Effect of exercise on aversion to acute opioid withdrawal in mice

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Abstract

Each year, in the United States, approximately 750,000 people are dependent on heroin (Kosten, 2003), the most commonly abused opioid, making heroin dependence a pressing issue. Continued opioid use may involve both positive reinforcement (reward) and negative reinforcement (avoiding the negative consequences of drug withdrawal). The focus of this study was acute opioid withdrawal, which can be produced by the administration of the opiate antagonist naloxone following a single dose or short-term exposure to morphine. The present study explored the effect of a behavioral manipulation (exercise) on the aversive effects of acute morphine withdrawal in mice. Conditioned place aversion (CPA) was chosen as the animal model to study this effect. Separate groups of mice were either given two hour access to running wheels for eight days before CPA and then tested (Group Before), or given access to running wheels after CPA, and then tested (Group After). Both groups went through a CPA schedule of two pairings of morphine (10 mg/kg) followed by the opioid antagonist naloxone (0.32 mg/kg) four hours later. As expected, acute opioid withdrawal produced a reliable CPA effect. The magnitude of CPA was decreased in the wheel running groups (Before and After). Thus, exercise appears to decrease the aversive properties of opioid withdrawal. Given the results of this study, it can be hypothesized that exercise might be a useful treatment for formally opioid-dependent patients who are in an abstinence period.
Introduction

Exercise is a popular activity for various reasons; it is a way to maintain physical health, make people feel more energetic, make people look fit and feel attractive, etc. Exercising may have additional benefits outside of traditional results, such as an adjunctive therapy for psychological problems like depression, stress, or drug dependence (Fox 1999; Paluska & Schwenk, 2000; Raglin, 1990). In addition, there is a considerable amount of evidence that suggests that not unlike humans, exercise is rewarding in rodents (Sherwin, 1998). The aim of the current project is to begin to explore the effects of exercise on the development of drug dependence.

Each year, in the United States, approximately 750,000 people are dependent on heroin (Kosten, 2003), the most commonly abused opioid, making heroin dependence a pressing issue. In many instances, the initial use of drugs such as heroin is for the euphoric (rewarding) effects of the drug. However, continued use of the drug may involve both positive reinforcement (reward) and negative reinforcement (avoiding the negative consequences of drug withdrawal) (Koob & Le Moal, 2001, 2005). Dependence on opiates and the occurrence of withdrawal symptoms upon abstinence is most commonly studied under conditions of chronic exposure to opiate agonists. However, signs characteristic of the opiate withdrawal syndrome can be precipitated by injection of an opiate antagonist after pretreatment with a single dose of an opiate agonist. For example, studies in humans have indicated that administration of the opiate antagonist naloxone following exposure to a single dose of morphine (Heishman, Stizter, Bigelow, & Liebson, 1989 a,b) can produce physiological signs, subjective ratings, and objective
(somatic) symptoms characteristic of withdrawal from chronic opiates. This phenomenon has been referred to as “acute dependence” (Matine & Eades, 1964).

The three most commonly used methods for studying opiate withdrawal are visual characterization of observable (somatic) signs of withdrawal (e.g., jumping, tremor, wet-dog shake) (Gellert & Holtzman, 1978), suppression of operant behavior (Schulteis, Heyser, & Koob, 1997) and conditioned place aversion, or CPA (Azar, Jones, & Schulteis, 2003). The latter two methods (suppression of operant behavior and CPA) are used to explore the motivational significance of opiate withdrawal. Given that the aim of this project is to look more directly at the motivational effects of opiate withdrawal and the effects of exercise on this process, the CPA model was chosen for the following reasons. First, CPA has been established as highly sensitive procedure to indicate the aversive motivational qualities of withdrawal from a chronic [or acute] state of opioid dependence (Azar et al., 2003). Second, this model does not require food or water deprivation, which is necessary in the suppression of operant responding method.

