2006

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Your file Votre référence
ISBN: 978-0-494-16742-7
Our file Notre référence
ISBN: 978-0-494-16742-7

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THESIS

Submitted to the Department of Psychology

In partial fulfillment of the requirements for

Master of Science, Psychology

Wilfrid Laurier University

2006

Abstract

Two experiments were conducted to determine whether cross-sensitization/tolerance between wheel running and the drugs amphetamine and morphine is possible in male Sprague Dawley rats. Each experiment compared a non-wheel control group and a chronic wheel access group of rats. Following a 24 day period of wheel access all animals were presented with a drug and saline challenge test (counterbalanced) with either 1 mg/kg of amphetamine (Experiment 1) or 10 mg/kg of morphine (Experiment 2). Prior to the challenge tests all animals were habituated to the novel testing environment in two 1 hr sessions (Experiment 1) or one 2 hr session (Experiment 2) to attenuate the acute motoric response to a novel environment. Behavioral sensitization/tolerance was measured by locomotion (cm) within long narrow activity boxes with the Ethovision video tracking system. In the first experiment the wheel access rats were significantly more active during the 1 hr amphetamine challenge test than the non-wheel rats thus showing sensitization. In Experiment 2, the wheel access rats demonstrated a reduced hypoactivity in the first hour and an augmented hyperactivity in the second hour of the 2 hour drug challenge test in comparison to the non-wheel rats after morphine administration thus showing both tolerance and sensitization. These two experiments provide strong support that cross-sensitization and cross-tolerance exists between wheel-running and amphetamine or morphine in rats. In both experiments the final level of wheel running, which varied greatly, was not correlated with the degree of cross-sensitization/tolerance to either amphetamine or morphine suggesting that the changes induced by wheel running were similar in all animals. In Experiment 2 it was also found that levels of both initial and final levels of wheel running were uncorrelated with a prior 2 hour baseline locomotor activity test suggesting that an enhanced vulnerability to the

addicting behavior of wheel running could not be demonstrated by looking at the level of wheel running in rats.

Acknowledgements

First and foremost I would like to thank Dr. Roelof Eikelboom, my advisor, for his guidance, encouragement, patience, and for his valuable time that he has given over the course of my studies. I am thankful for the opportunity to work within his lab.

My sincere thanks go also to my committee members, Dr. Linda Parker and Dr. Todd Ferretti, for their precious help, suggestions, and support. I appreciate the time they spent reading and commenting on my thesis.

I am also thankful to Kelly and Kristin for maintaining the lab and looking after the animals. I would also like to thank my lab-mate Graham Parfeniuk for his assistance in my experiments.

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The pattern of escalating wheel running seen in rats may have some parallels to the escalating consumption seen in human addictive behaviors (Eikelboom & Lattanzio, 2003). When given ad libitum wheel access young adult male rats voluntarily increase their running, beginning at low levels and over a few weeks reaching a peak of several thousand wheel turns per day (approximately 6 kms) (Eayrs, 1954; Eikelboom & Mills, 1988; Looy & Eikelboom, 1989). If this escalating wheel running behavior in rats can be considered an addiction-like behavior then it should exhibit features similar to those found with other escalating addictions such as drug abuse. It suggests that changes in behavior or neurobiology observed with other addictions should be evident in rats given wheel running experience. Changes evident in response to repeated administration of addicting drugs should also be evident after continuous chronic wheel access.

An increasing "consumption" is characteristic of both wheel running and drug self-administration and is believed to be an important defining feature of addiction (Ahmed & Koob, 1998). This escalating drug consumption has been suggested as being due to changes in drug effectiveness after repeated exposure. Sensitization (increased effectiveness of the drug) and tolerance (decreased effectiveness of the drug) have both been argued to be critical to the development of addiction (Anagnostaras & Robinson, 1996; Hinson & Siegel, 1982; Siegel, 1979, 1983). The escalating wheel running pattern seen in rats with ad-libitum wheel access may be due to, or cause, similar underlying physiological changes and serve as an efficient model of the transition from occasional drug use to addiction (Werne, Thoren, Olsen, & Brene, 1999, 2000). The high levels of wheel running behavior seen with ad libitum wheel access rats may be due to a sensitization or tolerance-like process. If this hypothesis is correct, then cross-sensitization or tolerance between wheel running and drugs of abuse might be evident.

This would suggest that behavioral addictions in humans (ie; exercise addiction) may also be due to processes responsible for drug sensitization.

If wheel running is a non-drug addiction, running should also be rewarding. Two paradigms have been suggested for the investigation of the rewarding properties of wheel running (Belke & Wagner, 2005). Making the opportunity to run dependent upon engaging in an operant behavior such as lever pressing is one way to show that wheel running is rewarding and animals will readily learn to lever press for wheel access (Belke & Heyman, 1994; Iverson, 1993; Kagan & Berkun, 1954). The second paradigm is conditioned place preference testing which involves pairing a specific context with wheel running and a different context with no wheel access. If the rats subsequently prefer the context previously paired with the wheel, then wheel running is a reinforcer. Belke and Wagner (2005) showed that rats would learn to press a lever for access to a wheel. Furthermore, the afteraffect of wheel running was sufficient to produce a preference for a chamber with which they were previously paired. There is now a considerable amount of additional support for the notion that during place preference testing rats will spend more time in a context that has been paired with wheel running as opposed to a context that has not been paired with wheel running (Lett, Grant, Byrne, & Koh, 2000; Lett, Grant, & Koh, 2001; Lett, Grant, Koh, & Flynn, 2002).

Previous investigations of wheel running in our lab have indicated that a rat's wheel running behavior becomes excessive but has also revealed a large degree of variability in the wheel running behavior of rats. When given wheel access, young male rats in our laboratory start to run about 1000 wheel turns per day. Over a period of several weeks, running increases until it plateaus at around 5000-6000 wheel turns per day, a distance of 5-6 km (Afonso & Eikelboom, 2003; Eikelboom & Mills, 1988; Looy

& Eikelboom, 1989; Mueller, Herman, & Eikelboom, 1999; Mueller, Loft, & Eikelboom, 1997). It has also been observed that in experienced runners some rats run over 12000 wheel turns per day while others run less than 1000 (Eikelboom, 2001). While the running difference between animals is large, it is stable for individual animals (Afonso & Eikelboom, 2003). Therefore, it is evident that wheel running in rats develops into an excessive behavior and that there is a significant degree of variability in this excessive behavior as is also evident in human addictive behaviors.

A cross-study comparison between Ahmed and Koob (1998) and Eikelboom and Lattanzio (2003) supports the hypothesis that wheel running in rats is an addiction which may parallel the self-administration behavior characteristic of addicting drugs in rats. Ahmed and Koob (1998) reported that, for rats receiving short access to cocaine during the night, intake remained low and stable. For rats that were provided with long cocaine access periods, however, their intake gradually increased over nights with no sign of stabilization after 22 sessions. Eikelboom and Lattanzio (2003) reported that during the night, which is when rats engage in the majority of their wheel running behavior, wheel access periods of 2 hours or more produced, over days, increases in running, whereas a night time one hour access period resulted in low, stable running which did not increase over days. Thus, manipulating access duration had similar effects for drugs of abuse and wheel running.

Wheel running has been shown to affect systems that are important in the actions of abused drugs. Oral intake of amphetamine (Kanarek, Marks-Kaufman, D'anci, & Pryzpek, 1995) and the intravenous self-administration of cocaine (Cosgrove, Hunter, & Carroll, 2002) in rats are attenuated in rats given wheel running experience. Wheel running also increased the extracellular level of dopamine in the nucleus accumbens

(Freed & Yamamoto, 1985; Wilson & Marsden, 1995). In a cross study comparison it appeared that wheel running (Robinson & Kolb, 1999) and morphine self-administration (Robinson, Gorny, Savage, & Kolb, 2002) produced similar morphological changes in the nucleus accumbens. These examples support the notion that systems important to drug addiction also play a crucial role in wheel running in rats.

Wheel running attenuates the antinoceptive properties of morphine and, therefore, decreases the sensitivity to morphine-induced analgesia in rats (Kanerek, Gerstein, Wildman, Mathes, & D'Anci 1998; Mathes & Kanerek, 2001). Tail-flick latency to a painful stimuli was utilized in this research in order to test the possibility of cross-tolerance between wheel running and morphine. Kanerek et al. (1998) and D'Anci, Gerstein, and Kanarek (2000) found that rats running in activity wheels were less sensitive than sedentary controls to the antinoceptive effects of the u-opioid agonist, morphine, suggesting that running had induced a degree of tolerance to morphine. In addition to the decreased sensitivity, the duration of drug antinoceptive action was shorter in active rats than in inactive rats. Therefore, it was concluded that the decrease in antinoceptive responding to morphine in exercised animals was evidence for the development of cross-tolerance between endogenous opioid peptides released during exercise and the exogenously administered opioid agonist morphine.

Sensitization and Tolerance

To better understand the drug induced changes seen in the transition to addiction and how they might be evident in wheel experienced animals, a discussion of sensitization and tolerance would be helpful. In behavioral pharmacology sensitization is defined as a progressive and persistent increase in a drug effect as a result of repeated drug administration (Anagnostaras & Robinson, 1996). Tolerance can be understood as

the decreased effect of a drug following repeated administration (Tiffany & Maude-Griffin, 1988). Therefore, with tolerance higher doses of the drug are needed to produce the same effects. With sensitization, a constant drug dose will produce an escalated behavioral response with each administration, or the same behavioral response can be induced by a continuously decreasing dose. Another way to look at tolerance is that it represents a horizontal shift in the dose-effect curve to the right (Tiffany & Maude-Griffin, 1988) and sensitization represents a horizontal shift in the dose effect curve to the left (Ahmed & Koob, 2004).

Sensitization and tolerance are both processes that can only be observed in terms of some observable drug effect, but are thought to reflect underlying changes in the animal. It is not clear if all changes in an observed drug effect (either in terms of tolerance or sensitization) are due to the same internal change. Drugs may have multiple effects and each may change independently showing either tolerance or sensitization. In fact, in some instances both processes of sensitization and tolerance may be evident for the same behavioral measure. This is the case with morphine administration as the initial, immediate effects of morphine on motor activity are depressant (hypoactivity), which then are followed by a delayed excitatory response (hyperactivity) (Schnur, 1984). Therefore, the initial effects of morphine on motor activity in the naïve animal are biphasic. These motor effects of morphine are dose dependant with larger doses producing more depression and with the hyperactivity occurring later after the injection. After repeated spaced morphine injections tolerance occurs to the depressant effects but the excitatory effect becomes more pronounced and occurs earlier (Schnur, 1984). Thus, with repeated exposure to the same morphine dose, one will see a decrease in the initial hypoactivity and an increase in the subsequent hyperactivity with it occurring earlier in

time. Depending on what part of the process one is concerned with one could argue that the motor effects of morphine show tolerance (to the hypoactivity) or sensitization (to the hyperactivity).

While many drug effects show tolerance or sensitization, the changes in the motor effects of drugs of abuse like those discussed for morphine have received considerable attention. It is assumed that changes in these motor effects are reflective of the changes that may be evident in the transition from consumption to addiction. Since the motor effects of common drugs of abuse like amphetamine (Browman, Badiani, & Robinson, 1998) and morphine (Babbini & Davis, 1972) are well characterized, this will be the measure that I use to explore how prior wheel running changes the animal's drug response. In other words, do rats with running experience show a motor response to drugs reflective of a rat with prior drug experience?

Measurement for sensitization/tolerance induced changes in behavioral locomotion requires repeated spaced administration of abused drugs. This research commonly involves the repeated and intermittent administration of drugs once a day or once every few days. In these studies intraperatoneal, intravenous, and subcutaneous injections have all been used with similar results (after dosage correction for the route of administration so that brain levels are more or less equivalent) (Crombag, Badiani, Maren, & Robinson, 2000; Olausson, Engel, & Soderpalm, 2000; Vanderschuren, Schoffelmeer, Mulder, De Vries, 1999). Cannulas directed at specific brain structures have also been used to measure the role of these brain structures in the motor effects (Pacchioni, Gionio, Assis, & Cancela, 2002). Behavioral motor responses are often measured after each drug administration with an emphasis on a challenge test dose at the end of a sequence of administrations to determine the resulting sensitization/tolerance

evident. The apparatus most often involves some type of activity arena or box where the animal's behavioral movement is measured. Researchers commonly use a long narrow activity box (for example 60x25x25 cm) to measure a rat's behavioral locomotion by measuring movement along the long axis (Fraioli, Crombag, Badiani, & Robinson, 1999; Vanderschuren, De Vries, Wardeh, Hogenboom, & Schoffelmeer, 2001; Vanderschuren, Schmidt, De Vries, Van Moorsel, Tilders, & Schoffelmeer, 1999; Vezina, Lorrain, Arnold, Austin, & Suto, 2002). The most commonly used data acquisition systems have been photobeams where data is collected each time an animal breaks a light beam or a camera with software that allows for the calculation of total distance traveled (Fraioli et al., 1999; Vanderschuren et al., 2001; Vanderschuren et al., 1999; Vezina et al., 2002). It is the underlying physiological changes producing the change in locomotion that is thought to be responsible for the addiction occurring with repeated drug administration. If wheel running is a non-drug addiction these physiological changes and changes in locomotion should also be evident in animals with chronic wheel exposure. Therefore, both because of its simplicity of measurement and because it is well characterized I have chosen to measure sensitization/tolerance by means of the behavioral locomotion that occurs within an activity testing apparatus and induced by a drug injection.

