarding properties (Bossert & Franklin, 2003), may increase response rates in seizure prone electrode locations (Reid, Gibson, Gledhill, & Porter, 1964).

Most importantly, many manipulations have invalidated the assumption that with all things being equal, response rate is proportional to reward efficacy. The discrepancy has some relation with the issues discussed above. For example, Hodos & Valenstein, (1962) showed that response rate with different intensities and electrode placement did not correlate with rats preference in a choice paradigm. It is readily apparent in the multitude of papers on the subject that response rate, though an indication of reinforcing properties, is not well correlated with reward efficacy (for review see Valenstein, 1964).

1.5. ICSS dependant measures: Rate-Independent Measures.

One of the first rate-independent methods was created by Valenstein & Meyers (1964) . Rats were allowed a free choice between two platforms that delivered either positive or no brain stimulation reward. Time spent on the positive platform was plotted as a function of the randomly varied current intensity to determine a threshold. The authors also investigated bar pressing response rate as a function of varying current intensities. Results showed that this function was sensitive to changes in current and lesions of the hypothalamic area (Valenstein & Meyers, 1964). They believed that this method presented a more accurate evaluation of the reinforcing properties of BSR than the traditional rate measures. More importantly, this paper highlighted both the

usefulness of describing ICSS by trade off functions and the need to devolop alternative dependent measures.

A series of three papers investigated both the theory and application of using trade-off functions to isolate a rate-independent "reward effect" from the parameters of ICSS. In the first paper, Gallistel et al. (1974) investigated whether priming and rewarding (response-contingent) effects differentially varied with changing stimulus parameters. The authors used the previously mentioned runway paradigm to allow for separation of prime and reward. This paradigm allowed them to systematically vary the BSR parameters during either prime or reward while holding the parameters for the other constant. They found that the prime instigated an instantaneous and transient adjustment in running speed, whereas an animal needed several exposures to the response-contingent stimuli to elicit an effect, which, conversely, was long lasting. Gallistel et al. (1974) believed that the properties of the "reward effect" constituted a memory-like event. It follows that activation of the memory-encoded magnitude of reward then facilitated a bar pressing rate congruent with the learned reward efficacy. The reward strength and subsequent response rate were relearned after several exposures to specific stimulation parameters. Furthermore, it was determined that in order to isolate the reward-contingent response to changing stimulus parameters, a "time-out" had to be employed after administration of the prime to dissipate its transient effect.

The second paper hypothesized that the reward of BSR was a bi-product of the capacity of the tissue underlying the brain-based reward system to temporally summate excitatory input from said rewards (Edmonds et al., 1974). The evidence for this was that response rate varied as stimulation pulse frequency varied. Firstly, the authors stated that

when the potential for temporal summation was eliminated by using one-pulse trains, it was impossible to train a rat to press a lever for BSR. However, when train length exceeded one, the response rate increased as a logarithmic function of pulse per train until eventually achieving an asymptote. The same relationship also occurred when current was varied and frequency kept constant. Furthermore, Edmonds et al. (1974) hypothesized that the true indication of reward efficacy lay not in the highly variable asymptotic value of response rate, but in the relatively stable threshold frequency (θ_0) and half-maximal rate frequency (M_{50}). To calculate reward summation, the authors suggested using the common psychophysical technique of trade-off functions, which is a method to determine to amount of variation in one parameter needed to mirror the change caused in a dependent variable by varying a second parameter. When a trade-off function was employed, it removed the absolute response rate from analysis, eliminating performance effects. Finally, the authors showed that when the performance requirement level was kept constant, the function was consistent and should theoretically predict the reward efficacy based on temporal summation.

In the third set of experiments, Edmonds & Gallistel (1974) set out to investigate whether θ_0 and M_{50} indices were truly devoid of influence from reward-independent priming and performance effect. Using the runway paradigm, Edmonds & Gallistel (1974) systematically varied physical load and magnitude of prime in addition to the administration of performance-debilitating drugs, such as methocarbamol. Each of these manipulations affected the asymptotic value but had little effect on the θ_0 and M_{50} indices. This paper once again highlighted the shortcomings of extrapolating response rate to

reward efficacy. On the other hand, it appeared that use of the rate-frequency measure seemed to sensitively extract reward efficacy from performance effects.

The question then arose of whether the trade-off functions would generalize from the runway paradigm to the more commonly used operant chamber bar-pressing paradigm. The belief was that the runway paradigm effectively separated reward from prime; however, priming effects were still apparent in the bar-pressing paradigm. Miliaressis, Rompre, Laviolette, Philippe, & Coulombe, (1986) found that even though frequency-thresholds are relatively stable over time, performance-affecting manipulations, such as increasing workload and changing reinforcement schedules, shifted M_{50} by between .05 and .20 log units. Chlormazine, pimozide and methocarbamol also caused similar shifts in M_{50}. Similar results were found using train-duration thresholds while manipulating workload (Frank & Williams, 1985). However, there was debate whether these shifts were significantly meaningful differences, and whether or not the function could be adjusted to eliminate these effects.

In an analysis of currently used θ_0 and M_{50} approximations, Coulombe & Miliaressis (1987) hypothesized that because of the sensitivity of this area of the curve, exact experimental value of these indices had too much inter-trial variability. Thus, the frequently used statistical technique of analyzing only the quasi-linear part of the frequency or current threshold curve (e.g. Edmonds & Gallistel, 1974) to arrive at θ_0 and M_{50} indices was insufficient. They suggested that the field should co-opt the biological and pharmacological technique of growth curve models. The underlying assumption was that the rate frequency function had to be analyzed as a whole, from θ_0 to the asymptote, by fitting a sigmoidal curve to the data. They also proposed that different growth curve

models could be used based on the rate of increase of the function. The authors found that with all models, growth-curve estimated θ_0 and M_{50} values are significantly more stable and less prone to performance effects than non-transformed indices.

1.6. A Brief Note on Rate-Intensity versus Rate- Frequency Functions

One of the debates when using trade off functions is whether to employ a rate-frequency or a rate-current function. The relative strength of a train of stimulation is a function of both the current and frequency of application, but varying each has different effects at the neuronal level (Easterling & Holtzman, 1997). The empirically supported data shows that changes in current affect the number of reward-relevant neurons recruited by BSR (Miliaressis et al., 1986). On the other hand, there is support that BSR pulse frequency has a direct linear relationship with the reward-relevant neuronal firing rate (Easterling & Holtzman, 1997). Furthermore, increasing pulse frequency does not recruit additional neurons, it simply increases excitation (Easterling & Holtzman, 1997). Under this assumption, varying pulse frequency simply varies the temporal summation of a fixed set of neurons, while varying current may vary both the temporal summation and the field of neurons recruited (Easterling & Holtzman, 1997). Thus, since trade-off functions are based on varying a single parameter while keeping all other parameters constant, varying pulse frequency better fits the theoretical constraint. Additionally, change in excitation field may recruit neurons with different excitation properties. Exciting these nuerons might change the function relating threshold to response rate which in turn may violate the assumptions of trade-functions. Experimental analysis of

the stability of rate-current and rate-frequency curves supports lower inter-trial variance with threshold measures (Konkle, Bielajew, Fouriezos, & Thrasher, 2001). Rate-frequency thresholds are also more stable than rate-current thresholds when using a titration method (Easterling & Holtzman, 1997).

1.7. A Brief Note on Ratio schedules versus Interval Schedules of Reinforcement.

Another methodological question is what reinforcement schedule should be used with ICSS. Fixed ratio (FR) scales normalize the number of responses necessary to achieve reinforcement. On the other hand, fixed interval (FI) scales normalize the time between successive reinforcements. Boye & Rompre (1996) showed that these two manipulations of reinforcements have differential effects on the magnitude of shift when rats were administered pimozide prior to testing. The authors found that the performance-debilitating drug had less effect on θ_0 and M_{50} when using FI, and thus these schedules were more sensitive in isolating reward efficacy change. The results were attributed to the inherent standardized reward densities in FI, regardless of response rate. Due to priming decrement, increased time between rewards increased rate-frequency thresholds (Miliaressis et al., 1986), and thus interval schedules help to eliminate this artifact present with FR.