In the CPA model, animals are trained in an environment in which one distinctive room is paired with opioid withdrawal, induced by an injection of opiate followed by an opioid antagonist (i.e. naloxone), while a second distinctive room is paired with an injection of a vehicle, such as saline, which will have no aversive effects. Due to the aversive consequences of the induced opioid withdrawal, the animal will avoid the room paired with the withdrawal when given free access to both rooms, after only a few pairings (Azar et al., 2003; Parker & Joshi, 1998). This CPA effect is measured by the amount of time the animal spends in each room when tested (given free access to both rooms). Prior research using the CPA model (described below) has found various other
experimental manipulations that affect aversion to opioid withdrawal. For example, isolation (Broseta, Rodriguez-Arias, Aguilar, & Minarro, 2005), GHB, a naturally occurring substance in the brain (Maldonado, 2004), glutamate receptor antagonists (Kawasaki et al., 2005), and buprenorphine and CRF1 antagonists (Stinus, Cador, Zorrilla, & Koob, 2005) have all been shown to attenuate aversion to opioid withdrawal. To my knowledge, however, the effect of wheel running on aversion to opioid withdrawal has not been examined, to date. Therefore, the current study has the potential to determine if exercise will have an effect, either to lessen or intensify aversion to opioid withdrawal.

Wheel running is a common model for exercise in rodents and has been found to be self-reinforcing in rats (Sherwin, 1998). Previous research showed that naloxone, attenuates the conditioned place preference induced by wheel running in rats (Lett, Grant, & Koh, 2001). Also, it has been observed that prior experience with wheel running produces cross-tolerance to the rewarding effect of morphine (Lett, Grant, Koh, & Flynn, 2002). Thus, the hypothesis that the rewarding effect of wheel running can be attributed to the involvement of exercise in the endogenous opioid system has emerged from the literature. Since morphine acts on the opioid system, it is plausible to hypothesize that exercise will have an effect on aversion to opioid withdrawal. More specifically, in the present study, the morphine/naloxone CPA training is assumed to produce conditioned place aversion, of which wheel running will either potentiate or attenuate.

An additional point worth emphasizing is that the ability to quantify the occurrence of these adaptive changes, with indices that are well established as markers of withdrawal in chronically opiate dependent rats, suggests that they may reflect the
beginning stages in the development of a chronic opiate dependent state (Schulteis et al., 1997). Thus, the results could generate a basis for the exploration of exercise as a non-traditional treatment for patients who are undergoing opioid withdrawal.

Methods

Subjects:

In this project, 64 adult male C57BL6J mice (60 - 80 days of age) were used. The mice were housed in groups of 3 - 4 per cage in a temperature-controlled room on a 12 h light/dark cycle (lights off at 10 am). The mice were given ad libitum access to food and water in their home cage. All mice were handled 3-5 days prior to the experiment.

Materials:

Running Wheels. Each of the eight stainless steel running wheels (Mini Mitter/Respironics, Bend, OR) had a circumference of 24.2 cm and a width of 8.0 cm. Each wheel was within a polycarbonate cage with water available and pine shavings on the floor.

CPA Apparatus. The CPA apparatus consisted of a rectangular wooden box divided into two chambers distinguished by different odors. Odors were prepared by soaking a cotton ball with 1.0 cc of the odor extract, either mint (McCormick Pure Mint Extract, Hunt Valley, MD) or almond (McCormick Pure Almond Extract, Hunt Valley, MD). The cotton ball was attached near the top of the chamber, so it could not be reached by the mice. Each chamber (30 x 20 x 29 cm) was separated from the other by a wall containing a hole (diameter 6 cm) for crossing between chambers, which was blocked during training.
Drug Solutions. Morphine and naloxone were obtained from Sigma Chemicals, St. Louis, MO. All drugs were dissolved in physiological saline, and the concentrations were based on the weight of the salt.

Procedure:

The entire group of mice was separated into two groups Before and After, which were each sub-divided into four groups (in total, four groups of eight mice in both Before and After). Both Groups Before and After went through four, identical phases, except in different orders (see Experimental Design Table), all taking place during the dark cycle. Group Before had access to the running wheels prior to pre-conditioning testing, then CPA training, and then a post-conditioning test immediately after the last conditioning session. Group After went through pre-conditioning testing, CPA training, followed by wheel running, and then post-conditioning testing.