Even when looking at changes in drug induced motor effects it would seem that there may be multiple underlying mechanisms for the induction of sensitization/tolerance. In particular, one mechanism is learned and regarded as associative whereas another mechanism involves non-associative processes (Anagnostaras & Robinson, 1996). The associative type of tolerance/sensitization involves learning processes as it is more evident in situations where there are cues predictive of the drug (Anagnostaras & Robinson, 1996). The non-associative type of sensitization is seen as a progressive

increase in the unconditional response to a drug due to drug-induced changes in the neural substrate that affects the unconditioned response (Anagnostaras & Robinson, 1996). Similar to sensitization, the non-associative tolerance is simply the occurrence of tolerance in the absence of context-specific predictive cues (Tiffany & Maude-Griffin, 1988).

Associative Sensitization/Tolerance. The associative type of sensitization/tolerance is seen as the result of associative learning processes involving drug-environment conditioning (Anagnostaras & Robinson, 1996). Associative sensitization involves the pairing of drug administration with a unique environment allowing contextual cues to acquire the properties of a conditioned stimulus (CS). The drug acts as the unconditioned stimulus (US) and following the pairing of the CS with the US the CS itself acquires the potential to produce druglike effects (Anagnostaras & Robinson, 1996). Therefore, with this type of sensitization it is possible that sensitization involves the acquisition of a progressively increasing conditioned response (CR), which in addition to the unaltered unconditioned response (UR) produced by the drug results in an increased observed effect (Anagnostaras & Robinson, 1996).

Associative theories of tolerance are similar to those for associative sensitization. However, here the conditioned response is opposite to the direct effects of the drug. A prominent associative model was proposed by Siegel (1975). This model has been called the conditioned compensatory model and suggests that environmental cues that are paired with drug administration will become CSs that evoke a CR that is opposite in direction to, or compensatory for, the direct effect produced by the drug (Siegel, 1975). Therefore, tolerance develops over conditioning trials due to the compensatory CR that grows in strength and counteracts the direct effects of the drug (Siegel, 1975).

The associative model of sensitization/tolerance has been researched extensively and there is considerable empirical support that it is an important mechanism for changes in drug effect. Fraioli et al. (1999) found strong support for the context-specific associative model of sensitization. In this study, rats who received repeated intravenous (IV) infusions of .375 mg/kg of amphetamine with no environmental cues predictive of drug administration did not show behavioral sensitization to the amphetamine (the drug was administered through a chronic IV line in their home cage). However, rats that were administered amphetamine in the same way in a novel test environment (which could act as a CS predictive of the drug) did show sensitization to amphetamine-induced locomotor activity (Fraioli et al., 1999). In a more or less similar type of experiment Vezina and Stewart (1984) confirmed that the morphine-induced increase in locomotor activity could be elicited directly by an environment associated with morphine administration. In addition, they concluded that the morphine-induced increase in locomotor activity and therefore, sensitization to the effects of morphine, was specific to the administration environment. Thus, it is evident that at least some forms of sensitization (and tolerance) are associative in nature.

Non-associative Sensitization/Tolerance. Another type of sensitization/tolerance is referred to as non-associative and suggests that sensitization/tolerance can occur independent of existing environmental cues predictive of drug administration. Vanderschuren et al. (1999) and Vanderschuren et al. (2001) provide strong empirical support that sensitization can occur in a context-independent manner. Vanderschuren et al. (1999) administered a single dose of 5 mg/kg of amphetamine (IP) in the rat's home cage, a separate environment from where the challenge test was administered. These rats were then separated into three groups and given an amphetamine (1 mg/kg) challenge test

in a novel environment either 3 days, 1 week, or 3 weeks after the initial drug injection and their motor behavior was measured. Significant sensitization occurred at all three test intervals with it being most evident in the three week challenge test (Vanderschuren et al., 1999). Thus, the only possible predictive cues for drug administration was the IP injection procedure as the first injection and the test phase occurred in different environments and it was concluded that the observed sensitization most likely represented a non-associative increase in the sensitivity to the locomotor effects of psychostimulants (Vanderschuren et al., 1999). The time course of this sensitization (maximized at 3 weeks) is also not consistent with associative models.

Vanderschuren et al. (2001) performed a similar study to investigate morphine sensitization. A single initial dose of 2, 10, or 30 mg/kg of morphine (in the home cage) was used. A challenge test of either 2 or 5 mg/kg of morphine was administered three weeks following the initial administration in a novel test environment. The animals preexposed to either 10 or 30 mg/kg of morphine displayed sensitization on challenge tests to both 2 and 5 mg/kg of morphine (Vanderschuren et al., 2001). Again as the only possible predictive cue for drug administration was the IP injection procedure (as the initial and challenge injections occurred in different environments) it was concluded that the observed sensitization most likely represented a non-associative increase in sensitivity to the locomotor effects of morphine (Vanderschuren et al., 2001).

Vanderschuren et al. (2001) also tested whether cross-sensitization existed between both morphine and amphetamine. This test was also of a non-associative nature as the testing environment was different from the environment used for the initial injection which suggests that virtually no predictive cues of drug administration would have existed. Within this portion of the study, one initial injection of morphine (2, 10, or

30 mg/kg) was administered in the home cage. Three weeks post-treatment, a challenge test in a different test environment was conducted with 1 mg/kg of amphetamine for each of the three dose groups of rats in the experiment. The result was that test animals pretreated with 2 mg/kg of morphine showed an augmented effect of amphetamine over the entire one hour test. Animals pretreated with 10 mg/kg of morphine displayed an increased locomotor effect to amphetamine during the 2^{nd}, 3^{rd}, and 4^{th} 10-min time blocks of the one hour activity test. In animals pre-exposed to 30 mg/kg morphine, the psychomotor effect of amphetamine was significantly increased only during the 6^{th} 10-min time block. Therefore, the result of this research shows that a certain level of non-associative cross-sensitization does exist between morphine and amphetamine.

Both studies by Vanderschuren et al. (1999, 2001) show that sensitization can occur non-associatively and this is important for the present investigation. In my proposed experiments the cross-sensitization/tolerance would also likely be non-associative in nature as testing occurs in a different place from where the expected changes are induced by wheel access as was the case in both experiments by Vanderschuren et al. (1999, 2001). This is due to the fact that the wheel running phase (the initial phase) in my experiments is conducted in the rats' home cage environment. The testing phase (challenge test) is performed in a separate, relatively novel environment after a short habituation period. The rats are tested for locomotor activity with the prediction that wheel access rats will display significantly higher locomotor activity in the testing phase compared to no wheel access rats who received the same amphetamine administration (sensitization). However, with morphine administration there is also the possibility that tolerance will be more evident with the wheel access rats displaying significantly less hypoactivity than the control animals after receiving morphine. A

tolerance/sensitization effect in animals with wheel experience would suggest that wheel running and morphine or amphetamine act upon similar systems. In these experiments rats experience only one drug administration and no external cues predictive of drug (or wheel access) are present at this administration. Thus, these experiments are tests of non-associative cross-sensitization/tolerance between wheel running and either amphetamine or morphine.

Practical Issues in the Experimental Design

Acute Response to a Novel Testing Environment. In a novel environment rats show an elevated locomotor behavior. This acute novel response creates a problem for our cross-sensitization/tolerance experiments as this elevated responsiveness might mask the sensitized response to amphetamine or morphine. In a pilot study with amphetamine it was observed that the locomotor response for both the experimental (wheel experienced) and control (wheel naïve) group of rats was almost identical and very high on the first day of testing. Sensitization may have occurred, but the elevated motor response to the novel environment over the one hour period made it impossible to observe whether sensitization was evident. Wheel experienced rats tested on a second exposure to the testing environment, when novelty was reduced, showed a larger effect of amphetamine than animals with no prior wheel experience.

Crombag, Badiani, Chan, Dell'Orco, Dineen, and Robinson (2001) found that one hour of habituation to the test environment significantly attenuated this acute novel motor response. Thus, a habituation period was incorporated into the present experiments in the expectation that this would aid in providing clearer data regarding sensitization to the effects of both amphetamine and morphine as the novelty locomotor ceiling problem would be less evident. The neurobiological mechanisms responsible for the acute

increase in locomotor response due to environmental novelty are not well characterized. However, it has been found that environmental novelty results in neuroendocrine and physiological changes that are usually associated with conditions of stress (Friedman & Ader, 1967).

Factors that Influence the Sensitization Process. The induction of sensitization by repeated drug exposure greatly depends on the nature of the administration regimen and dose of drug utilized (Vanderschuren et al., 1999). Russell and Pihl (1978) concluded that for amphetamine the dose administered repeatedly could result in either increases in locomotion or progressively increasing incidence of stereotyped behavior. Some of the stereotyped behaviors that rats will excessively engage in, in response to amphetamine injections, include; licking, scanning, perambulating, and head bobbing (Russell & Pihl, 1978). Higher doses such as 6.0 to 10.0 mg/kg of amphetamine produce greater levels of stereotyped behavior than do dose levels of 0.5 mg/kg and 1.0 mg/kg (Russell & Pihl, 1978). With repeated administration of amphetamine this stereotypy behavior begins to occur with lower doses. At the other end of the scale, Fraioli et al. (1999) report that 0.375 mg/kg is the lowest dose of amphetamine that will elicit a significant increase in locomotor activity. Therefore, in order to provide evidence for locomotor sensitization to amphetamine and avoid stereotypy behavior that can mask the measurement of this sensitization an appropriate moderate dose must be utilized. The dose of 1.0 mg/kg of amphetamine is low enough to avoid excessive stereotypy behavior and high enough to elicit an increase in locomotor activity and therefore, an appropriate dose to display the existence of locomotor sensitization between wheel running and amphetamine administration. Thus, in my first experiment this dose was utilized to look for wheel induced amphetamine sensitization.

Morphine has a biphasic effect on locomotor activity in hamsters (Schnur, 1984) and rats (Babbini & Davis, 1972). Morphine tends to produce a dose-related decrease in activity followed by a gradual dose-related recovery and finally, a period of sustained hyperactivity (Schnur, 1984). Schnur, Bravo, and Trujillo (1983) found that the repeated administration of low doses (0.5, 1.0, 2.5, and 5.0 mg/kg) of morphine causes two changes in the biphasic effect. Over repeated administration there is a decrease in the initial hypoactivity (tolerance) and an increase in the hyperactivity phase (sensitization) (Schnur et al., 1983). Schnur (1984) found that these changes in the effects of morphine also occur with higher doses such as 10, 20, and 40 mg/kg of morphine. These motor effects are most often evident in the two hour period following morphine administration. This is the justification for utilizing a two hour testing period in our cross-sensitization experiment with morphine. Babbini and Davis (1972) concluded that acute delivery of low doses (1.25, 2.5, and 5.0 mg/kg) of morphine produced primarily an excitatory effect in rats whereas high doses (20 and 40 mg/kg) delivered acutely had a depressant effect on locomotor activity. Thus, higher doses of morphine produce a more evident depressant effect (hypoactivity), where as low doses produce a more evident excitatory effect (hyperactivity). Babbini and Davis (1972) found that no substantial change in locomotor behavior occurred over a 30 day period with lower doses (1.25, 2.5, and 5.0 mg/kg) of morphine. However, motor behavior increased over a 30 day period with higher doses (10, 20, and 40 mg/kg) of morphine (Babbini & Davis, 1972). A dose that was capable of moving the dose response curve to the left or right and show sensitization and/or tolerance was desired for use in our cross-sensitization experiment with morphine. Babbini and Davis (1972) found that an acute delivery of 10 mg/kg of morphine has both

excitatory and depressant effects on motor behavior and therefore, this was the dose of morphine used in my cross-sensitization experiment with morphine.

Wheel-Drug Interval. A withdrawal period between the acquisition and challenge phases in drug studies can also influence the expression of sensitization/tolerance. Longer intervals between acquisition and test have been shown to enhance the sensitization induced by amphetamine (Vanderschuren et al., 1999). In a single injection acquisition study involving multiple groups of rats, Vanderschuren et al. (1999) showed that sensitization was moderately evident after a 3 day withdrawal period and was most pronounced following the 3 week withdrawal period (Vanderschuren et al., 1999). Thus, in my amphetamine cross sensitization study a one week withdrawal period was implemented to enhance the sensitization induced by the prior daily wheel access provided to the experimental group.