1.8. Hypothesis: Morphine Dependence and Rate-Frequency ICSS

Opioid agonists are particularly helpful in the study of drug dependence. Opioid dependence manifests both psychologically, evidenced by the associated euphoria achieved by their usage, and physiologically, evidenced by the physical withdrawal experienced upon discontinuation of a dosing regimen (Koob, 2006). Thus, opioid agonists give us insight into multiple factors that contribute to the complex pharmacopsychosocial phenomena of drug dependence. From an epidemiological standpoint, opioids are relevant to human drug addiction; 13.6% of Americans report lifetime non-medical use of prescription opioids and 1.5% report use of heroin (Mendelson, Flower, Pletcher, & Galloway, 2008). It is estimated that about 4.5% of Americans are dependent upon or abuse prescription opioids, and that just less than 1% meet the same DSM-IV criteria for heroin dependence (Mendelson et al., 2008). Morphine is a particular useful prototype for opioid agonists since it is both a prescribed opioid for pain medication and shares an appreciable amount of its pharmacological profile with heroine.

Morphine dependence in rats is an accepted model of opioid agonist addiction in humans (Kornetsky, 2004). Morphine, heroin, and other opioid agonists exhibit consistent addictive properties in rats as evaluated with traditional measures such as SA rates (Weeks & Collins, 1979), ICSS (Kornetsky, 2004), sucrose bottle preference (Stromberg, Meister, Volpicelli, & Ulm, 1997), conditioned taste preference (CTP) (Gaiardi et al., 1991), and conditioned place preference (CPP) (Mucha & Iversen, 1984). Maximal effects of morphine on naïve and sensitized non-dependent rats occur for ICSS

(Kornetsky, 2004), CPP (Mucha & Iversen, 1984), and CTP (Gaiardi et al., 1991), at subcutaneous (s.c) and intraperitoneal (i.p) doses of between 6 mg/kg and 8 mg/kg. Thus, the literature predicts that in order to obtain the optimal behavioral and motivational effects of morphine, the average self-administered dose would lie in this range. However, with unrestricted access, SA rates exceed a dose of 65 mg/kg and progress to the point of physical dependence as evidenced by loss of body weight and signs of withdrawal after extermination of the regimen (Weeks & Collins, 1979). Additionally, experimenter-administrated intermittent doses of 10 mg/kg or less do not facilitate physical dependence (Tjon et al., 1995), and doses of 20 mg/kg or more are traditionally used to maintain dependence (Vanderschuren, De Vries, Wardeh, Hogenboom, & Schoffelmeer, 2001, Cochin, 1964 #83). This is inconsistent with conditioned taste aversion (Gaiardi et al., 1991) studies which show that doses of 10 mg/kg and greater are aversive to naïve and sensitized non-dependent rats. Likewise, a majority of naïve humans find initial doses (10mg/kg – 70 mg/kg) of morphine and heroine unpleasant (Smith & Beecher, 1962), whereas post-addicts find similar doses euphorigenic (Martin & Fraser, 1961). Hence, if the basic characteristics of morphine do not predict high dose administration, then why does unrestricted SA lead to administration of increasingly large doses? One possibility is that repeated administration of morphine can shift its dose response curves to facilitate the process of dependence.

Beyond the scope of this paper, there is a large literature that shows that chronic morphine exposure can cause receptor-level desensitization (for a review see (Gintzler & Chakrabarti, 2006) leading to tolerance. These changes suggests that a rightward shift in the dose response curve accompanies dependence; in other words, increasing doses of

morphine would be necessary to achieve the same pharmacological effects previously associated with lower doses. Counter intuitively, in rat models, receptor desensitization is often accompanied by behavioral sensitization. This is most frequently evidenced by increased locomotion at higher doses (Bartoletti, Gaiardi, Gubellini, Bacchi, & Babbini, 1987; Vanderschuren et al., 2001). Additionally, sensitization can be observed when dependant rats are conditioned using a low dose that do not facilitate CPP in naïve rats (Shippenberg, Chefer, & Thompson, 2009), but an extensive morphine dose-response analysis of the motivational differences between dependent and non-dependent rats has not been performed. Due to the discrepancy between pharmacological and behavioral data, the aforementioned paradigms cannot be assumed to indicate the direction of the motivational changes that accompany dependence. Some studies have found a shift in morphine salience with increased SA in dependent rats (Weeks & Collins, 1979); however, for reasons previously mentioned in this paper, SA cannot accurately measure changes in reward efficacy. Thus, a characterization of the shift in reward efficacy accompanying morphine dependence is warranted in order to assess its relationship with behavioral sensitization and receptor desensitization. To address this question, Cooper, Truong, Shi, & Woods (2008) investigated the differences between morphine dependant and non-dependant rats on self-administration of the non-dependence causing mu-opioid agonist remifentanil. The purpose of this experiment was to investigate changes in reward efficacy in opioid self-administration, while at the same time controlling for difference in levels of exposure to morphine between the groups, effectively removing one of the pitfalls of the SA paradigm. A leftward shift in the ascending and descending limbs of the dose-response curve for remifentanil between dependant animals in withdrawal and non-

dependant animals was observed (Cooper et al., 2008), whereas there was no difference between dependant animals not fully in withdrawal and non-dependant animals. Likewise, Schaefer & Michael (1986) found that inducing withdrawal in morphine dependent rats causes an increase in ICSS current thresholds compared to control animals and precipitated withdrawal animals. However, Schaefer & Michael (1986) also found that both withdrawal groups had significant decreases in response rate, which highlights that the experiments done by Cooper et al. (2008) may be overly influenced by response rate.

This literature suggests that continued re-administration of morphine in SA might occur because of a shift in reward efficacy accompanying dependence. However, no systematic analysis of the difference between the morphine reward salience dose response curve in dependant and non-dependant animals has been executed. One may postulate that rats re-administer in order to remove the negative effects of withdrawal and/or because of a withdrawal-precipitated shift in the dose response curve increases the salience of morphine. Bechara & van der Kooy (1992) results suggested that the effect of withdrawal may cause experiments to produce a shift in the reward efficacy of morphine accompanying dependence when no motivational tolerance occurs. Thus, like Cooper et al. (2008), Bechara & van der Kooy (1992) suggest that increased reward efficacy is withdrawal dependent. Alternately, there may be a general increase in the salience of morphine in dependant animals not specific to withdrawal. Bechara & van der Kooy (1992) also reported that sensitization may occur at higher doses of morphine but not at low doses. The question can then be posed whether there is a change in reward efficacy of morphine accompanying dependence and whether this shift is withdrawal-dependent.

We believe that the motivational properties of the preceding questions can be more accurately addressed using the rate-frequency function of ICSS. We hypothesize that withdrawal in morphine-dependent animals causes an increase in the reward efficacy of morphine compared to non-dependant animals. Thus, we expect that morphine will cause a greater facilitation of ICSS M50 values in dependent animals when compared to control groups.

2. Methods

2.1 Subjects

The subjects were 23 Long Evans rats received at weights between 225g and 250g from Charles Rivers, Montreal. The rats were kept in a colony room maintained on a 12-h light/ 12-h dark cycle and were given unlimited access to food and water unless otherwise noted. Rats were acclimatized and handled for 7 days prior to surgery.

2.2 Surgery

All animals weighed at least 250g on the day of surgery. Rats were anesthetized Under 2.0-5.0 ppm Isoflurane (Baxter, Mississauga ON.) and then implanted with a unilateral bipolar electrode (Plastic One, Roanoke, VD.) aimed at the lateral hypothalamus. Stereotaxic co-ordinates were as follows: 2.7 posterior to bregma, 1.7

lateral to the midsagital sinus, and 8.7 below the dura. The electrode was secured with a dental cement skull cap bolted to the skull with stainless steel screws. Rats were allowed a minimum of five days recovery before training

2.3 Apparatus

Clear Plexiglas operant boxes (dimensions 29.5cm wide, by 28cm deep, by 27.5cm high) were used for all screening, training, and experimental conditions (Figure 1). One wall of the box had a metal siding with a centrally located metal retractable lever 6.5cm above the metal rod floor. The 3 other sides of the boxes were made of clear Plexiglas. The operant boxes were individually housed in a sound- and light- attenuating chamber (65cm by 50cm by 52cm) that contained a small house light 41cm above the floor. Rats' leads were connected to the stimulator output via a commutator (Plastic One, Roanoke, VA) mounted at the top of the operant chamber. Stimulation trains were 600ms of 0.15ms monophasic square-wave pulses ranging from 0 Hz to 712 Hz. They were generated by electrically isolated constant-current stimulators, driven by a computer-controlled, variable-frequency oscillator. To prevent a buildup of charge at the interface of the brain and electrode, electrodes were short circuited during the inter-pulse interval. A personal computer set the pulse frequency and reinforcement schedule and recorded the resulting responses. Currents were adjusted on the variable-frequency oscillator.