Experimental Design Table: order of phases for Group Before and Group After

<table>
<thead>
<tr>
<th>Group</th>
<th>Wheel running (8 days)</th>
<th>Pre-conditioning test (1 day)</th>
<th>CPA training (4 days)</th>
<th>Post-conditioning test (1 day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>After</td>
<td>Pre-conditioning test (1 day)</td>
<td>CPA training (4 days)</td>
<td>Wheel running (8 days)</td>
<td>Post-conditioning test (1 day)</td>
</tr>
</tbody>
</table>

In the wheel running phase, two of the four groups (Wheel Runners, WR) received access to a running wheel two hours a day for eight days. The other two groups (Non Runners, NR) were placed in a cage with a wheel that was locked in a stationary position for the same amount of time, which served as a control for wheel running. Water was available during running.
A pre-conditioning test preceded the CPA training. During the pre-conditioning test, which was identical to the post-conditioning test after CPA training, the mice were placed into the CPA apparatus, and given free access to both chambers, one with mint odor and the other with almond odor, for ten minutes. The amount of time spent in each chamber was measured to confirm that no bias for either chamber existed, for all mice. The time spent in each chamber was calculated as a difference score (time in mint – time in almond).

CPA training consisted of 4 days of a conditioning phase. During conditioning, on days 1 and 3, mice from one WR group and one NR group received an injection of the opioid antagonist naloxone (0.32 mg/kg administered subcutaneously (SC)), 4 hours after receiving an injection of morphine (10.0 mg/kg, SC). Immediately after the injection of naloxone, the mice were individually confined to the mint-odored chamber CPA apparatus, to be paired with that drug, for 30 minutes. On days 2 and 4, the mice were confined to the almond-odored chamber, paired with a vehicle injection (1.0 ml/100 g saline, SC), 4 hours after vehicle (1.0 ml/100 g saline, SC), and remained there for 30 minutes. The other WR group and NR group were given the same CPA schedule, except they received vehicle/vehicle on days 1 and 3 as well, serving as a control for the morphine/naloxone treatment.

Post-conditioning testing either occurred directly after CPA training for Group Before, or after wheel running for Group After. As with the pre-conditioning testing, the mice were placed into the CPA apparatus, and given free access to both chambers, one with mint odor and the other with almond odor, for ten minutes. The amount of time spent in each chamber, along with the number of crosses between chambers, was
measured for all mice. Aversion for the morphine/naloxone chamber was calculated as a difference score (time in mint – time in almond). The mean difference scores of each group for both the pre-conditioning test and post-conditioning test, the mean number of crosses between chambers of each group for the post-conditioning test, and the number of wheel rotations for the WR groups was analyzed in SPSS using an ANOVA for Groups Before and After, to determine the effect of exercise on the aversive properties of opioid withdrawal.

**Data Analysis:**

All data analysis for Group Before and Group After were done separately. The number of wheel rotations was analyzed by a 2 (drug condition) x 8 (days) mixed analysis of variance (ANOVA). Drug condition (vehicle or morphine/naloxone) was a between-subjects factor and days of access to the wheels served as a within-subjects (repeated) factor in the ANOVA. The number of crosses between chambers was analyzed by a 2 (exercise [wheel running or no wheel running]) x 2 (drug condition) ANOVA. It is possible to present the pre- and post-conditioning test data in three ways: absolute time in the mint chamber, proportion of time in the mint chamber relative to the almond chamber, or as a difference score (time in the mint-scented chamber minus the time in the almond-scented chamber). The difference score was selected, as it is more illustrative of odor preference or aversion (i.e., positive scores reflect a preference for the odor whereas a negative score reflects an aversion to that odor). Therefore, in the analysis of odor preference or aversion, the difference score was used as the dependent variable. These data were analyzed by a 2 (exercise) x 2 (drug condition) ANOVA. A Bonferoni test for multiple comparisons and simple main effects analyses were used to
determine the locus of significant main effects and interactions. A significance level of $p < 0.05$ was used for all statistical analyses.