Research on the sensitization and tolerance seen with morphine suggest that a short or a nonexistent withdrawal period produces the best and often increased evidence for sensitization or tolerance. Vanderschuren et al. (2001) conducted challenge tests with 5 mg/kg of morphine at 1 day, 3 weeks, and 9 week intervals following an acquisition phase and concluded that sensitization was most apparent with a one day withdrawal period.

Research on the effects of prior wheel running in rats on morphine-induced analgesia involves no withdrawal period between conditioning and testing phases. Kanarek et al.(1998) tested antinoceptive responses of rats using the tail-flick latency method immediately following a 20 day wheel access phase with no withdrawal period from wheel access to the testing phase. The sensitivity to morphine-induced analgesia was significantly decreased at morphine dose levels of 2.5, 7.5, and 12.5 mg/kg by prior

wheel experience. Mathes and Kanarek (2001) conducted a similar experiment testing antinoceptive responses in rats exposed to 3 weeks of wheel access and no withdrawal period between the running and testing phases. This experiment showed that with a 10 mg/kg dose of morphine prior wheel running significantly attenuated the antinoceptive properties of morphine. In these studies investigators showed tolerance with no withdrawal time between the acquisition and testing phases. Thus, I did not incorporate a withdrawal period in my cross-sensitization experiment with morphine.

Purpose of Investigation

If wheel running in rats becomes addicting with chronic exposure, then the underlying physiological mechanisms and changes which occur may be similar to those observed in other types of addictions, such as in drug abuse. The present study investigates whether there is a change in the effect of drugs of abuse due to preceding wheel exposure that is similar to changes seen after repeated drug exposure. The occurrence of sensitization and/or tolerance to abused drugs may be reflective of the underlying physiological mechanisms responsible for the escalation from general drug "consumption" through to drug addiction. Utilizing drugs of abuse such as amphetamine and morphine to test if the changes induced by wheel running are similar to those induced by prior drug exposure allows for a parallel to be drawn between drug addiction and other behavioral addictions. It has been suggested that the physiological process of neural sensitization is an important component in the transition to drug addiction (Anagnostaras & Robinson, 1996; Crombag & Robinson, 2004). Thus, it may be possible that the process of sensitization/tolerance is also a major factor in behavioral addictions such as exercise addiction. Therefore, the use of wheel running in the present investigation allows one to measure cross-sensitization/tolerance between a behavioral addiction and

the effects of both amphetamine and morphine. If cross-sensitization/tolerance exists between these two addictive components then this would suggest that sensitization/tolerance processes are occurring as a consequence of wheel running and therefore, possibly a major factor in the transition to behavioral addictions. This result would support the notion that behaviors such as excessive exercise may be due to a common addictive process also seen in drug addictions. In this case, with prior wheel exposure cross-sensitization/tolerance should be seen at drug testing in a way similar to that seen after repeated administration of these drugs of abuse.

When a drug like amphetamine is administered repeatedly there is an escalation in motor behavior over trials (sensitization). Therefore, rats chronically exposed to running wheels should show an elevated level of locomotion to an amphetamine challenge when compared to control animals with no prior wheel experience. This result would suggest that a sensitization-like process may be common to addictions and be the driving force behind the escalation in "consumption" that is characteristic of all addictions.

Both experiments in this study also involved the investigation into whether a relation exists between the level of wheel running and a rat's response to either amphetamine or morphine. In other words, is it the case that rats with a high response to wheel running also have an augmented response to either amphetamine or morphine administration. If this is evident, then it would suggest that certain animals are more susceptible to the effects of abused drugs as has been suggested for the initial locomotor responses to amphetamine (Piazza, Deminiere, Moal, & Simon, 1989).

Experiment 1: Amphetamine

Method

Subjects. Forty-eight male Sprague-Dawley rats (200-225 grams, 47 to 49 days old at arrival) were ordered from Charles River, Canada. Animals were individually housed and allowed to habituate for one week to our animal room conditions (12:12 light/dark cycle with lights on at 07:00, 50% relative humidity, and 21-22 degrees Celsius). All procedures and housing conditions in all experiments were approved by the Animal Care Committee of Wilfrid Laurier University.

Apparatus. Research animals were individually housed in Polycarbonate cages (47 x 26 x 20 cm) for the duration of the experiments. Rats in the experimental groups had a Nalgene running wheel (33 cm in diameter and 11 cm in width) within their home cage. The wheel turns for each rat in the experimental groups were recorded at 1 second intervals with electromagnetic reed switches via Vital View 4.01, a Mini-Mitter data collection system. The lids of each cage were made of wire mesh that held standard lab pellets (Rat Diet 5012, PMI Feeds 3.11 kcal/g metabolize energy) and a water bottle. Both the experimental group and control group rats were housed in a common colony room.

The testing arena consisted of eight individual black plexiglass activity boxes (60x25x25 cm) with wire mesh lids. All eight boxes were strategically placed on a table beneath a camera that allowed the tracking of each animal's movement in centimeters traveled. For this experiment, four of the eight activity boxes had white stripped walls the other 4 were solid black (these two activity boxes were counterbalanced across groups and found to make no difference in locomotor response). The camera sent a signal to a

computer where the data was recorded and subsequently analysed by the Noldus Etho-Vision videotracking system (Noldus Information Technology, Sterling, VA).

Drugs. D-Amphetamine Sulfate was prepared in a solution of 1 mg/ml and injected intraperitoneally in a volume of 1 ml/kg (1 mg/kg) for the challenge test. Physiological saline was administered intraperitoneally at 1 ml/kg.

Procedures. Following the acclimatization period (Day 1 through 7) 24 rats in the wheel group were housed in cages with the Nalgene wheels. The other 24 rats in the no wheel group were housed in regular cages with no wheel access and served as the controls. All rats remained in these housing conditions for 24 days between Days 8 to 31 and were weighed daily at 09:00. Following this wheel access period a wheel withdrawal period of 7 days was implemented for the experimental group, Days 32 to 38. Five days after wheels were removed a two day habituation period to the testing environment was carried out (Days 37 and 38). This habituation period involved providing all rats with one hour of access to the testing arena on each of the two day habituation period. Rats were moved into the activity boxes without injections and were returned immediately to the animal colony following each habituation session. Following these habituation days a two day testing period occurred (Days 39 and 40). On Day 39 half of both the experimental and control group rats were given 1 mg/kg of amphetamine, while the other half of both the experimental and control groups were administered 1 ml/kg of saline 10 minutes prior to being placed in the testing environment. On the second day of testing the injected drugs were reversed for all animals. Locomotor behavior (cm traveled) was monitored via the Ethovision video tracking system over the one hour motor behavior test. The rats were always tested throughout the day starting at 07:00 and eight rats were tested at one time for the one hour motor activity test. All rats were tested at the same

time of day for both the habituation and testing periods. Following each one hour motor behavior test the testing arena was cleaned with hot soapy water and the animals were returned to the colony room.

All statistical tests were carried out using the Statistical Package for the Social Sciences (SPSS) Version 13, with a $p < .05$ used as the level of significance. On any repeated measure analysis of variance the results are only reported as significant if also significant ($p < .05$) using the Greenhouse – Geisser correction. If any interactions were significant appropriate follow-up tests were carried out.

Results

Figure 1 displays both the individual and mean daily wheel running of the wheel access group averaged over both the initial four days and the final four days of wheel access. It is evident that rats show a wide range of wheel running in this experiment. The mean wheel running for the first four days of access was 1507 wheel turns per day and this progressed to an average of 8611 wheel turns per day for the final four days of wheel access. Comparing the wheel running over the initial four and final four days of wheel access indicates that animals significantly increased their wheel running over the 24 days of wheel access, $F(1, 22) = 79.47$, $p < .001$. Variation in running is evident as at the end of wheel access some animals engaged in over 12000 wheel turns per day, while others engaged in less than 4000 wheel turns per day. The variation in running permits subsequent correlation analysis with other behaviors as running has sufficient variation, so any correlation involving running will not suffer from range restriction. In looking at the change in running from the initial four to the last four days a correlation analysis revealed no correlation between early and late running, $r = .056$, $p = .799$. Thus, it is the

case that rats initially considered "high" runners did not necessarily remain "high" runners through to the end of the experiment.

All animals were given two one hour habituation sessions to the testing arena over a two day period. These habituation sessions occurred five days following the cessation of wheel access for the wheel access rats. Figure 2 displays the results of the habituation sessions and a days by group mixed ANOVA indicates that no significant difference in motor behavior existed between the control and wheel access groups during the habituation period, $F(1, 45) = .17, p = .684$. It also appears that locomotion was reduced from the first to the second habituation session in both groups, but this difference was not significant.

Figure 3 displays the raw locomotor score results of the one hour testing period for rats receiving saline or amphetamine in a cross over design just before being tested. A drug order by drug by group ANOVA revealed only a significant main effect of drug, $F(1, 43) = 163.70, p < .01$, and a significant drug by group interaction, $F(1, 43) = 9.42, p < .01$. It is clear from Figure 3 that amphetamine induced a significant increase in locomotor activity and the significant interaction suggests that the wheel access group experienced a greater change after drug (relative to saline) administration than did the control group. Simple main effects of the amphetamine test results revealed a between group difference existed after amphetamine administration, $F(1, 45) = 4.32, p < .05$. This difference in activity after amphetamine administration suggests that the wheel access group experienced a sensitization-like effect to amphetamine as an augmented drug response is evident in Figure 3 for the wheel access rats. There was no main effect under saline administration between groups, $F(1, 45) = 2.69, p = .108$. The order of drug

administration during the two day challenge test had no significant impact on the results reported in this experiment.

Another method of measuring the effect of amphetamine is to compare the percentage activity increase under amphetamine administration (difference between amphetamine and saline) while using each rat's saline response as the baseline (ratio score was equal to 100 x [actual response under amphetamine – actual response under saline] / response under saline). Figure 4 indicates that the wheel access group rats engaged in significantly more activity after amphetamine administration than did animals without wheel access, the control group, $F(1, 45) = 7.39, p < .01$. This result showing the relative increase after amphetamine supports the sensitization-like response to amphetamine amongst the rats with wheel experience compared to those without.

If wheel running is an addiction, then one might expect amphetamine sensitization to be larger in animals showing high levels of running. In order to measure whether or not the level of running has any relation with the degree of amphetamine sensitization, a correlation between wheel running levels and the effects of amphetamine on locomotion was calculated. This correlation was determined between the average daily wheel turns for each animal in the wheel access group, calculated from the final four days of wheel access, and the degree of activity increase under amphetamine relative to saline administration (the % locomotion increase). The scatterplot shown in Figure 5 indicates that this correlation was not significant, $r = .138, p = .529$. This suggests that animals who engaged in elevated amounts of wheel running behavior were not necessarily more susceptible to the effects of amphetamine. The difference in running activity between the first four days and the final four days of wheel access (final four day average – initial four

day average) also had no relation with the effects of amphetamine on locomotion during the first hour of testing, $r = .157$, $p = .474$.

Discussion

Figure 2 displays the result of the two initial habituation sessions given in this experiment. The habituation period revealed no significant motor activity differences between groups with and without prior wheel experience. Thus, the motor activity of the control and the wheel access groups were very similar over both habituation sessions. This activity level for both groups during the habituation period was similar to the activity results under saline administration during the testing period which could be seen as a third habituation trial. It should be noted that the habituation period in this experiment followed a five day wheel withdrawal period for the wheel access group.

Experiment 1 ultimately indicates that wheel running has a similar physiological effect on rats as prior experience with amphetamine. This is the motor sensitization effect where prior experience with amphetamine induces sensitization of the locomotion effects as measured with a subsequent challenge dose. This experiment suggests that the augmentation evident, with prior wheel experience, during the locomotor effect of the amphetamine challenge test is comparable to that seen in traditional sensitization experiments.

The "cross-sensitization" between wheel running and amphetamine in this experiment is evident in both Figures 3 and 4. Figure 3 reveals a significant interaction, indicating that the wheel experienced group experienced a greater degree of change in motor activity under amphetamine administration in comparison to the control group. Figure 4 displays this degree of change under amphetamine administration when the results under saline administration were used as the baseline for each group. Both graphs

use the same data, however, Figure 4 allows for one to view the effects of amphetamine taking into account any baseline differences in motor activity. These two graphs suggest that the amphetamine sensitization that is evident because of prior wheel experience with a single challenge test of amphetamine is similar to the sensitization seen in experiments utilizing prior amphetamine administration to demonstrate sensitization. The results are comparable in the direction of the response but do not permit a comparison between wheel running access and amphetamine administration in terms of the amount of sensitization. It is not clear at this time about how much ad-lib wheel access is required to induce the same degree of sensitization response as a particular schedule of repeated amphetamine administrations. This comparison will require more parametric work looking to see how much wheel running is necessary to induce sensitization and if the sensitization can be augmented with increased wheel experience. It is also necessary to see if repeated amphetamine in animals with wheel experience will result in further sensitization which would suggest differing mechanisms for sensitization.