Figure 1: *The self-stimulation apparatus*. A single self-stimulation box and the surrounding sound attenuating chamber are shown.

2.4 Procedure

2.4.1 Screening

Rats were screened by delivering 600 ms trains of 0.15 ms pulses at a frequency of 400 Hz and an experimenter-adjusted amplitude of between 260 μA and 400 μA. Reinforcement was on a FR-1 schedule. During screening, rats were placed in the operant boxes with their electrodes attached to the leads. During testing one of electrodes was designated as the ground. A few priming trains were administered by the experimenter at which point the boxes were closed and the rats left to learn the lever-pressing response. Rats that exhibited aversive or stimulation-induced motor effects were excluded from further screening and testing. Rats who passed the exclusion criteria, but who did not learn the response during the first 60-min training period were given an identical second screening period with the alternate electrode designated as ground. After successful screening the ground electrode designated as ground was fixed. Rats who did not learn the response in either of the screening session were not further tested.

2.4.2 Training

Rats exhibiting spontaneous responding were trained for 45 minutes on a FR-2 reinforcement schedule. On the following day they were trained for 45 minutes on a FR-5 reinforcement schedule. During the FR-5 training session, currents were adjusted to

maintain an optimal response rate of approximately 100 responses/min. Next, the rats were trained for five days on a multiple schedule (MS). MS consisted of 3-minute trials alternating random interval reinforcement trials alternating with extinction (0HZ) trials. An RI=2-sec was chosen to maintain a constant reinforcement density across a wide range of response rates. A Multiple schedule was employed to establish rapid response decline during extinction trials, and rapid reinstatement of responding during RI periods. To progress from a RI trial to an extinction trial, rats must have made more than ten responses in the final two minutes of the trial. In order to return from the extinction schedule to the RI schedule, rats must have made fewer than two responses in the final two minutes of the trial. Trials were separated by a 10-sec timeout period during which the lever was retracted. Once the lever was re-extended a single non-contingent priming pulse was delivered at the optimal current stimulus frequency (for RI trials) or at 0 Hz (Extinction trials). The timeout and priming pulse were used to dissociate the current trial from priming effects of the previous trial.

A four-day RF training period followed the MS training period. RF testing maintained the same 3-min trial structure, RI schedule, time-out period, and subsequent prime upon resumption. Each RF session was 40 minutes long and consisted of a four-min warm-up period at the optimal frequency followed by initiation of the RF procedure, which began at the same optimal frequency as the warm-up. The RF procedure consisted of twelve 3-min periods separated by the aforementioned timeout. On each succeeding 3-min trial the log of frequency decreased by 3 percent of the previous trial until the rat met the extinction criterion (less than two responses in the final two minutes of the session). At this point, the frequency was reset to optimal frequency and the decrements in pulse

frequency began again. Rats were trained twice per day with the second session occurring two hours after the beginning of the first.

9.4.3 Testing

All testing periods utilized the same RF procedure. Rats were weighed each day and were then injected subcutaneously with saline or drug and tested on the RF protocol at 1-hr and 3-hr post injection. 1-h and 3-h time points were analyzed separately. Two time points were utilized for two reasons. Firstly, we wanted to have sensitivity to time-dependent withdrawal effects. Secondly, multiple time points allow us to assess morphine reward efficacy at both peak morphine activity and during the descending limb of the drug effect which have been shown to cause different effects on ICSS (Hand & Franklin, 1986). Morphine activity peak is estimated to occur between 45 and 95 minutes following morphine administration (Porreca, Cowan, & Tallarida, 1981). Morphine's half-life is estimated to be between two and half and three hours, hence the second testing period occurred after approximately one half-life (Porreca et al., 1981).

After completion of the training procedure, baselines were determined as follows. For four consecutive days rats were injected with saline and then subjected to the RF procedure at 1-h and 3-h post injection. The mean of the 4 days' M_{50} and Rmax is the baseline line value. Rats who did not meet the Stability criterion (first two-day M_{50} differed from the second two-day M_{50} average by no more than 3% of their average baseline RO value) were not further tested. The mean M_{50} of the final two-day of the baseline-testing period was designated as the naïve baseline.

Rats were then sensitized to morphine over 5 days. During this period rats were given 3mg/kg of morphine 1-hr prior to testing each day. Since dependent rats would receive morphine before the dose-response curve begins, all subjects were sensitized to morphine to eliminate the possible that differences could be due to previous morphine exposure and not specifically morphine dependence.

After the sensitization phase, rats had one day of rest before the beginning of the dose-response regimen. Rats were given increasing doses of morphine on a 3X dose escalation regimen (saline, 1 mg/kg, 3mg/kg, 10 mg/kg for all groups and additionally 30 mg/kg in dependent rats only). Rats were tested for 3 days at each dose followed by a day of rest. This regimen allows us to evaluate a sub-maximal doses (1 and 3 mg) that have been shown in the CPP literature to have different effects on dependent and non-dependent rats (Shippenberg et al., 2009). Also, we wanted to evaluate a high-dose (10 mg) that was on the descending limb of the dose response curve (Tjon et al., 1995) We did not test non-dependent or food-deprived rats at 30 mg/kg because that dose is above the LD50 for non-dependent rats (Davis & Khalsa, 1973). The first day of testing at each dose was eliminated from analysis as an acclimatization day. The mean M_{50} and Rmax from the second and third days were determined to give a singular value for both time points and dependent measures for each dose. Thirty days after their final dose in the dose regimen, rats received a saline injection and were tested for three days to determine their post-treatment baseline.

2.5 Non-Dependent v. Dependent dosing regimens

Dependent (D) (N=8) and non-dependent (ND) (N=8) groups received identical treatment until day one of sensitization. Thirty minutes following their final RF session this day, D rats received a maintenance injection of morphine while ND rats received an injection of saline. The maintenance dose was escalated over 5 days (10, 15, 20, 25, 30 mg/kg morphine) coinciding with the 5 days of sensitization. Thereafter D rats were maintained on a daily 30mg/kg dose of morphine and ND rats maintained on daily saline. Physical dependence was assessed by body weight change; failure to gain weight has been shown to be indicative of dependence (Tjon et al., 1995). Testing occurred approximately eighteen hours after the maintenance dose. Since 4 half-lives are conventionally needed to clear morphine from the bloodstream, the 6 ½ half-life interval between doses was sufficient to do so. This dosing regimen was based on previously reports that physical dependence can be achieved with intermittent doses of 30 mg/kg (Tjon et al., 1995).

2.6 Non-Dependent v food-deprived animals

Morphine dependence causes weight loss in rats, which itself has been shown to produce ICSS facilitation (Abrahamsen, Berman, & Carr, 1995; Blundell & Herberg, 1968)}. To control for the effect of weight lose we employed a second control group of food-deprived rats (FD) (N=7). Food deprived animals were maintained on exactly the same testing and living conditions as ND rats with the exception of food availability. FD

animals were food deprived for 24 hrs after sensitization day one, after which their food was restricted so that their daily percentage weight change mirrored that of D groups (see figure 2, 3). Rats were fed 30 minutes after their daily testing session.

2.7 Drugs

Morphine sulphate (Sigma-Aldrich, St. Louis, MO) was dissolved in saline to create 1ML/kg solutions for each dose of morphine. All injections were administered subcutaneously.

2.8 Histology

Rats were sacrificed under an overdose of pentobarbital. Brains were removed and stored in 10% formal saline for a minimum of 24h, then sliced in 30 mm sections in a cryostat. Sections were stained with thionine and examined under a microscope to identify the site of the electrode tip with reference to Paximos and Watson (1998).