Results

**Group Before:**

*Wheel Running.* The ANOVA conducted on the number of wheel rotations revealed a significant main effect of days $F(7,98) = 54.08, p < 0.05$ and drug condition $F(1,14) = 5.49, p < 0.05$, along with a significant days x drug condition interaction $F(7,98) = 6.58, p < 0.05$. Further analyses indicated that wheel running increased significantly for the first 3 days after which asymptotic levels of running were reached (see Figure 1). No differences in wheel running were observed on day 1 between the groups. Mice assigned to the vehicle group ran significantly more than those assigned to the morphine/naloxone condition. The locus of the interaction appears to be mainly due to a sudden increase in running in the mice assigned to the vehicle group on day 4 relative to the mice assigned to the morphine/naloxone drug condition on Day 4. Although wheel running decreased in the mice assigned to the vehicle condition on the next day, Day 5, these mice continued to have higher wheel running totals than those mice assigned to the morphine/naloxone treatment group (see Figure 1).
Figure 1. Mean (+ SE) number of wheel rotations made by all 8 mice in Group Before (wheel running before CPA) vehicle and morphine treated groups during each 2-hr session.

**Crosses.** The ANOVA conducted on the number of crosses between the chambers during the post-conditioning test revealed a significant main effect of drug condition (vehicle or morphine/naloxone) $F(1,28) = 29.56, p < 0.05$ and exercise (no wheel running or wheel running) $F(1, 28) = 4.77, p < 0.05$. Further analyses indicated that vehicle-treated mice made more crosses than morphine/naloxone-treated mice (see Table 1). In addition, mice with wheel-running experience made more crosses than mice that were not able to run on the wheel.
Table 1

Mean (+ SE) number of crosses made by all 8 mice between chambers during 10 min. post-conditioning test in all groups, vehicle and morphine, Groups Before and After.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Crosses Before</th>
<th>Mean Crosses After</th>
</tr>
</thead>
<tbody>
<tr>
<td>NR veh</td>
<td>55.4 ± 2.0</td>
<td>67.9 ± 3.1</td>
</tr>
<tr>
<td>WR veh</td>
<td>70.0 ± 5.9</td>
<td>42.1 ± 2.1</td>
</tr>
<tr>
<td>NR morph</td>
<td>40.1 ± 3.0</td>
<td>34.4 ± 4.8</td>
</tr>
<tr>
<td>WR morph</td>
<td>42.6 ± 3.7</td>
<td>53.0 ± 3.4</td>
</tr>
</tbody>
</table>

Pre-conditioning Test. During the pre-conditioning test, there was no significant difference in the time spent in the mint-scented chamber and the time spent in the almond-scented chamber (see Figure 2). Given that all difference scores are close to zero, it can be concluded that there were no initial preference for either odor in any of the groups prior to conditioning. As such, this can be considered an unbiased design.
Figure 2. Mean (+ SE) difference score (time in mint – time in almond) of all 8 mice in all groups during a 10 min. pre-conditioning test period. Time spend in the mint-scented chamber was not significantly different than time in the almond-scented chamber.

Post-conditioning Test. The statistical analysis of the post-conditioning difference scores (time in mint – time in almond) revealed a significant main effect of drug condition $F(1, 28) = 71.64, p < 0.05$, along with a significant interaction of drug condition and exercise $F(1, 28) = 5.95, p < 0.05$. Here it should be noted that a negative difference score refers to an aversion for mint odor, whereas a positive score would reflect a preference for the mint-scented chamber. Mice treated with vehicle continue to show no aversion for either odor. In contrast, mice treated with morphine/naloxone spent significantly less time on the mint chamber relative to the almond chamber (see Figure 3). This is indicative of conditioned place aversion. Further analysis of the interaction indicated that for the morphine/naloxone condition, the wheel running group exhibited significantly less aversion than mice in the no wheel running group (see Figure 3).
Figure 3. Mean (+ SE) difference score (time in mint – time in almond) of all 8 mice in Group Before (wheel running before CPA) during a 10 min. post-conditioning test period, which occurred following CPA training. Wheel running group exhibited significantly less aversion than mice in the no wheel running group (*p < 0.05).