Another important aspect of this experiment was the determination of whether the level of wheel running has any influence on the susceptibility to addiction. In other words, is there a relationship between the level of wheel running and the motor response to an initial amphetamine administration? A relationship indicating that a high level of wheel running correlates with an augmented response to an initial amphetamine administration might suggest that certain animals have a greater susceptibility to addiction than others. The correlation between the ultimate wheel running levels of the wheel access group and the degree of activity change under amphetamine administration proved to be non-significant (Figure 5). This suggests that the influence of amphetamine on motor behavior is independent of the level of prior wheel running in animals with

extensive running experience. However, a positive relationship may still exist between these two measures as a period of "sensitivity" may be apparent with more or less ad-lib wheel exposure. It may also be the case that animals who engage in the highest levels of wheel running are not necessarily more susceptible to the effects of amphetamine and more importantly, possibly addiction in general, than are animals with lower overall levels of running.

Experiment 2: Morphine

The second experiment involved the use of morphine to measure any possible tolerance and/or sensitization induced by prior wheel running experience. The use of morphine in the second experiment examines the generality of this relationship as morphine is an opiate whereas amphetamine is a stimulant. In order to provide support for the possibility that sensitization and/or tolerance are common in both behavioral and drug addictions it is helpful to investigate the influence of wheel running on multiple classes of drugs, the primary reason for the use of morphine in the second experiment.

Chronic exposure to morphine results in changes in morphine induced activity in rats (Babbini & Davis, 1972). As detailed earlier, morphine typically has a biphasic effect on motor response. Repeated administration of morphine results in a decrease of the original hypoactivity (tolerance) and an increase in the subsequent delayed hyperactivity response (sensitization). With repeated administration this hyperactivity also occurs earlier when compared to the motor effects after the initial administration (Babbini & Davis, 1972).

The present experiment between wheel running experience and morphine administration should yield a similar response to that seen in rats with only a history of

repeated morphine administration. Thus, a cross-sensitization/tolerance study between prior wheel running experience and morphine should result in rats with a wheel history experiencing a reduced initial hypoactivity effect and an augmented hyperactivity effect after an initial morphine administration, in comparison to a control group that has received no prior wheel exposure. The primary focus of this research is to investigate the possibility that processes responsible for sensitization and/or tolerance to drugs also occur with non-drug behavioral addictions.

This second experiment provided for the opportunity to investigate whether certain animals were more vulnerable to the addicting behavior of wheel running. Piazza et al. (1989) concluded that rats classified as "high" responders to a novel environment were more vulnerable to the effects of amphetamine than rats classified as "low" responders to a novel environment. Therefore, a baseline locomotor activity test was conducted in this second experiment in order to explore whether "high" responders to novelty are also more susceptible to wheel running behavior. In other words, locomotor activity during this baseline test was to be correlated with both the initial and final four days of wheel access.

Methods

Subjects. Thirty-six male Sprague-Dawley rats (200-225 grams at arrival) were ordered and housed as in Experiment 1.

Apparatus. The same apparatus in Experiment 1 was utilized for this experiment except for the testing room contained 8 activity boxes that were entirely black in color.

Drugs. Morphine sulphate dissolved in a physiological saline solution (10 mg/ml) was administered intraperitoneally at a dose of 10 mg/kg. Physiological saline was administered intraperitoneally at 1 ml/kg.

Procedures. All animals were allowed to acclimatize to the animal colony for seven days (Day 1 to 7) in regular polycarbonate cages. After this acclimatization period, a two hour locomotor test (cm traveled) was implemented on Day 8 for all animals using the testing apparatus for this experiment. Each animal was placed in the testing arena for two hours and the testing arena was cleaned between trials. This initial motor test began at 08:00 during the day providing baseline locomotor behavior for each animal and allowed for the later separation of animals into the groups of either high or low activity. Following this initial motor activity test, 24 rats in the wheel group were given access to the Nalgene wheels. The other 12 rats in the non-wheel group were housed in regular cages with no wheel and served as the controls. All rats remained in these housing conditions for 24 days between Day 9 to 32 and were weighed daily at 09:00.

A one day habituation period to the testing environment occurred on Day 33. This habituation day involved providing all rats with two hours of access to the testing arena with no injections. All rats were returned to their assigned housing conditions following the habituation session so that no wheel withdrawal period existed before the testing phase. All animals were given access to the testing arena in the same manner as the initial motor test. Following the habituation test, a two day testing period occurred on Days 34 and 35. On day one of testing half of both the experimental and control group animals were administered 10 mg/kg of morphine while the other half of both the experimental and control groups were administered 1 ml/kg of saline (these injections were reversed on the next day). All drugs were administered intraperitoneally 10 minutes prior to being placed in the testing arena. On the second day of testing the drugs were reversed for all animals. The rats were tested during the day time starting at 08:00 with eight rats at a time for the two hour motor activity test, except for the last trial which

consisted of four animals. Therefore, there were five trials each testing day and each trial consisted of a balanced number of animals from both the wheel and non-wheel groups. All rats were tested at the same time of day for both the habituation and testing periods. All animals were returned to their assigned housing conditions following each testing session. Locomotor behavior (cm traveled) was monitored via the Ethovision video tracking system over a two hour motor behavior test. The testing arena was thoroughly cleaned following each two hour motor behavior test.

Results

Figure 6 displays both the individual and mean daily wheel running of the wheel access group averaged over both the initial four days and the final four days of wheel access prior to the day with the habituation session. As in the previous experiment, Figure 6 indicates that a wide range of wheel running occurred in this experiment. The mean daily wheel running through the first four days was 1209 wheel turns per day which increased to an average of 4466 wheel turns per day over the final four days of wheel access. The variation in the running is further evident as some animals engaged in over 8000 wheel turns per day, while others engaged in less than 2000 wheel turns per day during the final four days of wheel access. This suggests that the running has a large variation which then can be used to correlate with other measures. Similar to the previous experiment, a comparison of the average wheel running between the initial four and final four days of wheel access prior to the habituation period suggests that these animals substantially increased their wheel running behavior over the 24 days of wheel access, $F(1, 23) = 43.61, p < .01$. In looking at the change in running from the initial four to the last four days in this experiment, a correlation analysis revealed a significant correlation in running, $r = .43, p < .05$, between these periods. Thus, contrary to the previous

experiment, it was the case that rats initially considered "high" runners did remain "high" runners through to the end of the experiment, but this accounted for less than 20% of the variation in running.

A baseline locomotor activity test prior to wheel access was conducted to determine whether rats who engaged in elevated levels of baseline activity were more susceptible to high levels of wheel running. The locomotor scores for animals going into the wheel did not differ from those staying in the non-wheel cages, $F(1, 34) = 1.91$, $p = .176$. The scatterplot in Figure 7 reveals that the correlation between the wheel running of the wheel access rats, calculated from the first four days of wheel access, and the locomotor behavior in the activity test, conducted prior to wheel access, was not significant, $r = .284$, $p = .178$. It should be pointed out that both locomotion in the activity boxes and wheel running showed considerable variability. This non-significant correlation indicates that animals who engaged in more locomotion during the activity test did not necessarily engage in a higher level of wheel running. A similar correlation involving the mean daily wheel turns from the final four days of wheel access and locomotor scores was also non-significant, $r = .242$, $p = .254$, (data not shown). Therefore, animals that could be considered "high" or "low" responders from the activity test were not more susceptible to the behavioral addiction of wheel running as determined by the amount of wheel running. The difference in running activity between the first four days and the final four days of wheel access (final four day average – initial four day average) was correlated with the locomotor behavior in the activity test and also proved to be non-significant, $r = .196$, $p = .359$.

All animals received one two hour habituation session to the testing arena, conducted over one day but without a wheel withdrawal period. That is, animals were

removed from wheel equipped cages (and home cages), tested for 2 hours in the testing arena and immediately returned to their wheel cages (and home cages). Figure 8 shows the results for each hour of the single habituation period and demonstrates a significant difference in activity between rats with and without wheel experience. A mixed ANOVA comparing the two groups over each hour revealed both a significant hour effect, $F(1, 34) = 45.04, p < .01$, and a significant group difference, $F(1, 34) = 33.64, p < .01$. Therefore, the wheel access rats engaged in significantly less motor activity during this habituation period in comparison to the control group animals. It was also evident that both groups of rats habituated to the testing arena during this period as locomotion significantly decreased from the first to the second hour of the habituation period.

Morphine typically has a biphasic effect on locomotion involving both an initial hypoactivity and subsequent hyperactivity phase. Thus, the testing period was analyzed in one hour intervals over the two hour testing period allowing for the biphasic effect of morphine to be more accurately demonstrated. All the results from the first hour of testing will be discussed prior to the results pertaining to the second hour of testing.

Figure 9 displays the results of the first hour of testing. A drug order by drug by group ANOVA revealed a significant drug effect, $F(1, 32) = 47.78, p < .01$, a drug by group interaction, $F(1, 32) = 10.60, p < .01$, and a drug by drug order interaction, $F(1, 32) = 6.11, p < .02$. Simple main effects revealed that only the difference in locomotor activity after saline administration was significant, $F(1, 34) = 10.46, p < .01$. The simple main effect of morphine during the first hour of testing was non-significant, $F(1, 34) = .431, p = .516$. During the first hour of testing the control group experienced a greater drop in activity under morphine administration when compared to saline, $F(1, 11) = 29.59, p < .001$, than the wheel access rats, $F(1, 23) = 10.27, p < .01$. This suggests that

the wheel access group experienced a degree of tolerance to the sedating effect of morphine. However, it should be noted that the difference was due to the reduced running under saline in the wheel access group. The significant drug by drug order effect is apparent as the hypoactivity effect of morphine is more evident on the second day of the drug challenge test (5729 ± 573 on day one and 3807 ± 526 on day two) the saline challenge did not result in a difference over days (7400 ± 494 on day one and 7337 ± 454 on day two) and indicates that two separate habituation sessions used in Experiment 1 may be more effective in attenuating the effects of a novel environment on locomotor activity.

As the expected initial effect of morphine is a period of hypoactivity, another way to measure the first hour results of the testing period is to compare the percentage activity decrease under morphine administration (difference between morphine and saline) while using each rat's saline response as the baseline (ratio score was equal to 100 x [actual response under morphine – actual response under saline] / response under saline). Figure 10 indicates that the wheel access group experienced tolerance to morphine during the first hour of the locomotor activity test as the morphine induced hypoactivity was smaller than that exhibited in the control group under morphine administration, $F(1, 34) = 6.77$, $p < .05$. Therefore, prior wheel experience has an effect on a rat's initial response to morphine suggesting it induces a degree of morphine tolerance.

If wheel running is an addiction, then one might expect any morphine tolerance to be larger in animals showing high levels of running. To investigate whether the level of running has any relation with the degree of morphine tolerance, a correlation between wheel running levels and the effects of morphine on locomotion during the first hour of testing was calculated. This correlation was determined from the average daily wheel

turns for each animal in the wheel access group, calculated from the final four days of wheel access, and the degree of change in locomotor activity under morphine relative to saline administration (the % locomotion decrease). The scatterplot shown in Figure 11 reveals that this correlation was non-significant, $r = .201$, $p = .346$. This suggests that animals who engaged in elevated wheel running were not necessarily more tolerant to the hypoactivity inducing effects of morphine as there was no correlation with the effects under morphine administration for this time period. The difference in running activity between the first four days and the final four days of wheel access (final four day average – initial four day average) also had no relation with the effects of morphine on locomotion during the first hour of testing, $r = .206$, $p = .334$.

Figure 12 displays the raw data score results for the second hour of the testing period. A drug order by drug by group ANOVA revealed a main effect of drug that approached significance, $F(1, 32) = 3.58$, $p = .067$, and an effect of drug order, $F(1, 32) = 5.46$, $p < .03$. The effect of drug order is due to the locomotion being reduced under both saline and morphine administration on the second day of the drug challenge test. However, Figure 13 indicates that when the effect of morphine is compared to the result under saline administration as the baseline (the % locomotion increase), a difference in locomotor activity exists between groups during the second hour of the testing period, $F(1, 34) = 4.25$, $p < .05$. This difference in locomotor activity increase under morphine compared to saline administration suggests an increased hyperactivity effect in the second hour of testing for the wheel access group under morphine administration. This hyperactivity effect is the second phase of the biphasic effect of morphine and Figure 13 indicates that the wheel access group experienced a sensitization-like response to morphine during the second hour of the locomotor activity test.

If wheel running is an addiction then one might expect any morphine sensitization to be larger in animals showing high levels of running. In order to measure whether or not the level of running has a relation with the degree of morphine sensitization, a correlation between wheel running levels and the effects of morphine on locomotion during the second hour of testing was calculated. This correlation was determined from the average daily wheel turns for each animal in the wheel access group, calculated from the final four days of wheel access prior to the habituation period, and the degree of activity increase under morphine relative to saline administration during the second hour of testing. The scatterplot shown in Figure 14 reveals that this correlation for the second hour of testing was not significant, $r = .207$, $p = .331$. This suggests that animals who engaged in elevated amounts of wheel running were not necessarily more susceptible to the stimulating effects of morphine during this time period. The difference in running activity between the first four days and the final four days of wheel access (final four day average – initial four day average) also had no relation with the effects of morphine on locomotion during the second hour of testing, $r = .238$, $p = .262$.