2.9 Statistics

The response rate at each frequency was calculated as the average of the response rate per minute for the final 2-min of each trial. A rate frequency curve was then calculated with a 4-parameter logistic regression (Sigma-Plot 11.0). This method fitted the growth curves suggested by Coulombe & Miliaressis (1987) and has been previously

shown to generate smoother curves with limited data points (Bossert & Franklin, 2003). The M_{50} and Rmax values were then extracted from this curve. As previously noted, data from day one of each dose were discarded. The M_{50} and Rmax values from days two and three were averaged to obtain single M_{50} and Rmax values for each rat at each dose (saline, 1, 3, 10, 30 and post-regimen saline). These values were then converted to percentage change from baseline in order to adjust for differences in initial baselines. 1h and 3h data were analyzed separately. A one-way mixed-design ANOVA was performed for all analyses using Statistica 9 for Windows with Group (dependent, non-dependent and food-deprived) as a between factor and drug dose or time point as the repeated measure as indicated in text. Interaction effects were broken down by performing one-way ANOVAs at each dose or time point. Pair-wise Fischer's LSD post-hoc test was used for between cell comparisons. By using Fischer's LSD as the post-hoc test we preserve the experiment type I error rate at a nominal level of significance since we have exactly 3 experimental groups (Meier, 2006). Statistical significance is set for all tests at an alpha level of $p < .05$.

3. Results

3.1. Baseline Performance

The brain stimulation current for each rat established during baseline testing onward, the ranged from 250 μA to 350 μA. The mean naive baseline M50 for D, ND

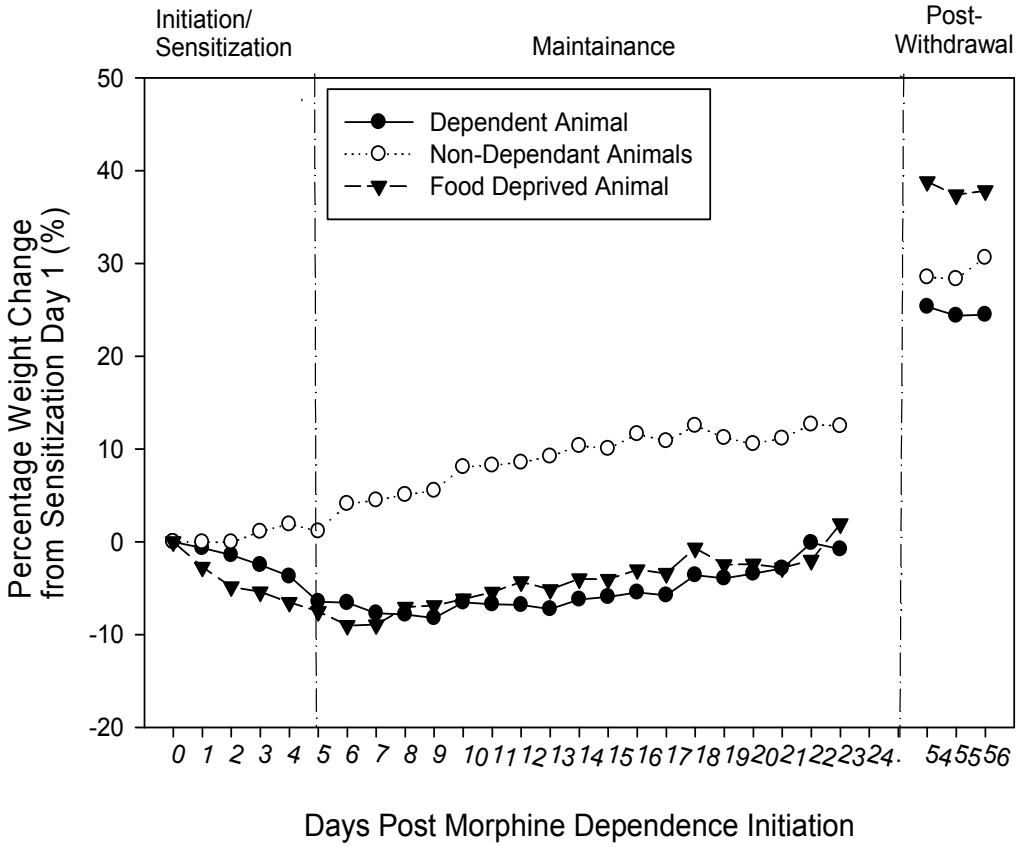

Figure 2: *Timeline of the Effect of Morphine Dependence and Food Deprivation on Body Weight.* Daily cumulative weight change as a percentage of weight on sensitization day 1 is plotted against days after sensitization day 1.

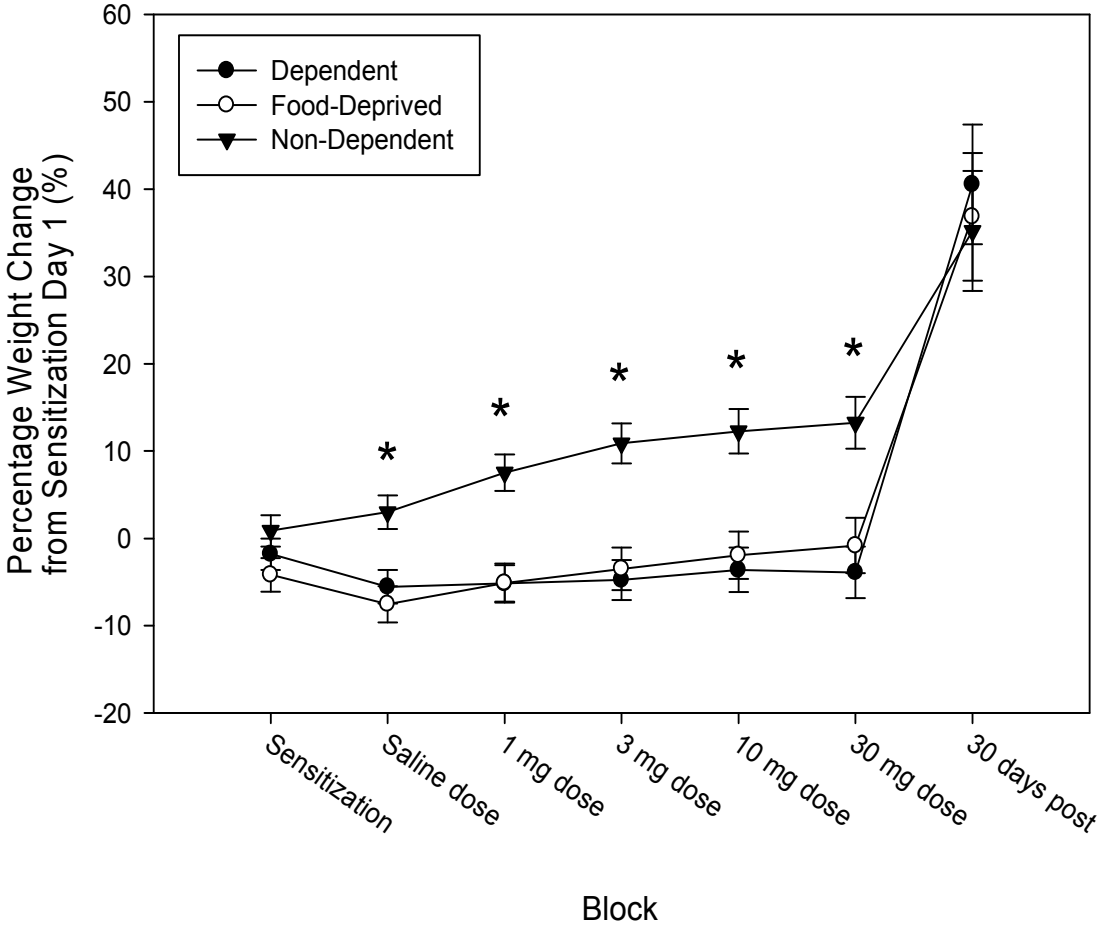

Figure 3: *Effect of Morphine Dependence and Food Deprivation on Body Weight*. Average cumulative weight change for each block is expressed as a percentage of weight on Sensitization Day1. Blocks encompass the 3-day period spent at each dose and day off following, if any. Error bars represent SEM and asterisks indicate significant between group difference to an alpha level of $p<.05$.

and FD rats were 2.184, 2.139 and 2.172 log units respectively at 1-h and 2.200, 2.145, 2.204 respectively at 3-h; these values were not significantly different (see Figure 4,5). In accordance with the stabilization criteria, the mean two-day differential during baseline testing was 2.26% and 1.62% at 1-h and 3-h respectively. The mean baseline Rmax for D, ND and FD rats were 142, 140 and 128 responses/min respectively at 1-h and 123, 121, 119 respectively at 3-h; these values were not significantly different.