**Group After:**

*Wheel Running*: The ANOVA conducted on the number of wheel rotations revealed a significant main effect of days F (7,98) = 25.19, p < 0.05, along with a significant days x drug condition interaction F (7,98) = 4.37, p < 0.05. Further analyses indicated that wheel running increased significantly for the first 3 days after which asymptotic levels of running were reached (see Figure 1). No differences in wheel running were observed on day 1 between the groups. However as the experiment progressed across days, vehicle-treated mice ran significantly less than morphine/naloxone-treated mice (see Figure 4).
Figure 4. Mean (+ SE) number of wheel rotations made by all 8 mice in Group After (wheel running after CPA) vehicle and morphine/naloxone treated groups during each 2-hr session.

Crosses. The ANOVA conducted on the number of crosses between the chambers during the post-conditioning test revealed a significant main effect of drug condition $F(1,28) = 10.43, p < 0.05$. There was also a significant interaction between drug condition and exercise on the number of crosses $F(1,28) = 40.10, p < 0.05$. Vehicle-treated mice in the no wheel group made significantly more crosses than all other groups (see Table 1). In contrast, morphine/naloxone-treated mice in the no wheel group made significantly fewer crosses than all other groups (see Table 1).

Pre-conditioning Test. During the pre-conditioning test, there was no significant difference in the time spent in the mint-scented chamber and the time spent in the almond-scented chamber (see Figure 2). Given that all values are close to zero, it can be
concluded that there were no initial preferences for either odor in any of the groups prior to conditioning. As such, this can be considered an unbiased design.

*Post-conditioning Test.* The statistical analysis of the post-conditioning difference scores (time in mint – time in almond) revealed a significant main effect of drug condition $F(1, 28) = 74.40, p < 0.05$, along with a significant interaction of drug condition and exercise $F(1, 28) = 4.00, p = 0.05$. As stated before, it should be noted that a negative difference score refers to an avoidance of the mint odor, whereas a positive score would reflect a preference for the mint-scented chamber. Mice treated with vehicle continue to show no aversion for either odor. In contrast, mice treated with morphine/naloxone spent significantly less time on the mint chamber relative to the almond chamber (see Figure 5). This is evidence for conditioned place aversion. Further analysis of the interaction indicated that for the morphine/naloxone condition, the wheel running groups exhibited significantly less aversion than mice in the no wheel running group (see Figure 5).
Figure 5. Mean (+ SE) difference score (time in mint – time in almond) of all 8 mice in Group After (wheel running after CPA) during a 10 min. post-conditioning test period, which occurred following wheel running. Wheel running group exhibited significantly less aversion than mice in the no wheel running group (*p = 0.05).

Discussion

In all four wheel running groups, the number of wheel rotations per two-hour session increased during days 1-3, reaching an approximate asymptotic level by day 4. The increase in wheel running can be attributed to the self-reinforcing properties that are associated with wheel running (Sherwin, 1998). In addition, it is a plausible explanation that the wheel running not only increased due to the rewarding effect, but also in response to an increase in practice with the wheel after repeated exposure, and thus increasing coordination and ability to run on the wheel.

Ideally, the mean number of wheel rotations per day, during the eight two-hour wheel running sessions, would have increased to the same asymptotic level for all four
wheel groups, thus allowing the effect of wheel running to be equally compared across all groups. However, several differences among the groups were significant.

For Group Before, the significant difference in the mean number of wheel rotations between mice assigned to the vehicle-treated and morphine/naloxone-treated groups is most apparent on day 4. There was a sudden increase in mean number of wheel rotations in the mice assigned to the vehicle-treated group on that day. It is important to note that both groups were not actually exposed to injections, of vehicle or drug, before wheel running. The significant difference in the mean number of wheel rotations between these groups may be due to random variance in activity levels of the two groups of mice, resulting in a selection bias (i.e. group assignment). It is also possible that this is simply a spurious effect on day 4, the cause of which remains unclear. Nonetheless, it is apparent is that mice in both groups increased wheel running across days, and reached an asymptotic level.

For Group After, the mean number of wheel rotations for the morphine group was significantly greater than the vehicle group. This could be attributed to ability of exercise to attenuate the aversive properties of acute opioid withdrawal, as was hypothesized. In other words, because the morphine/naloxone group was in withdrawal, they may have increased their running levels in attempt to lessen the negative aspects of opioid withdrawal. The decrease in activity, which may have been caused by injections, was possibly negated by the motivation to reduce opioid withdrawal, a motivation that the After vehicle group did not experience. However, as with Group Before, it is also reasonably possible that these differences in mean number of wheel rotations is entirely
due to random variance in activity levels of each group of mice. In spite of this, it is important to note that all groups did, in fact, wheel run during the eight day period.