Discussion

This experiment provided the opportunity to investigate whether certain animals are more vulnerable to the addictive properties of wheel running than others, as has been found in studies of drug addiction (Piazza et al., 1989). Piazza et al. (1989) suggested that an enhanced vulnerability exists between rats classified as "high" responders to a novel environment and their response to an initial administration of amphetamine compared to rats classified as "low" responders to a novel environment. In order to investigate this issue, a baseline activity test was conducted prior to wheel access for each animal. The activity results of this baseline test were correlated with the wheel running

results from the initial four days of wheel access. Figure 7 indicates that the correlation between these two measures was non-significant suggesting that the levels of wheel running where unrelated to the baseline activity results for the wheel access group. A similar correlation involving the final four days of wheel access prior to the habituation session also proved to be non-significant. These results suggest that animals who were considered "high" or "low" responders in the baseline activity test are not necessarily more susceptible to the addiction of wheel running as determined by the levels of wheel running. This suggests either that the level of wheel running is not a good measure of its addiction value, that even "low" levels of running can be viewed as addicting. Alternatively it may be that 4 day averages are not appropriate ways to measure running vulnerability. Perhaps shorter periods of running should be tested, or alternate measures such as time spent running used.

The habituation session in this experiment produced results that were different from those seen in the first experiment. A significant difference between wheel experienced and wheel naïve rats was evident throughout the two hour habituation period, the wheel access group rats engaged in less motor activity than the control group rats. It should be noted that in this experiment no wheel withdrawal period existed prior to the habituation period as the wheel access group had constant wheel access until immediately prior to the habituation session. This difference between the groups in habituation activity makes subsequent drug comparisons more difficult.

This experiment suggests that the wheel access group experienced a cross-tolerance and cross-sensitization response to an initial morphine administration relative to a group with no wheel experience. Figures 10 and 13 reveal the actual influence of morphine administration on both groups for the first and second hour of the testing

period, respectively. These two figures display the degree of change in motor activity under morphine administration when compared to the results under saline administration which was used as the baseline for this analysis. Figure 10 indicates that during the first hour of the testing under morphine administration the control group showed a significantly greater activity suppression or hypoactivity than the wheel access group. Figure 13 indicates that during the second hour of the testing period the wheel access group experienced an increased hyperactivity under morphine administration as they engaged in an elevated level of activity when compared to the control group. With repeated administration of a constant dose level of morphine a rat experiences an increasing tolerance or a reduced hypoactivity response and an augmented period of hyperactivity which occurs earlier in time with each subsequent administration (Babbini & Davis, 1972). The results of this experiment are similar to those seen in investigations involving repeated administration of morphine. Therefore, the degree of cross-tolerance and sensitization that is evident between wheel running and an initial administration of morphine is comparable to that seen in rats with a history of morphine experience. Once again, the similarity is found in the direction of response to a challenge test dose of morphine as a wheel running amount/repeated morphine comparison needs to be made.

This experiment allowed for further investigation on the influence of wheel running level for the susceptibility to addiction and the effects of morphine. Therefore, addressing the question is there a relationship between wheel running behavior and a rat's initial response to morphine administration. A positive relationship between high levels of running and both an enhanced tolerance and sensitization response to an initial morphine administration would suggest that certain animals are more susceptible to addiction than others. As in Experiment 1, the ultimate levels of wheel running were

compared with the actual response under morphine administration for both the first and second hours of the testing period as morphine typically has a biphasic effect. Figures 11 and 14 indicate that a correlation between these two measures was non-significant for both hours of the testing period. These results suggest that the prior level of wheel running did not influence the effect of morphine on motor activity. However, as pointed to before, it is still possible that a relationship exists between these two measures as a period of "sensitivity" may be apparent with more or less ad-lib wheel exposure. With a extended wheel access this experiment supports the idea that animals who engage in elevated levels of wheel running behavior are not necessarily more susceptible to the effects of morphine and more importantly, possibly addiction in general.

General Discussion

This investigation indicates that rats with chronic wheel experience showed a response to an initial administration of either amphetamine or morphine similar to that seen in rats given repeated spaced administration of these drugs. Experiment 1 demonstrated that following a period of chronic wheel exposure rats showed an augmented locomotor response (sensitization) to an initial administration of amphetamine. As the effects of morphine are biphasic, in Experiment 2 rats with chronic wheel experience showed both a reduced hypoactivity (tolerance) followed by an augmented hyperactivity (sensitization) to an initial morphine administration. The present study also demonstrated that differences in daily running totals and the effects of either amphetamine or morphine do not seem to be related. The baseline locomotor activity test conducted in the second experiment was also not predictive of the amount of running. A comparison between the habituation periods of both experiments suggests

that wheel running may have a profound short term suppressant effect on a rat's general motor behavior.

These important issues and their implications will be elaborated on in the order that they occurred during these investigations. I will begin with a comparison of the results from the habituation period in the two experiments, followed by a discussion on whether certain rats are more susceptible to the addicting behavior of wheel running. Then the effects of amphetamine or morphine will be explored and I will conclude with a review of the sensitization and/or tolerance evident to an initial administration of either amphetamine or morphine in rats with prior chronic wheel exposure.

A comparison between the two experiments revealed an interesting effect that wheel running has on the general motor behavior of rats in a novel environment. The two 1 hour habituation periods in the first experiment found no difference in motor behavior between the wheel access rats and the control no wheel rats. However, a significant difference in locomotor behavior was evident between these two groups for the 2 hour habituation period of the second experiment. The habituation period in both experiments followed 24 days of ad-libitum wheel access. The only important difference in methodology between the two experiments was that a five day wheel withdrawal period was implemented prior to the habituation in the first experiment. No such wheel withdrawal period occurred in the second experiment; all animals in the experimental group had constant wheel access throughout the habituation and testing period. It should be noted that the no wheel control rat's locomotor behavior was similar in both experiments.

The results from these two habituation periods raises the question of why a motor difference between groups is evident in a novel environment immediately following ad-

libitum wheel access and not following a few days of wheel abstinence. The difference in locomotor activity between the wheel access and control group during the habituation period of the second experiment may be due to a reduced corticosterone level in the wheel access group resulting in a reduced locomotor response to novelty as it has been suggested that activity is positively correlated to corticosterone levels (Dellu et al., 1996). If this was the case, then the first experiment suggests that a 5 day wheel withdrawal period allows animals to "recover" from any influence wheel running may have on corticosterone levels. If rats vary in their stress levels and a novel environment induces stress (Dantzer & Mormede, 1983), then it may be possible that chronic wheel access has an immediate positive impact on a rat's ability to handle stress. Stress and corticosterone levels may be lower in animals with wheel access and is something that could be experimentally tested. Elliott and Grunberg (2005) showed that both social enrichment and physical enrichment enhance a rat's ability to habituate to a novel open field environment. Therefore, it may also be the case that wheel access is a significant form of environmental enrichment and responsible for the difference in motor activity between groups evident in the habituation session of Experiment 2.

The difference in locomotor response during the habituation period of the second experiment may also be due to simple fatigue. It is possible that the enhanced physical activity of the wheel access rats had an impact on their physical endurance resulting in a reduced locomotor response during the habituation trial. Whatever the case, a comparison of the results between the habituation periods of both experiments indicates that wheel running has a direct impact on a rat's general motor activity, whether it is a physiological effect of fatigue, stress, or a direct effect on exploratory motivation.

Further research is required in order to determine why wheel running has this impact on the general motor behavior of rats. Comparing these two experiments reveals that the period of time between wheel access and exposure to the novel testing environment obviously has a strong influence. Thus, manipulating the period of wheel withdrawal prior to exposure to the novel testing environment will reveal how long this effect lasts after wheel exposure and how much running is necessary to see the effect. Manipulating the wheel access, both its duration and the length of daily access, for rats will help determine when wheel running will have this effect on the general motor behavior of rats and will also reveal whether this effect can be enhanced or decreased. It may also be interesting to investigate whether this effect is induced solely by wheel running or whether other behavioral manipulations reducing stress can elicit a similar response. Nevertheless, further research on this issue is needed in order to determine why wheel running has this effect on locomotion in a novel environment for rats and if this effect has any impact on a rat's sensitization and/or tolerance response to drugs of abuse following chronic wheel running experience.

This set of experiments also investigated whether certain animals are more susceptible to wheel running addiction than others. Piazza et al. (1989) suggested that such a vulnerability to addiction varies in rats. They conducted a baseline activity test to measure the individual reactivity (locomotor response) of rats to a novel environment. From this baseline test rats were separated into two groups and classified as either "low" responders or "high" responders to novelty. The next day all rats were again habituated for 3 hours to the same environment and then immediately tested in this environment for their 3 hour motor response to 1.5 mg/kg of amphetamine. The group of rats classified as "high" responders to the novel environment had a significantly higher response to the

amphetamine than did the "low" responder group, and this difference was most marked during the first 30 minutes after amphetamine. They suggest that "high" responders were thus more vulnerable to the addicting effects of amphetamine. The investigators conducted a second experiment repeating the same procedures, however, in this second experiment half the animals in the "high" and "low" responder groups received four injections of 1.5 mg/kg of amphetamine at three day intervals to test for the acquisition of amphetamine sensitization. This experiment revealed that the "high" responder group responded more robustly to the first amphetamine injection than the "low" responder group and only by the fourth amphetamine injection did the "low" responders reach the locomotor response level of the "high" responder group. Following these administrations all rats were implanted with intravenous cannulas and then allowed to freely self-administer amphetamine for a limited time period. During the self-administration period, only the rats in the "high" responder group pre-exposed to saline injections started self-administration whereas saline pre-exposed "low" responder rats did not. Self administration rates did not differ between "high" and "low" responder groups in animals pre-exposed to amphetamine injections. These experiments indicated that there was a significant positive correlation between the magnitude of the novelty response of individual animals and their subsequent response to amphetamine during the first 30 minutes of observation as indicated by locomotion and if the first exposure was in a self administration procedure. The conclusion of these experiments is that the significantly different response displayed by the "high" responder group to both initial and repeated amphetamine treatments suggests that a high vulnerability to drug addiction may be evident for this particular group of rats but that this difference may be lost after repeated drug experience (Piazza et al., 1989). It should be noted that individual differences

evident between "high" and "low" responders to a novel environment may be reflective of stress differences (Bornstein & Sigman, 1986). This is suggested by findings that (i) previous mild stress (such as handling) is enough to enhance exploratory locomotor activity (West & Michael, 1988); (ii) the novel environment is as potent as electric footshock in raising plasma corticosterone levels (Dantzer & Mormede, 1983); and (iii) rats with higher locomotor responses to novelty (High responders) have higher basal levels of corticosterone in comparison to rats classified as "low" locomotor responders to novelty (Dellu, Mayo, Vallee, Maccari, & Piazza, 1996).

My two experiments in the present investigation revealed results that were inconsistent with this way of exploring the differential vulnerability to addiction. A baseline activity test, similar to the one performed by Piazza et al. (1989), was conducted in the second experiment prior to any wheel access. The purpose of this baseline activity test was to investigate whether animal's vulnerability to behavioral addictions like wheel running might be evident in initial response to this novel environment. Figure 7 indicates that there was no relationship between the baseline activity test and the wheel running levels averaged over the first 4 days of wheel access. There was also no relationship between the baseline locomotor activity and the wheel running average for the final four days of wheel access prior to the habituation period. These results suggests that "high" responders to a novel environment are not more likely to be vulnerable to the behavioral addiction of wheel running as measured by the amount of wheel running. Erb and Parker (1994) using the place conditioning paradigm also showed that "high" responders to a novel environment were not more susceptible to the strength of a preference formed for an amphetamine-paired place.

In both of my experiments, wheel running behavior was also compared with the wheel access rat's individual responses to an initial administration of amphetamine or morphine. Figure 5 indicates that a correlation between the final levels of wheel running and the response of the wheel access group to an initial amphetamine administration was non-significant. In the second experiment, Figures 9 and 14 reveal that the relation between the final levels of wheel running and the response of the wheel access group to an initial administration of morphine was non-significant for both the first (hypoactive) and second (hyperactive) hours of the testing period with morphine. Therefore, these results suggest that high running rats are not more susceptible to the effects of either amphetamine or morphine.