3.2 Effect of withdrawal and abstinence on M_{50}

The 5-day sensitization period had no significant effect on naive baseline M_{50} values at 1-h ($F(2,36) = 2.428$, NS) (Figure 4). There was also no effect of group suggesting that daily withdrawal and food restriction had no significant effect on baseline M_{50} values ($F(2,18) = .837$, NS). Likewise, there was no change in 1-h naïve baseline M_{50} values after the 30-day abstinence period that followed the termination of the morphine-dosing regimen ($F(2,36) = 2.273$, NS) (Figure 4).

After 5-days of sensitization there was a significant increase in M_{50} from naïve M_{50} values at 3-h ($F(2,36) = 4.92$, $p < .05$) (Figure 5). Daily withdrawal and food restriction did not significantly modulate the increase in M50 values ($F(2,18) = .837$, NS). M_{50} values after the 30-day abstinence period decreased from the post-sensitization baseline (Pair wise Fischer LSD, $p < .05$) but were not significantly different from the naïve baselines ($p > .05$)

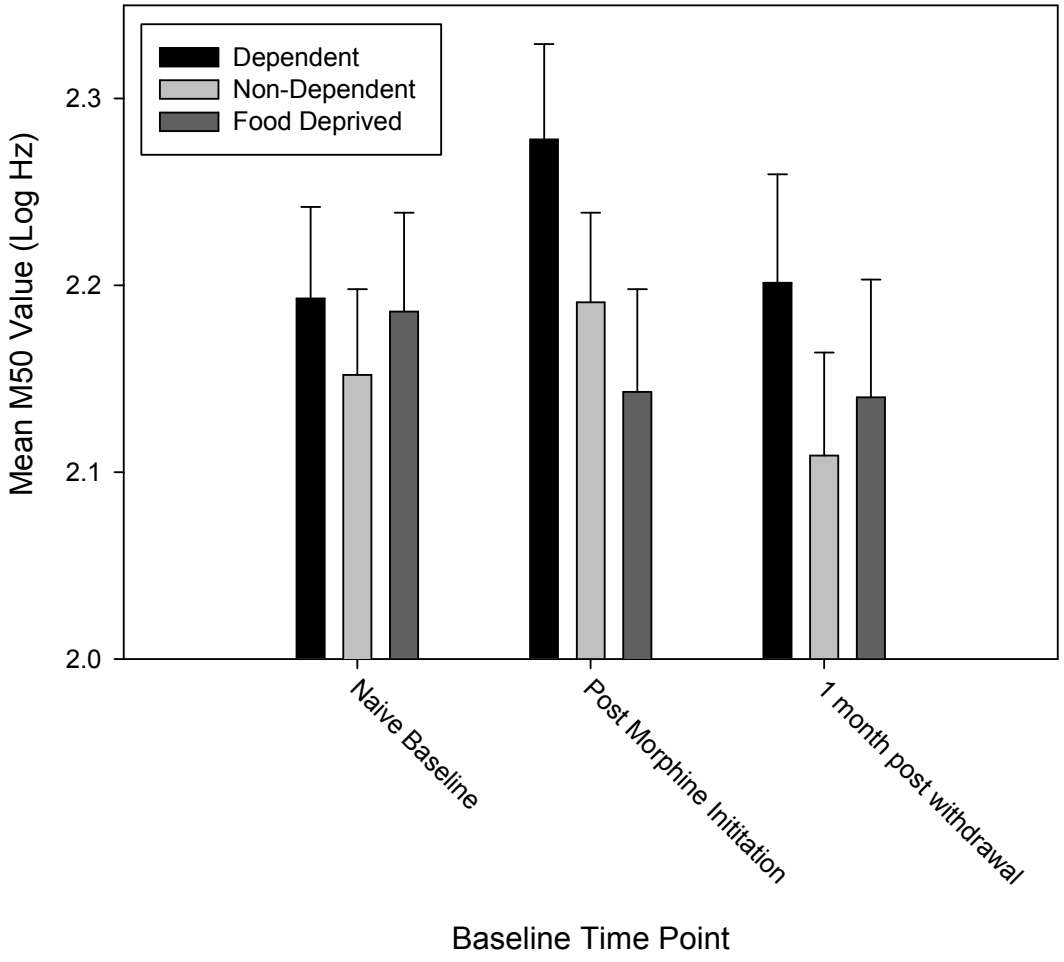

Figure 4: *Effect of Sensitization, Withdrawal and Abstinence on M50 Values at 1 hr Post Injection.* The log of the mean M50 for naïve baseline, post morphine initiation and one moth post withdrawal for each group is represented. Error bars indicated SEM.

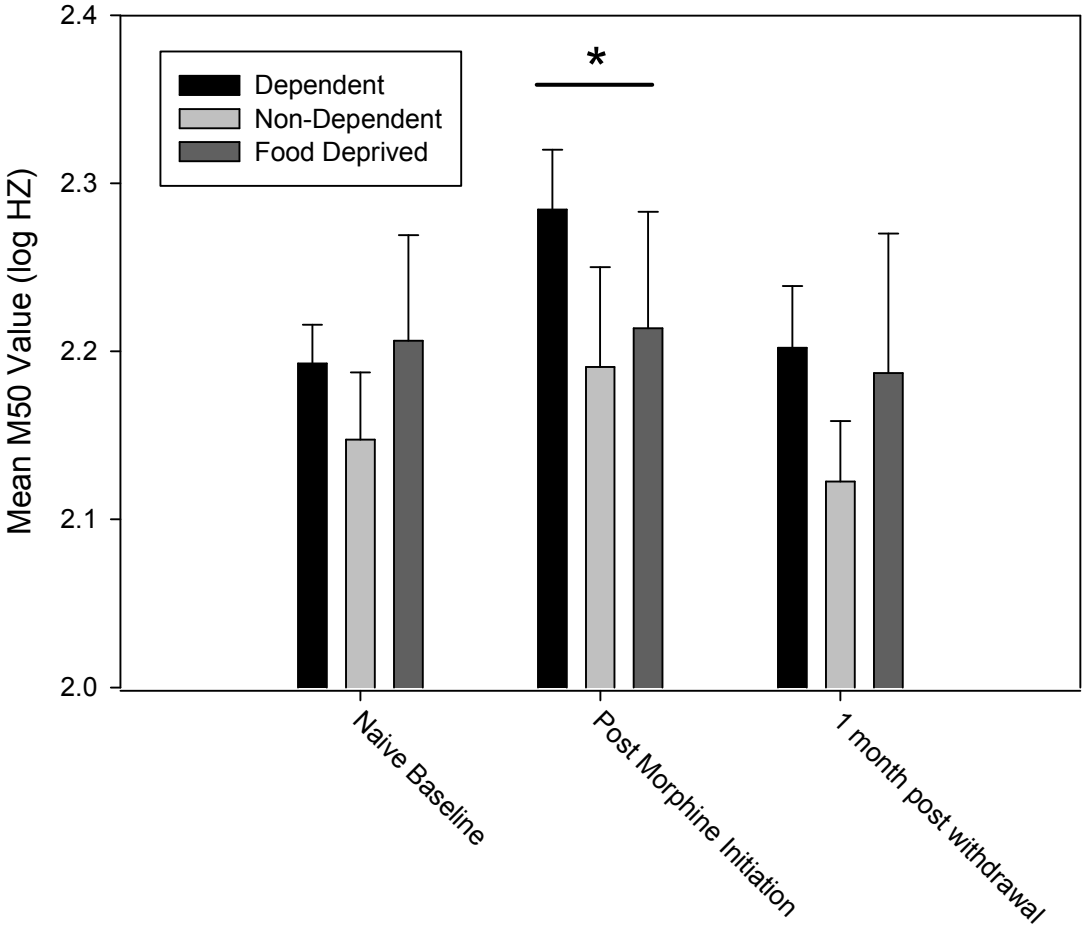

Figure 5: *Effect of Sensitization, Withdrawal and Abstinence on M50 values at Three-Hour post injection*. The log of the mean M50 for naïve baseline, post morphine initiation and one month post withdrawal for each group is represented. Error bars indicated SEM. Asterisks indicate significant effect of time point across groups to an alpha level of $p < .05$.