The number of chamber crosses during the post-conditioning test phase was recorded in the present study. Thus, all mice had gone through exercise (no wheel running or wheel running) and drug conditioning (vehicle or morphine/naloxone). Although the implications of chamber activity (i.e. crosses) have received little attention in the literature, there are several reasons for its inclusion in the present study. First, chamber crosses can serve as a general index of exploratory behavior. For example, extremely low levels of activity might reflect an avoidance of a given chamber (a strong aversive response). However, extremely low levels of activity may also be the result of freezing in the aversive environment, resulting in the observation of preference for that environment. Although significant differences in chamber crosses were observed in the present study, these values are within normal range and provide evidence that the mice were actively exploring their environment.

A second reason for examining the number of chamber crosses is because it is highly conceivable that the two manipulations used (exercise and drug condition), lead to changes in activity levels (and patterns). In the present study, the mean number of crosses in the vehicle group was greater than that observed in the morphine/naloxone group. This would suggest that opiate withdrawal leads to a decrease in chamber crosses either by directly inhibiting activity or by simply reducing entries into the withdrawal chamber. In addition, the finding that in the Before Group, wheel running mice made a greater number of crosses than mice that did not run could be the result of an overall increase in activity as a result of wheel running (exercise). To answer these questions,
one would need to monitor activity within each chamber during conditioning and testing (Cunningham, Patel, & Milner, 2006).

The lack of significant difference between all eight groups in the mean difference scores during the pre-conditioning odor test shows that prior to CPA training, mice did not have a preference or aversion to either the mint or almond chambers. This reinforces the important fact that no bias exists within the apparatus, and the procedure can therefore be considered unbiased.

A consistent place aversion was observed in the mice given two morphine/naloxone pairings in the mint-scented chamber. As illustrated in the figures, the negative mean difference score showed that the morphine/naloxone CPA training did, indeed, successfully produce conditioned place aversion. This confirms the existing literature findings that conditioned place aversion can be precipitated by the treatment of an opioid antagonist after a single does or short term exposure to an opioid antagonist (Azar, et al., 2002; Parker & Joshi, 1998). Therefore, the no wheel running groups Before and After, provided a reliable baseline level of aversion with which to compare the wheel running groups to.

The experience of wheel running significantly affected the expression of conditioned place aversion. More specifically, the time spent in the mint “withdrawal” chamber was significantly less for the wheel running than the no wheel running morphine group, for both Group Before and Group After. As a result the mean difference score for the no wheel running group was more negative than the wheel running group. Therefore, aversion to the “withdrawal” chamber was decreased by wheel running, whether before or after conditioning. This confirms the hypothesis that exercise will attenuate the
aversive effects of acute opioid withdrawal. This effect is apparent regardless of whether wheel running is before conditioned withdrawal or after.

To my knowledge, this is the first demonstration of exercise (wheel running) attenuating the expression of conditioned place aversion. The neural mechanisms underlying this effect are unknown, as this study was conducted only on a behavioral basis. Further research on a molecular level, such as microdialysis, would have to be done during each of the phases of this study, pre-conditioning testing, wheel running, CPA training, and post-conditioning testing, for both Before and After groups, in order to reach a more conclusive answer. Nevertheless, there are several plausible explanations for this effect.