Is it still possible that a differential vulnerability to wheel running addiction exists in rats? The short answer would be it is still a possibility. As described earlier, Piazza et al. (1989) showed that rats classified as "high" responders to a novel environment showed sensitization to amphetamine more rapidly than rats classified as "low" responders. However, it was also evident that any difference in the sensitization response between these two groups was abolished once both groups of animals with this amphetamine pre-exposure were provided the opportunity to self-administer amphetamine (Piazza et al., 1989). This was evident as "high" and "low" responder groups pre-exposed to amphetamine did not differ in their amphetamine self-administration but did differ during their initial exposure to amphetamine (Piazza et al., 1989). Thus, it may be the case that the ad-lib wheel access provided to rats in my experiments acts in a similar manner on behavioral sensitization/tolerance to that seen in the two groups of rats allowed to self-administer amphetamine in the experiment by Piazza et al. (1989). In addition the difference between "high" and "low" responders was most evident in the first 30 minutes

of amphetamine exposure. Therefore it may be that my 4 day average wheel running experience is not sensitive to the vulnerability differences and the effect needs to be explored in the very early stages of running experience. Further investigation is required in order to determine whether rats classified as "high" responders to a novel environment are more vulnerable to the addicting behavior of wheel running and whether high wheel runners are more susceptible to the effects of either amphetamine or morphine. It would be beneficial in future studies to manipulate (shorten) the wheel exposure provided to the experimental group in order conclude whether a particular period of "sensitivity" exists for the expression of an enhanced vulnerability to either wheel running or the later amphetamine and morphine locomotor effects. Manipulating the duration of the baseline locomotor activity test may also prove beneficial as a shorter or longer period of novelty exposure may reveal an enhanced susceptibility to wheel running addiction in specific groups of rats. Therefore, future investigations should concentrate on manipulating the period of access both to baseline locomotor activity tests and to wheel running, which will help determine whether a period of "sensitivity" exists for the expression of an enhanced susceptibility to wheel running and the effects of either amphetamine or morphine.

This study indicates that chronic wheel experience elicits a sensitization or tolerance response to an initial administration of either amphetamine or morphine. Figure 3 indicates that the wheel access group experienced a significantly larger motor response compared to that of the control group following the initial amphetamine administration, suggesting that the wheel access group had a sensitization-like response to amphetamine. For the second experiment, Figure 8 reveals that the wheel access group experienced a significantly reduced hypoactivity (tolerance) during the first hour of testing with an

initial morphine administration. During the second hour of testing with an initial administration of morphine, Figure 12 indicates that the wheel access group experienced an augmented hyperactivity (sensitization) in comparison to the control group. Therefore, following a chronic period of wheel experience, the wheel access group in both experiments displayed a motor response, following an initial administration of either amphetamine or morphine, similar to that seen in rats with only a history of repeated spaced administrations with either of these drugs of abuse.

The behavioral responses to repeated administrations of either amphetamine or morphine have been well characterized in rats. Behavioral sensitization has been shown to occur with repeated spaced administrations of amphetamine as an augmented motor response is evident with each subsequent administration of amphetamine (Browman et al., 1998). Crombag et al. (2001) administered 0.5 mg/kg of amphetamine or saline daily over 12 consecutive days and then conducted a challenge test with 0.5 mg/kg of amphetamine following a 6 day abstinence from drug administration. The difference in motor activity between rats pretreated with saline and amphetamine during the challenge test is quite comparable to that seen during the testing session in Experiment 1 between wheel exposed and wheel naïve rats. This comparison suggests that 24 days of ad-libitum wheel access may have a similar impact on rats to that seen with multiple spaced administrations of low doses amphetamine. Morphine typically has a biphasic effect on motor response as the initial response is one of hypoactivity which is followed by a subsequent period of hyperactivity in motor behavior (Babbini & Davis, 1972; Schnur, 1984). However, with repeated spaced administrations of morphine the initial period of hypoactivity is reduced (tolerance) while the following period of hyperactivity is augmented (sensitization) and occurs earlier in time (Babbini & Davis, 1972; Schnur,

1984). Both of these responses to repeated administrations of morphine become more evident with each subsequent administration and may reflect a unitary underlying mechanism (Babbini & Davis, 1972; Schnur, 1984). Babbini and Davis (1972) administered various doses of morphine daily over a period of 30 days while measuring the resulting locomotor activity in rats. The tolerance response shown (the reduction in the degree of hypoactivity) by the wheel access group during the second experiment of my investigation is similar level to that seen in the first hour of testing with 8 administrations of 20 mg/kg of morphine delivered daily over 8 consecutive days (Babbini & Davis, 1972). The sensitization response shown (the increased hyperactivity) by the wheel access group is similar to that seen in rats with 4 administrations of 20 mg/kg of morphine delivered daily over four consecutive days and is evident during the second hour of locomotor testing (Babbini & Davis, 1972). This comparison suggests that 24 days of ad-libitum wheel access has a similar impact on rats to that seen with multiple administrations of a relatively high dose of morphine. However, more work must be done to compare the effects of wheel access with prior drug experience.

Both physiological processes underlying sensitization and tolerance have been suggested as playing a crucial role in the transition from occasional drug use and drug addiction (Anagnostaras & Robinson, 1996; Hinson & Siegel, 1982; Siegel, 1979, 1983). It is evident that the changes in drug responsivity that occur with each subsequent drug administration are important in the process of addiction. Therefore, the most important result of the present investigation is the fact that the behavioral addiction of wheel running can elicit both a sensitization and tolerance effect upon a challenge test with drugs of abuse like amphetamine and morphine. This set of experiments suggest that the physiological processes responsible for sensitization and/or tolerance may also play a

crucial role in the transition to behavioral addictions as has been suggested in the transition to drug addiction. It may be the case that processes underlying drug sensitization and tolerance are also responsible for the escalation in wheel running behavior of rats that is evident with chronic wheel exposure. It is possible that a rat experiences an augmented "sensitization-like" effect with each period of wheel running activity resulting in an increase in the rewarding value of wheel running that in turn motivates the rat to increase its wheel running behavior. It may also be possible that a rat becomes tolerant to the rewarding effects of wheel running causing the rat to increase its running activity in order to continue to experience the same rewarding effects that are produced by wheel running. Direct effects of the rewarding value of initial and chronic wheel running would be helpful. This could be carried out testing naïve and experienced runners in an operant chamber where lever pressing results in wheel access. Experienced runners should find wheel running more rewarding.

This investigation is a relatively novel study exploring an animal model of behavioral addictions and the results suggest further research on the underlying causes of behavioral addictions would be profitable. It would be beneficial to investigate the effects of prior exposure to drugs of abuse like amphetamine and morphine on the wheel running in rats. This could provide further support for the existence of sensitization and/or tolerance in the behavioral addiction of wheel running in rats. It is unknown at this time how amounts of wheel running relate to particular schedules of drug administration which would prove useful in understanding the levels of sensitization and/or tolerance that are elicited in behavioral addictions. This could be tested by comparing the sensitization/tolerance response produced by both various periods of wheel running and various schedules and amounts of drug administration. It would also be of

great interest to investigate if sensitization and/or tolerance change if the rewarding value of wheel running is altered. This could be tested by increasing or decreasing the effort required to run in the wheel. Stressful events such as tail pressure in rats have been shown to induce a sensitization response to a later injection of amphetamine (Antelman, Eichler, Black, & Kocan, 1980). Therefore, if wheel running is a behavioral addiction future investigations should result in a greater sensitization response to amphetamine following wheel running in comparison to more non-specific stressful events such as tail pressure or foot shock in rats. However, it is clear that the present investigation is only the first step in exploring behavioral addictions.

If the processes underlying sensitization and tolerance are responsible for the addicting behavior of wheel running in rats then it may be possible that similar processes are also important in human behavioral addictions. An escalation in behavior, like that seen in the wheel running of rats, is characteristic of human behavioral addictions like gambling, exercise, and sex addiction. Therefore, the sensitization and tolerance processes that are evident in drug addiction and the wheel running behavior of rats may also be responsible for the transition to addiction in these human behavioral addictions. Each time a human engages in one of these behaviors he or she may experience a "sensitization-like" effect that increases the behaviors rewarding value and therefore, promote the desire to increase ones activity in these behavioral addictions. Humans may become "tolerant" to the rewarding effects of these behavioral addictions which might cause the individual to increase the behavior to maintain the rewarding effects of these behavioral addictions. Whatever the reason for the behavioral addictions in humans this investigation proposes the possibility that the physiological processes underlying drug

sensitization and/or tolerance may play an important role in the transition to behavioral addiction.

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Figure Captions

Figure 1 – Individual and mean wheel running behavior of rats in the wheel access group over both the initial four and final four days of wheel access. Lines connect each rat's wheel running response during these two time periods.

Figure 2 – Mean (±SEM) locomotion of rats in the wheel access and control conditions over the two one hour habituation trials occurring 5 days after the wheel access rats were removed from the wheel.

Figure 3 – Mean (±SEM) locomotion of rats in the wheel access and control conditions under both initial saline and amphetamine administration during the two day cross-over design sensitization challenge test that occurred the two days following the final habituation sessions.

Figure 4 – Mean (±SEM) locomotion increase of rats in the wheel access and control conditions under amphetamine administration when compared to locomotion results under saline administration used as baseline for each group (ratio score was equal to 100 x [actual response under amphetamine – actual response under saline] / response under saline).

Figure 5 – Scatterplot revealing the relation between each individual rat's ultimate wheel running behavior, averaged over the final four days of wheel access, and their increase in

locomotion under amphetamine administration when compared to locomotion results under saline administration which was used as baseline.

Figure 6 – Individual and mean wheel running behavior of rats in the wheel access group over both the initial four and final four days of wheel access prior to the habituation period. Lines connect each rat's wheel running response during these two time periods.

Figure 7 – Scatterplot revealing the relation between each individual rat's initial wheel running behavior, averaged over the initial four days of wheel access, and their locomotion response to the prior baseline motor activity test.

Figure 8 – Mean (±SEM) locomotion of rats in the wheel access and control conditions over the one 2 hour habituation session that immediately followed 24 days of wheel access for the wheel access group of rats.

Figure 9 – Mean (±SEM) locomotion of rats in the wheel access and control conditions under both saline and morphine administration during the first hour of testing over the two day cross-over design drug challenge test. The insert reveals the drug by drug order (day 1 or day 2) effect where animals who received morphine on day 1 showed a larger response than the animals who received morphine on day 2.

Figure 10 - Mean (±SEM) locomotion decrease of rats in the wheel access and control conditions under morphine administration when compared to locomotion results under saline administration used as baseline for each group (ratio score was equal to 100 x

[actual response under morphine – actual response under saline] / response under saline). Data reveals the results during the first hour of testing during the two day cross-over design drug challenge test.

Figure 11 - Scatterplot revealing the relation between each individual rat's ultimate wheel running behavior, averaged over the final four days of wheel access, and their decrease in locomotion under morphine administration during the first hour of the drug challenge test when compared to locomotion results under saline administration which was used as baseline.

Figure 12 - Mean (±SEM) locomotion of rats in the wheel access and control conditions under both saline and morphine administration during the second hour of testing over the two day cross-over design drug challenge test.

Figure 13 - Mean (±SEM) locomotion increase of rats in the wheel access and control conditions under morphine administration when compared to locomotion results under saline administration used as baseline for each group (ratio score was equal to 100 x [actual response under morphine – actual response under saline] / response under saline). Data reveals the results during the second hour of testing during the two day cross-over design drug challenge test.

Figure 14 - Scatterplot revealing the relation between each individual rat's ultimate wheel running behavior, averaged over the final four days of wheel access, and their increase in locomotion under morphine administration for the second hour of testing during the drug

challenge test when compared to locomotion results under saline administration which was used as baseline.