3.3 Effect of Morphine on M50

There was an significant effect of dose ($F (3, 60) = 28.55$, $P < .05$) and a significant dose by group interaction on M50 values ($F (6, 60 = 11.44$, $P < .05$) at 1-h (Figure 6). The within group effect of morphine was tested using Fischer LSD. The dependent group exhibited decreased M_{50} values at each dose when compared to saline baseline (Fischer LSD, all $p < .05$). Additionally, the M50 value for D rats at each dose were significantly lower that the M_{50} value at the previous dose (all $p < .05$). Likewise, the ND rats showed decreased M_{50} values at each dose when compared to saline baseline (all $p < .05$). However, M_{50} values at 3mg/kg were significantly decreased from the 1 mg/kg dose ($P < .05$), whereas M_{50} values at 10 mg/kg were significantly increased from the 3 mg/kg dose, ($P < .05$) but were not significantly different from values at the 1 mg/kg dose (NS). In the FD group only the 3 mg/kg caused a significant lowering of M_{50} values ($P < .05$) when compared to saline baseline.

To investigate a significant dose by group interaction a simple effects analysis was performed which revealed a significant between group effects at the 10 mg/kg dose ($F (2,20) = 6.18$, $P < .05$). There were no significant between group differences in M_{50} values for saline ($F (2,20) = 3.14$, NS), 1mg/kg ($F (2,20) = 0.92$, NS) and the 3mg/kg ($F (2,20) = 1.15$, NS) doses. At the 10 mg/kg dose M_{50} values for the FD and ND groups were not significantly different from one another (Fischer LSD, NS), but both groups had significantly higher M_{50} values than the D group.

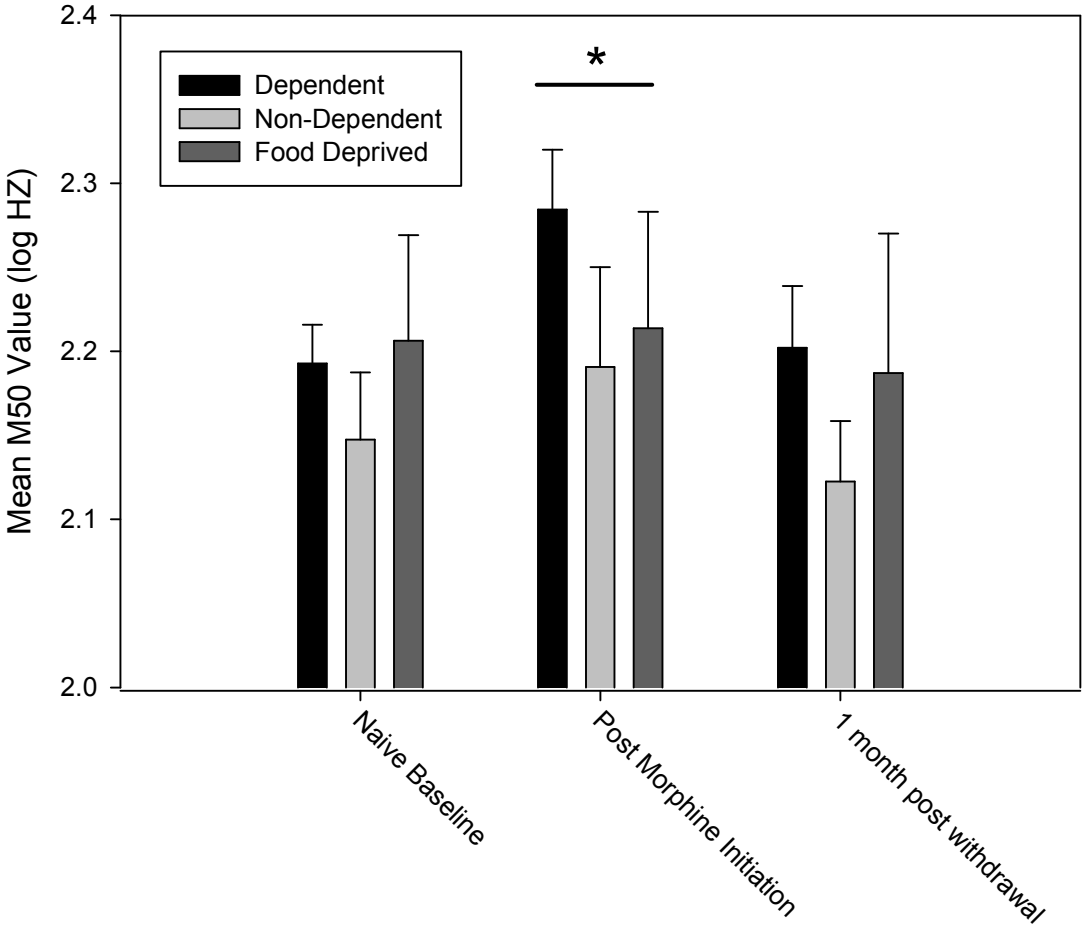

Figure 5: *Effect of Sensitization, Withdrawal and Abstinence on M50 values at Three-Hour post injection*. The log of the mean M50 for naïve baseline, post morphine initiation and one month post withdrawal for each group is represented. Error bars indicated SEM. Asterisks indicate significant effect of time point across groups to an alpha level of $p < .05$.

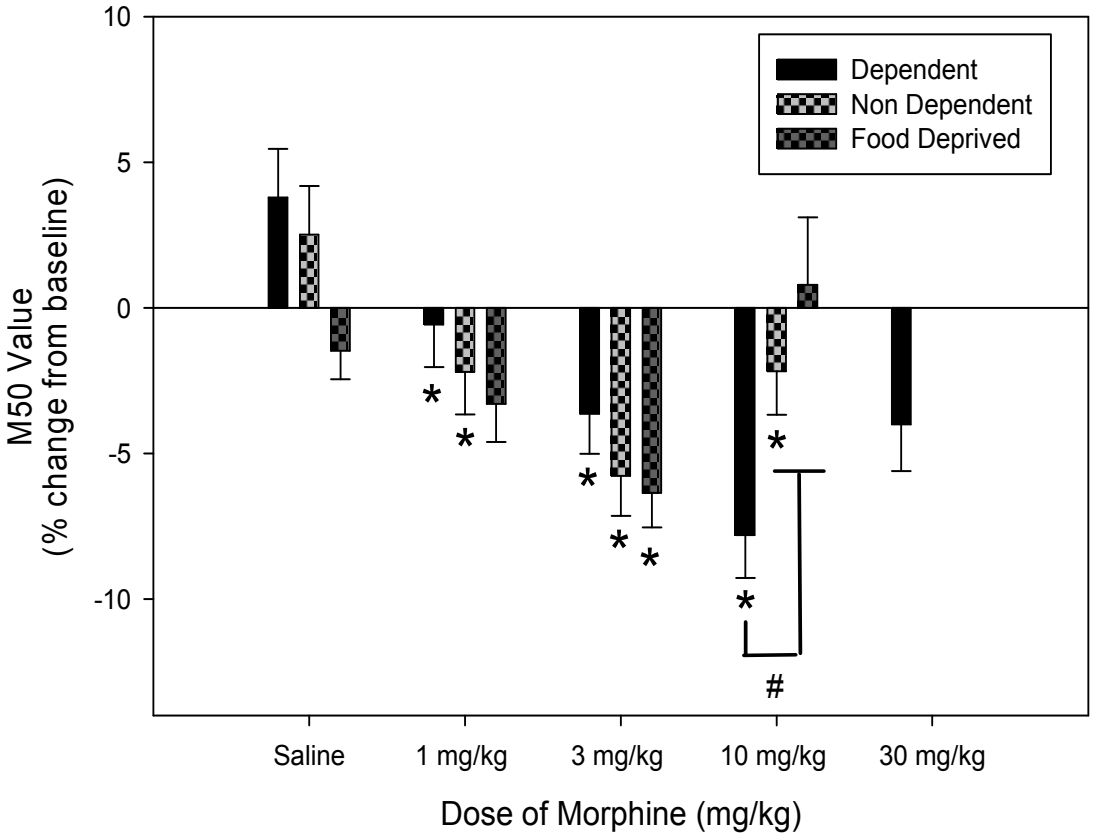

Figure 6: *Effect of Morphine on M50 Values at One-Hour Post Injections*. Mean change in M50 Values from baseline, represented as a percent of baseline values, is plotted against dose of morphine in mg/kg. Error bars represent SEM. Asterisks indicate significant within group difference from saline values to an alpha level of $p < .05$. Hash marks indicate significant between group differences to an alpha level of $p < .05$.

Morphine caused a significant dose-dependent decrease in M_{50} values at 3-h compared to baseline ($F(3, 60) = 14.98$, $P < .05$) (Figure 7). 10 mg/kg morphine caused a significant decrease in M_{50} values compared to saline baseline (Fischer LSD, $p < .05$). M_{50} values at the other two doses did not significantly differ from one another or from saline baseline (NS). Morphine dependence and food deprivation did not significantly modulate the effect of morphine on M_{50} values at 3-h ($F(2,20) = .260$, NS).