The rewarding actions of opiates appear to take place in several brain locations, primarily in the midbrain dopamine system projections to the VTA and nucleus accumbens, and also in the periaqueductal gray, hypothalamus, and hippocampus (Wise, 1989). Opioid dependence primarily deals with the mu receptor (Easterling & Holtzman, 2004) and secondarily the delta receptor (Harris & Gewirtz, 2004), on which endogenous and exogenous opioids act. Opioid withdrawal can be simulated by the administration of an opioid receptor agonist (i.e. morphine), followed by an opioid receptor antagonist (i.e. naloxone). At low doses, naloxone acts primarily on the mu receptor (Liu & Prather, 2001), and naloxone fails to produce a place aversion effect in mu-opioid receptor knockout mice (Skoubis, Matthes, Walwyn, Kieffer, & Maidment, 2001). Exercise, manifested through wheel running, has been shown to not only increase levels of endorphins (Hoffmann, Terenius, & Thoren, 1990), but dynorphin and enkephalin (Bjornebekk, Mathe, & Brene 2006) as well, implying that exercise produces changes in
the endogenous opioid systems. In addition, it has been observed that the aversive properties of naloxone are attenuated by lesioning of the primary site of central beta-endorphin synthesis (Mucah, Millan, & Herz, 1985), suggesting that naloxone may also be an antagonist of beta-endorphin activity in the brain. Therefore, it can be hypothesized that naloxone-precipitated opioid withdrawal is affected by exercise.

The mediation of the effect of exercise by the endogenous opioid system has been demonstrated in a variety of studies. The findings of Lett et al. (2001) illustrate that the conditioned place preference induced by the rewarding effects of wheel running (exercise), can be attenuated by naloxone. Buprenorphine, an opioid partial agonist at the mu opioid receptor, was found to block the acquisition of opiate withdrawal-induced conditioned place aversion (Stinus, Cador, et al., 2005). It was hypothesized that buprenorphine was effectively blocking the negative affective state associated with withdrawal. The explanation for the effect of this study can easily be translated to that of the current study. Here, it is plausible that exposure to wheel running both prior to CPA and following CPA, produces an increase in endogenous opiates, which then act to reduce the aversive effects of withdrawal.

As the procedure of this study utilized only two pairings of conditioned place aversion, it allowed examination of acute, as opposed to chronic, opioid withdrawal. Therefore, the hypothesis that development of opioid dependence is progressive and may be initiated with a single opiate exposure, as supported in the literature (Schulteis, et al., 1997), is further reinforced with the results of this study. Thus, acute withdrawal should be considered highly pertinent to chronic withdrawal, as it helps to evaluate the initial phases of induction of chronic dependence (Harris & Gewirtz, 2004). Research within
the field of acute dependence and withdrawal may help to explain how dependence is initiated and why it leads to chronic dependence.

The initial reasoning behind this study was to explore the effect of manipulations of the opioid system during a period of abstinence from opiate use. The research on the relevant topic of drug abstinence has produced particularly interesting and somewhat counterintuitive results. On the topic of drug craving, contrary to the view that cocaine craving decays progressively after the stopping of drug use, it has been observed that persistence of cocaine-seeking habits, tested from 1 to 60 days after the last day of self-administration of cocaine, increase linearly over time (Grimm, Hope, Wise, & Shaham, 2001; Liu, L., Hope, Dempsey, Liu S.Y., Bossert, & Shaham, 2005). Therefore, their study found an increase in craving during a period of abstinence, which became termed the “incubation of craving.” In comparison, in the present study, mice in Group Before were tested immediately after conditioning, where as Group After were tested eight days after conditioning. As was observed with cocaine craving, aversion to morphine withdrawal persists for at least one week after conditioning, as seen in this study, confirming the findings of a previous study that showed long retention of opiate withdrawal-induced conditioned place aversion (Stinus L, Caille S, & Koob, 2000).

These findings help to illustrate that a neutral stimulus, such as the mint-scented chamber, can take on aversive associations that can endure for long periods of abstinence, as a result of its pairings with opioid withdrawal. Therefore, relapse to drug taking, after a period of abstinence, may be the result of the stimulus acting as a cue that serves as a negative reinforcing stimulus, which in turn, increases the motivation to reduce the negative conditioned effects of the cue. For that reason, examination of abstinence from
drug-taking may be crucial to understanding the neural changes that are correlated with the development of dependence and susceptibility to relapse. Thus, therapy and treatment after a patient stops drug-taking is an extremely important component in the prevention of relapse to drug dependence. Given the results of this study, it can be hypothesized that exercise could be a useful treatment for patients who are in an abstinence period from opiate use.
References


Cunningham, C.L., Patel, P., & Milner, L. Spatial location is critical for conditioning place preference with visual but not tactile stimuli. *Behavioral Neuroscience, 120(5),* 1115-1132.