Figure 1

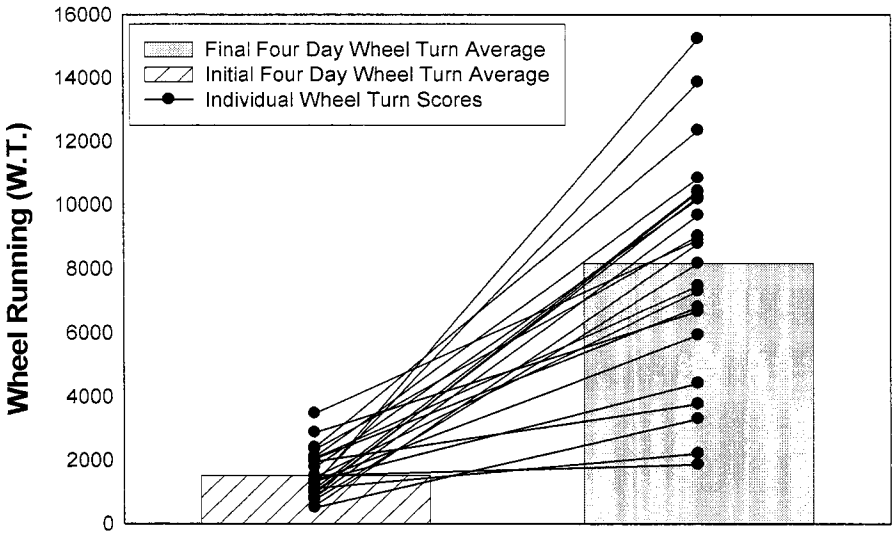

16000
14000
12000
10000
8000
6000
4000
2000
0
Wheel Running (W.T.)
Final Four Day Wheel Turn Average
Initial Four Day Wheel Turn Average
Individual Wheel Turn Scores

Figure 2

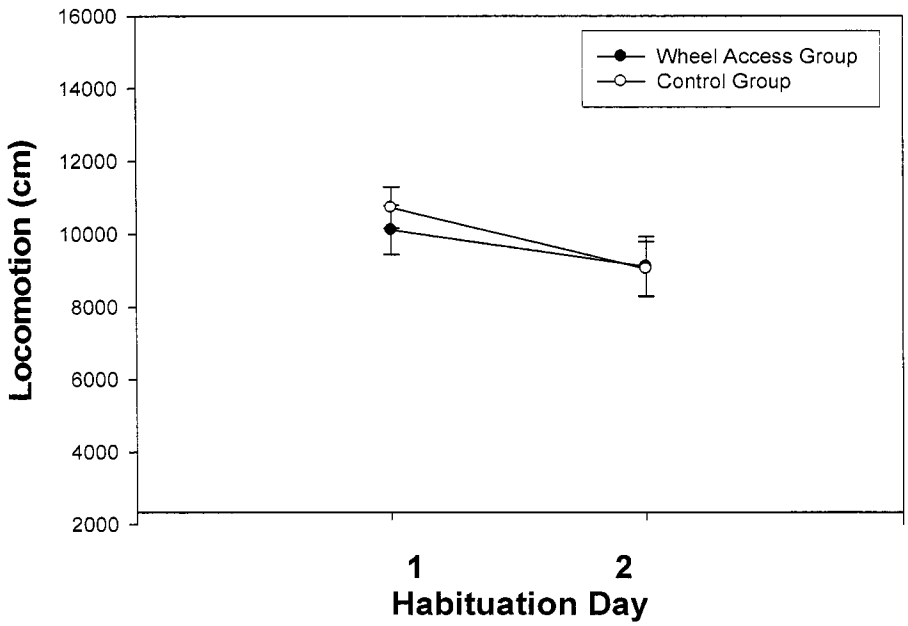

16000
14000
12000
10000
8000
6000
4000
2000
Locomotion (cm)
Wheel Access Group
Control Group
1
2
Habituation Day

Figure 3

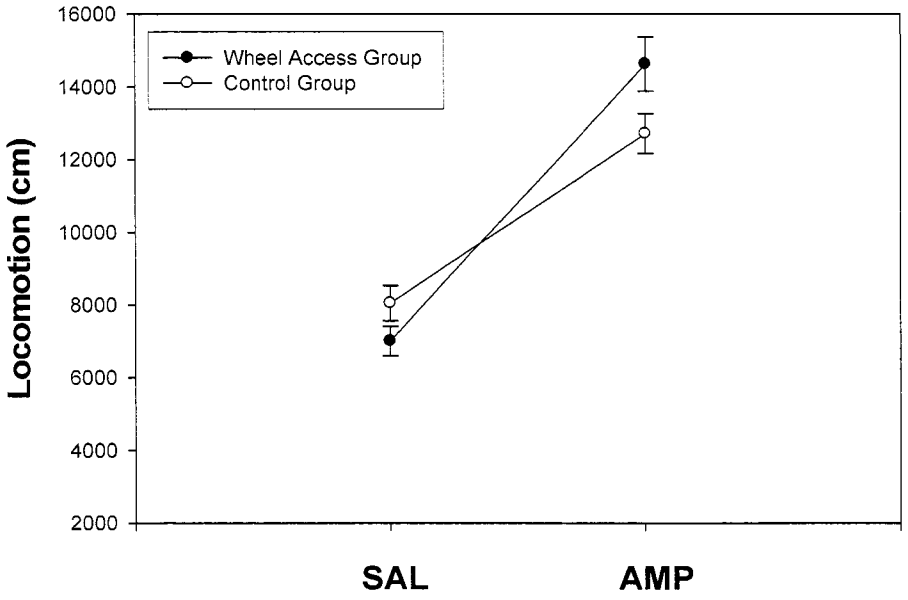

16000
14000
12000
10000
8000
6000
4000
2000
Locomotion (cm)
Wheel Access Group
Control Group
SAL
AMP

Figure 4

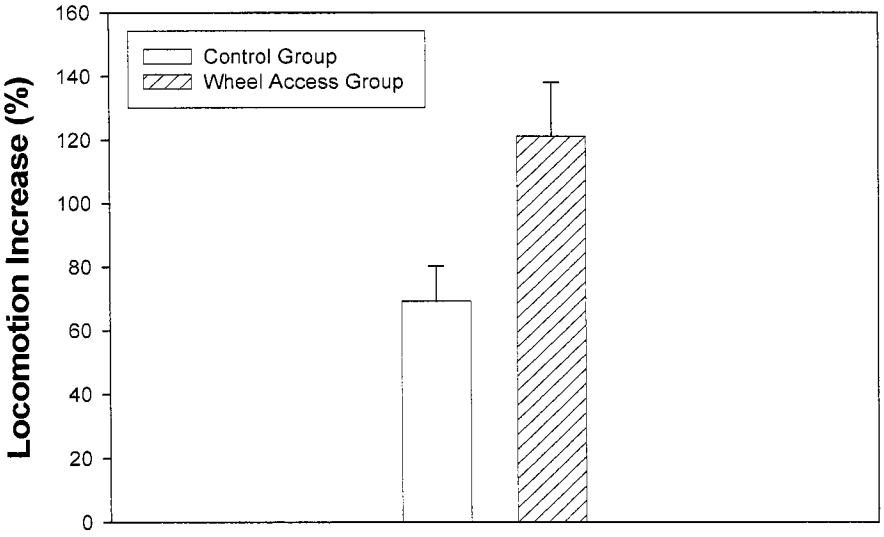

Locomotion Increase (%)
160
140
120
100
80
60
40
20
0
Control Group
Wheel Access Group

Figure 5

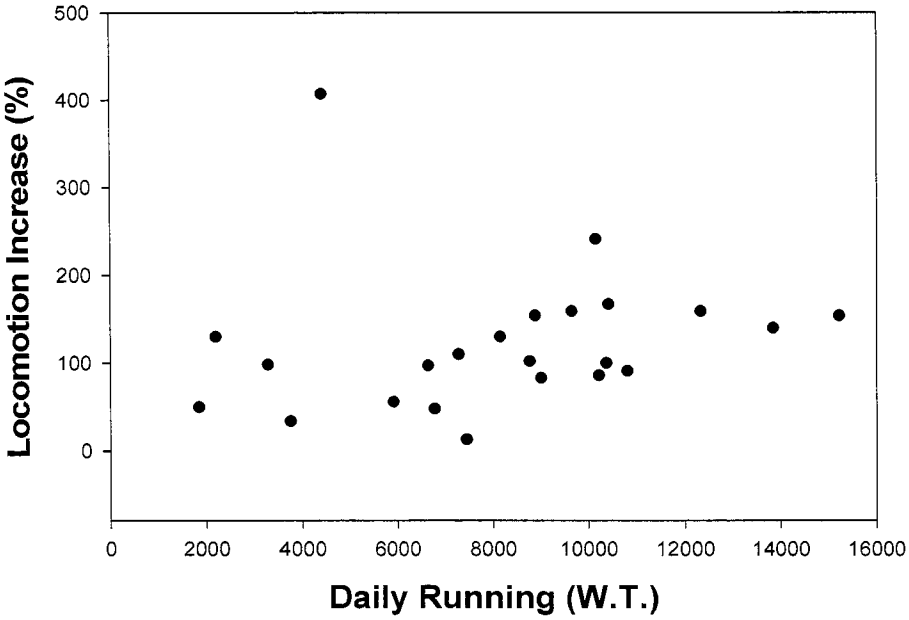

Locomotion Increase (%)
500
400
300
200
100
0
0
2000
4000
6000
8000
10000
12000
14000
16000
Daily Running (W.T.)

Figure 6

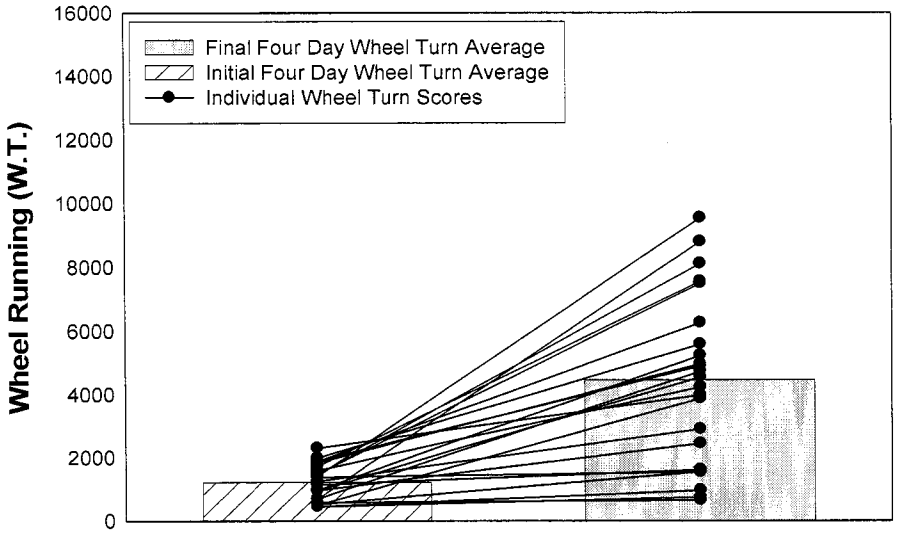

Final Four Day Wheel Turn Average
Initial Four Day Wheel Turn Average
Individual Wheel Turn Scores
Wheel Running (W.T.)
16000
14000
12000
10000
8000
6000
4000
2000
0

Figure 7

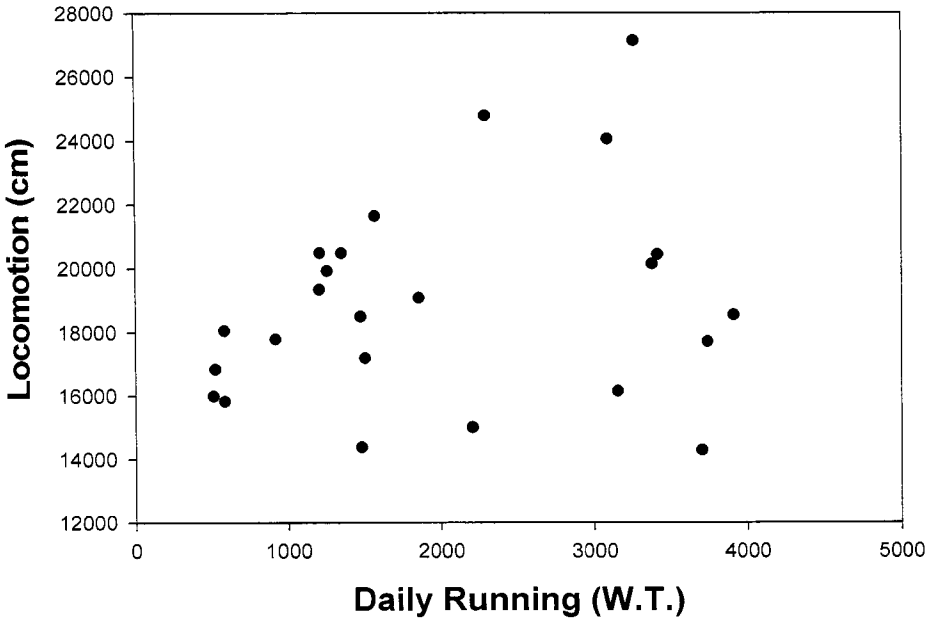

28000
26000
24000
22000
20000
18000
16000
14000
12000
Locomotion (cm)
0
1000
2000
3000
4000
5000
Daily Running (W.T.)