3.4 Effect of Morphine on Rmax

Morphine had no significant effect on Rmax values at 1-h post injection ($F(3,60) = 2.03$, NS) (Figure 8). On the other hand, morphine caused a dose dependent increase in Rmax values at 3-h post injection ($F(3,57) = 5.40$, $P < .05$) (Figure 9). Rmax values did not differ from saline value for the 1 mg and 3 mg dose (Fischer LSD, NS), however, the 10 mg dose cause a significant increase in Rmax compared to saline and the other 2 doses of morphine ($p < .05$). The effect of morphine was consistent across groups ($F(2,20) = .56$, NS).

3.5 Body Weight

Change in body weight as a percentage of weight on sensitization day 1 was tracked daily as a marker for morphine dependence (Figure 3). Body weight was then grouped into blocks corresponding with each dose; each block value represented the

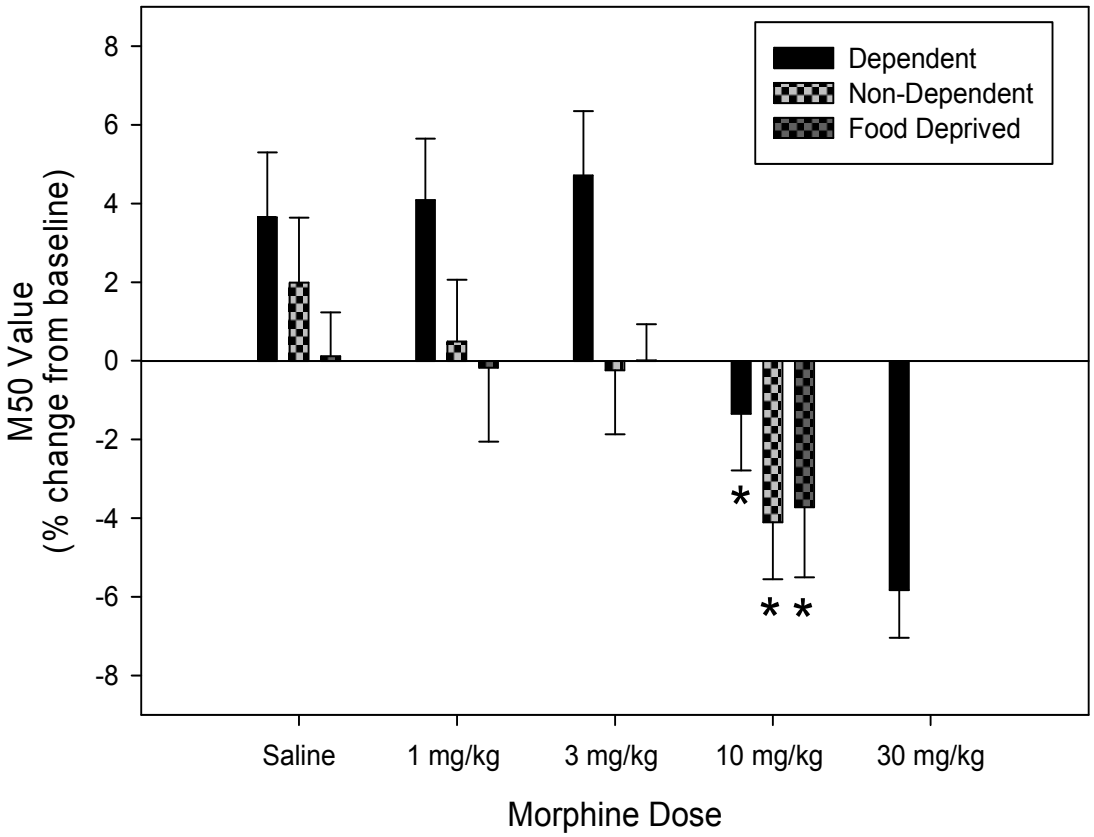

Figure 7*: Effect of Morphine on M50 Values at Three-Hour Post Injections*. Mean change from baseline in M50 Values, represented as a percent of baseline values, is plotted against dose of morphine in mg/kg. Error bars represent SEM. Asterisks indicate significant within group difference from saline values to an alpha level of $p < .05$.

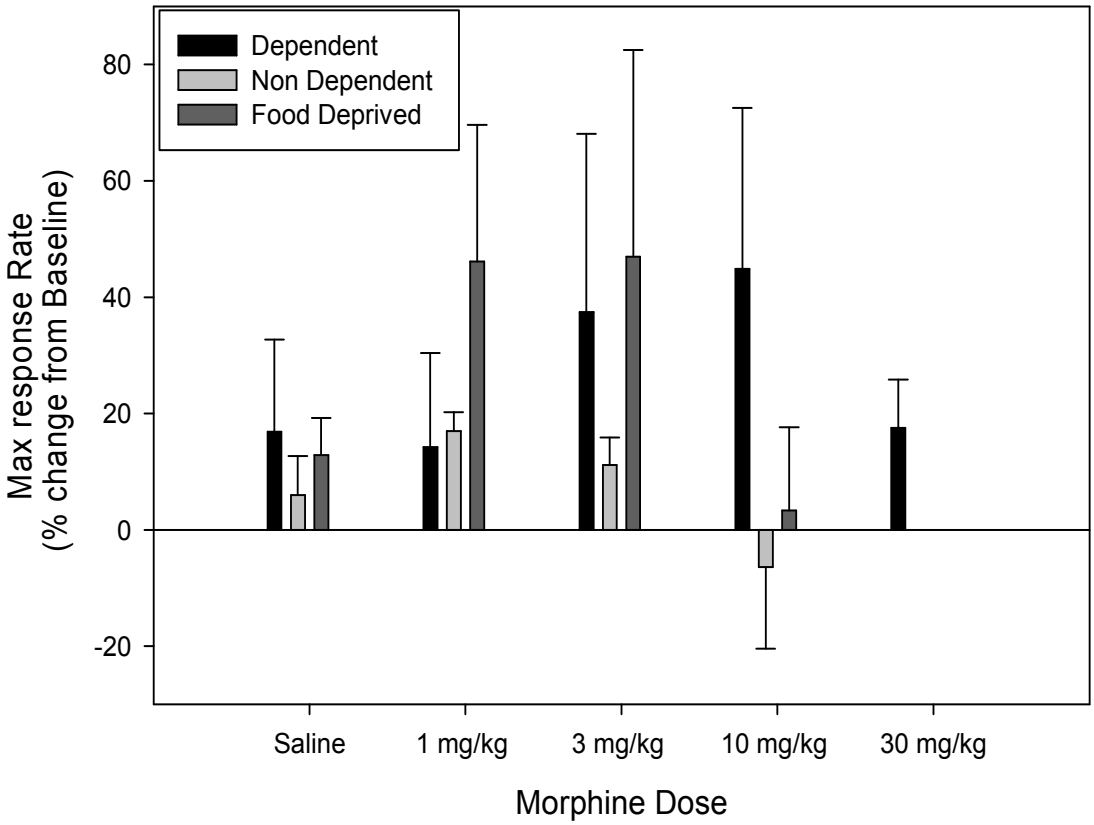

Figure 8: *Effect of Morphine on Rmax Values at One-Hour Post Injection*. Mean change from baseline in max response rate per minute, represented as a percent of baseline values, is plotted against dose of morphine in mg/kg. Error bars represent SEM.

mean weight for the 3 days spent at each individual dose and the subsequent off day if any (Figure 2). The dependence regimen caused a significant reduction in body weight in D animals when compared to ND animals from the start of the morphine-testing period (saline block) to the end (30 mg block) inclusive (Fischer LSD, all $p < .05$). Body weight matching of the FD to the D group was successful as body weights were not significantly different from one another at any time point (all NS). The FD animals also exhibited significant reduction in body weight from beginning to end of the morphine-testing period. Thirty days after termination of dependence and food restriction, the body weights of the D and FD animals recovered to be not significantly different from ND animals (all NS).

4. Discussion

Our results showed that morphine dependence modulates morphine-induced facilitation of ICSS. All groups showed dose-dependent decreases in M50 values at both time points with no difference between groups at the 3 hour time point. However, at 1-h D rats exhibited their largest decrease in M50 value at a higher dose and with greater magnitude than control groups. This change in the morphine-ICSS response curve cannot be accounted for by increased reward salience due to withdrawal or weight loss.