Figure 8

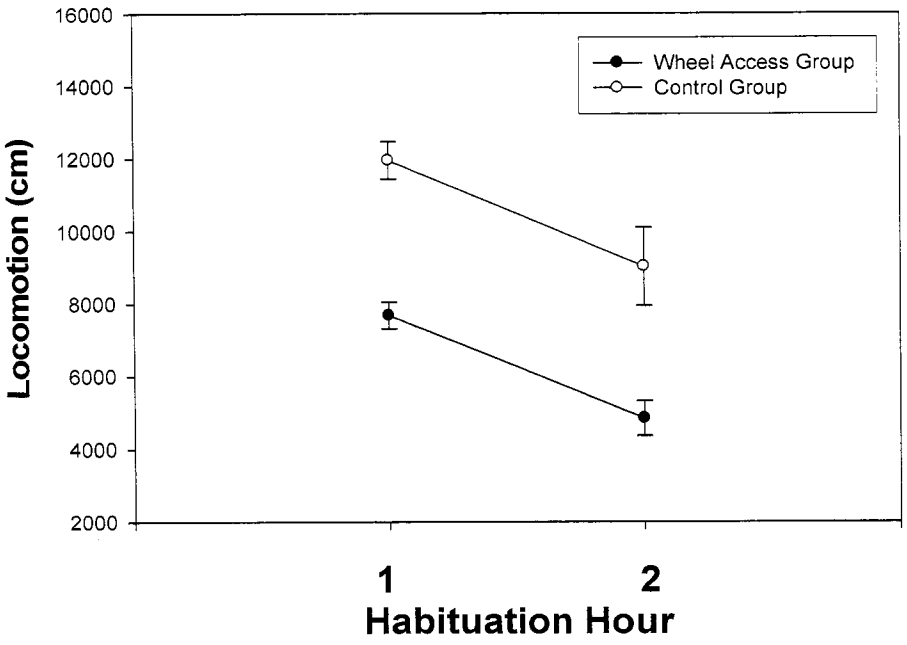

16000
14000
12000
10000
8000
6000
4000
2000
Locomotion (cm)
Wheel Access Group
Control Group
1
2
Habituation Hour

Figure 9

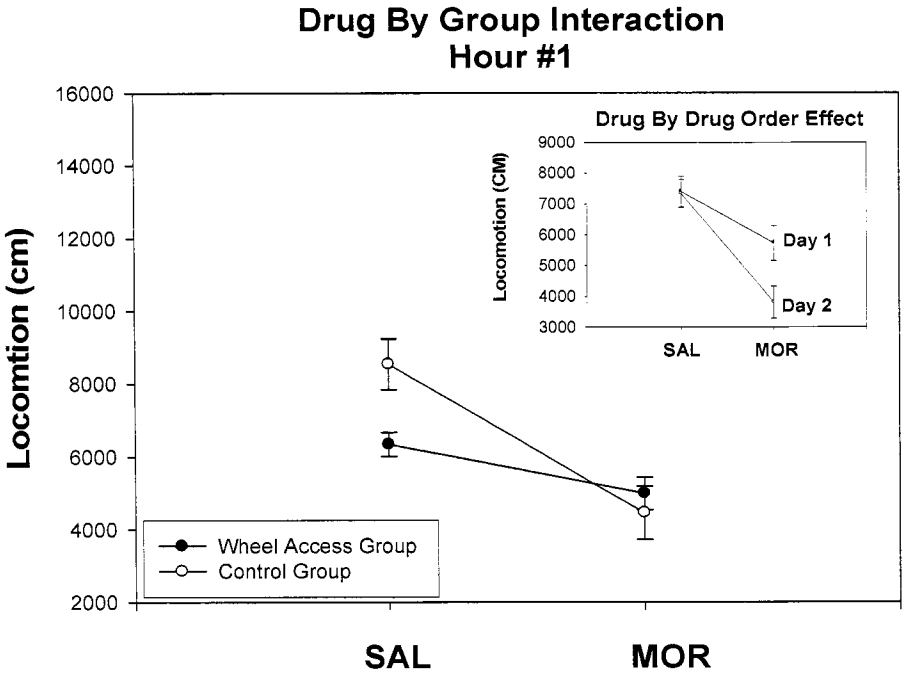

Drug By Group Interaction
Hour #1
Locomtion (cm)
16000
14000
12000
10000
8000
6000
4000
2000
SAL
MOR
Wheel Access Group
Control Group
Drug By Drug Order Effect
Locomotion (CM)
9000
8000
7000
6000
5000
4000
3000
Day 1
Day 2
SAL
MOR

Figure 10

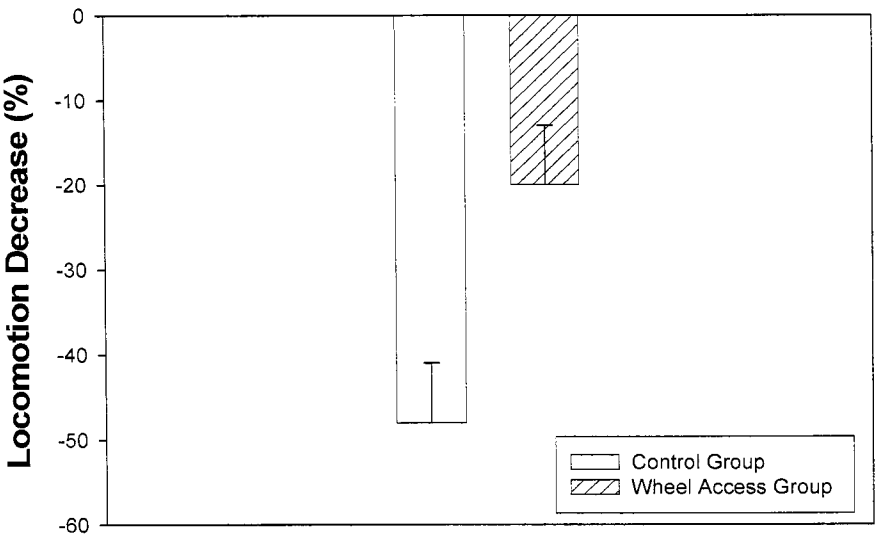

Locomotion Decrease (%)
0
-10
-20
-30
-40
-50
-60
Control Group
Wheel Access Group

Figure 11

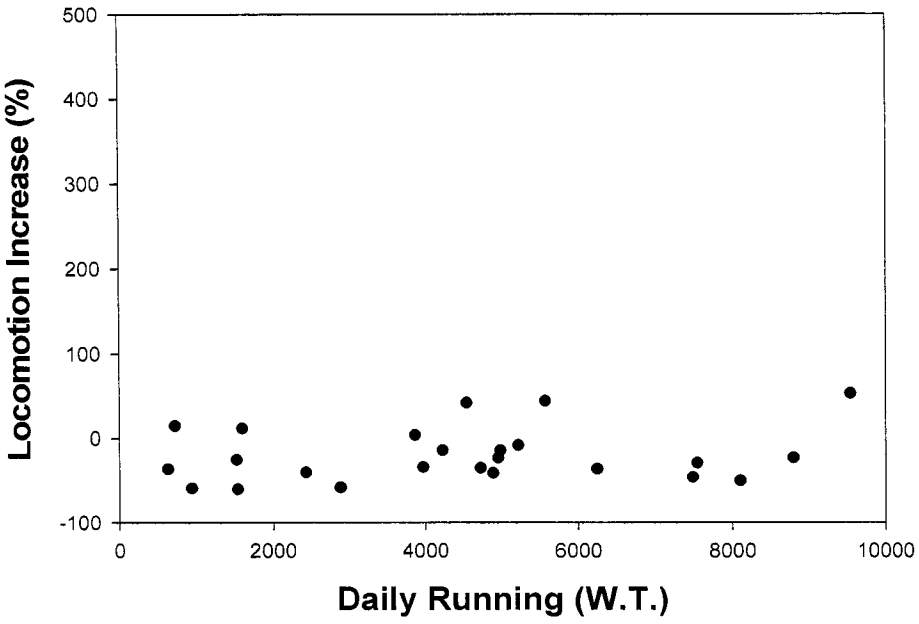

Locomotion Increase (%)
500
400
300
200
100
0
-100
0
2000
4000
6000
8000
10000
Daily Running (W.T.)

Figure 12

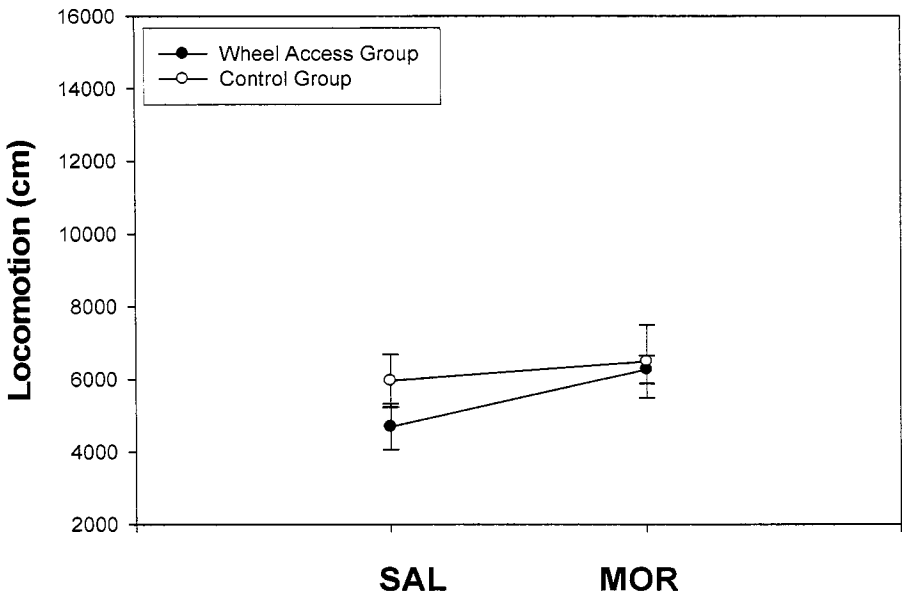

16000
14000
12000
10000
8000
6000
4000
2000
Locomotion (cm)
Wheel Access Group
Control Group
SAL
MOR

Figure 13

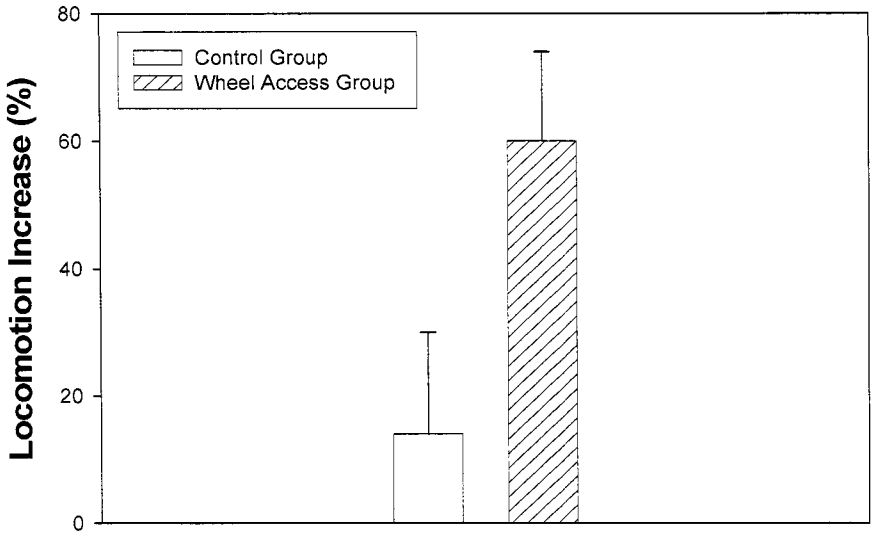
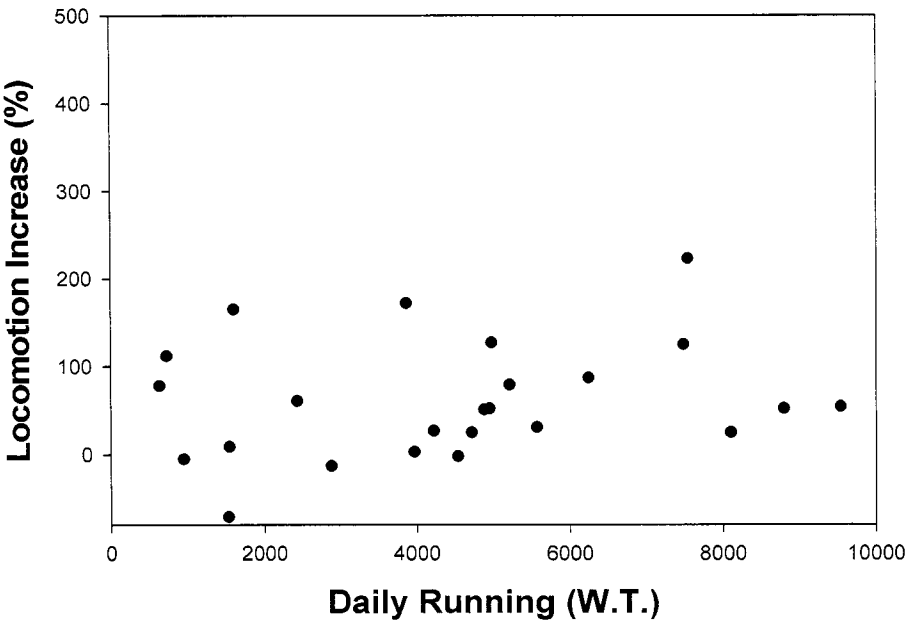

Locomotion Increase (%)
80
60
40
20
0
Control Group
Wheel Access Group

Figure 14


Locomotion Increase (%)
500
400
300
200
100
0
0
2000
4000
6000
8000
10000
Daily Running (W.T.)