Firstly, our results suggested that morphine dependence does not cause a withdrawal-induced increase in reward efficacy. Morphine dependence – induced by a empirically supported dosing regimen (Tjon et al., 1995) and evidenced by a statistical decrease in body weight compared to ND controls – did not cause a change in ICSS

responding during withdrawal. If withdrawal causes a shift in opioid reward efficacy, we would expect a corresponding shift in M_{50} values. However, M_{50} values for D animals in withdrawal at the early time point were equivalent to their pre-dependence baselines and did not differ from control groups. The increased M_{50} values for the later time point do not indicate a decrease in reward efficacy due to withdrawal since both control groups mirrored this effect. Instead, this effect may indicate a time-dependent effect of previous morphine experience on ICSS responding.

Secondly, dependence caused a dose-dependent rather that a generalized increased response to morphine. If dependence and subsequent withdrawal causes a generalized sensitization of the opioid reward system then there should be a greater morphine-induced facilitation at all doses when compared to controls. However, at 1mg/kg and 3mg/kg facilitation of ICSS was equivalent in all 3 groups. Only at a high dose, 10mg/kg, was a difference between groups observed. Thus there was no shift in the morphine reward dose-response curve. The failure to observe a difference at 3-h for the 10 mg dose is consistent with 1-h results; from morphine's half-life, the amount of morphine in the rats system at 3-h would be close to 3mg/kg – a dose at which we would not expect to see a group difference. In D rats there appears to be greater facilitation of ICSS at 30mg/kg dose at 3-h – when the concentration is close to 10 mg/kg – than at the 10mg/kg dose. This effect supports the results from the 1-h, since increased facilitation would be predicted at this dose in D but not FD or ND rats.

Since the M_{50} index is a rate-independent measure, shifts in values are independent of drug-induced behavioral changes. However, since Rmax is sensitive to non-specific effects, it would serve as a secondary measure to indicate behavioral

excitation or sedation. In turn, we could compare reward and behavioral effects of morphine by juxtaposing Rmax and M_{50}. This comparison could help address whether reward efficacy changes were due to removal of inhibitory- or increased excitatory-effects. However, observed Rmax variance was very high and thus we only had power to detect very large effects. There was an increase in response rate at 3-h in all groups for the 10mg/kg dose. This indicates that at this dose behavioral excitation accompanies the rewarding effect of morphine. It has been previously shown in the literature that moderate doses of morphine increase locomotion (Pearl & Glick, 1996). Note that, at 1-h the D group's mean Rmax value stays elevated from the 3 mg/kg dose to the 10 mg/kg dose whereas both ND and FD group's Rmax mean value decreases above the 3 mg/kg dose, and in the case of ND group the mean is depressed below baseline. It follows that at 1-h, the 10-mg/kg dose produces behavioral excitation along with morphine reward in dependent rats, whereas behavioral sedation accompanies morphine reward in non-dependent rats. It has also been previously shown that prior exposure to high doses of morphine facilitates increased locomotion at higher doses of morphine (Pearl & Glick, 1996). This would indicate that the apparent increase in morphine reward efficacy at high doses in D rats might be due to removal of an inhibitory effect present in ND rats.

It is of interest to note, that much like daily withdrawal, morphine abstinence had no significant effect on M50 values. At both 1-h and 3-h baselines, M50 values were unchanged from naïve baselines for all groups. This indicates that recovery from morphine dependence may not cause a shift in the value of ICSS rewards, or that 30 days is sufficient to recover from any abstinence-induced shift. Likewise, an intermittent morphine-dosing regimen that does not result in physical dependence may not cause a

long-term shift in baseline M50 values. In addition, the time-dependent effect of prior morphine exposure was not observed at this time point, indicating that the this effect may be transient.

We did not observe a significant effect of food deprivation on baseline M50. However, the FD group was the only group to show a decrease in post-sensitization baseline when compared to naïve baseline. Additionally, only the FD group failed to exhibit significant morphine-induced facilitation of ICSS at the 1 mg/kg and 10 mg/kg dose. Thus there was an effect of FD on ICSS in pre-empting morphine facilitation. Our failure to observe a significant effect of FD on baseline may be due to an effect of morphine exposure; it is possible that the sensitization to morphine may have masked the effect of food deprivation, since the other groups exhibited a decrease in mean M50 values in response to morphine sensitization.

In the context of morphine dependence, the associated loss in body weight may have caused an underestimation of the differences between the D and ND groups. In particular, there was no facilitation of ICSS at 10 mg/kg in the FD group whereas there was facilitation over baseline in the ND group. Then, it is possible that loss of body weight may increase the depressant effect of high morphine doses.

Our failure to show an effect of withdrawal on M50 does not conflict with the literature. Schaefer & Michael (1986) reported elevation of ICSS thresholds during precipitated withdrawal but not during spontaneous withdrawal. However, the increase in threshold values was only significant during the first testing period, which occurs 4 hours after discontinuation of the dosing regimen. At 28 hrs and thereafter there was no significant effect of withdrawal on reinforcement thresholds. Additionally, in

concordance with our results there were no long-term effects of abstinence on ICSS responding (Schaefer & Michael, 1986). It is therefore possible that withdrawal causes a transient decrease in reward efficacy that last only a few hours and was not detected in our experiment

In general, our experiment revealed two effects of morphine dependence. Firstly, despite the development of physiological tolerance in dependent rats there is neither a corresponding tolerance to the rewarding effects of morphine nor, a withdrawal-precipitated shift in reward efficacy. Secondly, tolerance to the sedative effects of high doses of morphine may unmask strong reward at these doses in dependent individuals. It is often assumed that administration of increased dose of morphine in dependent individuals is a byproduct of lower doses losing their original acute rewarding effects (Koob, 2000). Our results argue that the acute rewarding effects of morphine remain intact in dependent individuals. In agreement with this, CPP studies have reported that morphine reward is equivalent in opioid experienced and naïve individuals suggesting that physiological tolerance to morphine does not accompany tolerance to its reinforcing effects (Bechara & van der Kooy, 1992, Contarino, 1997 #112).

Another common hypothesis is that addicts re-administer opiates primarily to avoid the negative effects of withdrawal (Koob, 2000). The extreme form of this hypothesis is that tolerance causes loss of acute rewarding effects of opiates, and escape from withdrawal becomes the sole driving force behind re-administration (Bechara & van der Kooy, 1992). It is well established that withdrawal has negative physical and psychological effects. In humans and rats, precipitated withdrawal from opiates is accompanied by flu-like symptoms and reports of anhedonia and anxiety (Koob & Le

Moal, 2008). Some studies have reported increased morphine salience during withdrawal; for example, Cooper et al., (2008) associate the observed increased self-administration of remifentanil during full withdrawal (24-h after maintenance dose) but not present in an opioid deprived (12-h after maintenance dose) state as an indication of increased reward efficacy specific to the withdrawal state. We suggest that this may be a byproduct of decreased lethargy at later stages of withdrawal; Schaefer & Michael (1986) reported that the withdrawal related depression of response rate decreased at later time points independent of increased reward efficacy (as evidenced by ICSS thresholds). In contrast, our results show that the physiological effects of withdrawal do not increase morphine salience, and suggest that morphine may be equally rewarding to a dependent individual whether or not they have morphine in their system. It seems that withdrawal may cause a transient generalized depression of the reward system as evidenced by ICSS rates (Schaefer & Michael, 1986) and sucrose self-administration (Zhang et al., 2007) and no long term effect on the reward system as evidenced by our results.

Physical dependence itself increases morphine self-administration; in rats on a dosing regimen those that exhibited physical dependence had higher rates of SA (Weeks & Collins, 1979). Due to the interconnectivity of dependence and withdrawal, physical dependence may cause an overestimation of the role of withdrawal in the choice addicts make to re-administer opiates. In support of our results, Contarino et al. (1997) argue that tolerance to the negative effects of high doses increases net reward of these doses in dependent rats. Thus, we suggest that continued use and reuse of opioids during dependence may have less to do with escape from withdrawal, and more to do with physiological changes that enable the rewarding effect of high doses to be revealed. The

dose-effect curve for reward is not changed by dependence but tolerance enables the user to self-administer higher and more rewarding doses of morphine. Thus, the strong reward associated with high doses increase the likelihood of their administration and helps account for the increased motivation for opiates reported to accompany dependence.

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