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Escalation of Wheel Running: An Animal Model of Non-Drug Addiction

Ali Gheidi
Wilfrid Laurier University

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ESCALATION OF WHEEL RUNNING: AN ANIMAL MODEL OF NON-DRUG ADDICTION

by

Ali Gheidi

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THESIS

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Abstract

Animal studies using the self-administration paradigm suggest that duration of drug access (Ahmed, 2005) and individual phenotype (Piazza, Deminiere, Le Moal and Simon, 1989) are important factors in the development of drug addiction. Wheel running in rats has been proposed as a model to study non-drug addictions (Eikelboom & Lattanzio, 2003). When first given *ad lib* wheel access, young male rats initially run at low levels (about 1000 wheel turns per night) and gradually increase this distance to high levels (about 5000 wheel turns per night) (Afonso and Eikelboom, 2003). Like with self-administration, duration of wheel access has been found to be critical to the development of this excessive running (Eikelboom & Lattanzio, 2003). At this point it is not clear how individual phenotype and duration of access interact to result in the excessive behaviour seen in addiction. Understanding the mechanisms involved in this increase may shed light on the transition from regulated levels of behaviour to high and uncontrollable levels. The three experiments in this thesis manipulated the duration and time of wheel access to determine how these variables affect final running levels. In Experiment 1, 32 male rats had either 30, 45, 60 or 90 minutes of nightly wheel access either starting at 13:00 (one hour after lights went out) or ending at 14:30 for 24 days. Running increased only in the 90 minute group. Rats introduced to the wheel at 13:00 ran more and increased their running more than rats introduced later. This difference was determined to be due to baseline running differences prior to restricted access. In Experiment 2 duration of nightly wheel access was held constant (45 minutes) but time of wheel introduction was manipulated. From lights out to 6 hours after lights off all groups ran equally and did not increase their running significantly. In Experiment 3 time of wheel introduction was held constant and 72 rats were given either 30, 60, 90, or 180 minutes of wheel access 1 hour after lights out. While running increased for all groups the 180 minute

group showed the largest increase. Individual correlations suggested that final running levels could be predicted by the mean of the first 4 days of running. A multiple-regression using both duration of access, and the mean of first 4 days suggested that both are important in final running levels. Similar to drug addiction, both duration of access and individual differences may be important in non-drug addictions.

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The term addiction is usually used with reference to excessive drug consumption, but it appears that other behaviours may become excessive and addictive as well. These behaviours, which normally occur in a well regulated manner, can become compulsive and show a profile similar to that seen in drug addiction (Orford, 2001). Behaviours which have been discussed as "addiction like" include excessive exercise, compulsive shopping, excessive internet use, eating disorders, obsessive sexual behaviour, and pathological gambling (Davis & Claridge, 1998; Elbourne & Chen, 2007; Koh, Lett, & Grant, 2000; Koran, Faber, Aboujaoude, Large, & Serpe, 2006; Lobo & Kennedy, 2006; Myers, 1995). This thesis explores aspects of an animal model, wheel running in rats, which shares characteristics with these excessive non-drug addictions in humans.

Using the drug self-administration paradigm (a commonly used method to study drug intake in laboratory animals where drug infusions are contingent on operant responses), the transition from low to high drug consumption can be potentiated by long access sessions and so duration of access has been argued to be an important feature of drug addiction (Ahmed and Koob, 1998). Access duration may also be an important factor in non-drug addiction (Eikelboom and Lattanzio, 2003). This thesis studies the transition from low to high, and arguably excessive levels of running induced by varying the duration of wheel access.

Drug Abuse

Drug abuse poses a great burden on the Canadian economy. The Canadian Centre on Substance Abuse (CCSA) estimates that each year Canada spends $39.8 billion dollars on both direct and indirect costs of treating drug abuse (CCSA, 2006). This figure represents costs to each individual Canadian of $1,267 per year. This estimate can be further broken down with tobacco accounting for $17 billion, alcohol for $14.6 billion and illegal drugs for $8.2 billion.

This total amount is determined by taking into account costs of health care, law enforcement, loss of productivity in the workplace, or at home, and premature death and disability resulting from substance abuse. The above statistics do not, however, take into consideration non-drug 'addictions'; if they were included, the cost of 'addiction' would in fact be even higher.

Individuals who suffer from substance abuse, bulimia, pathological gambling or sexual addiction show many similar clinical features (Goodman, 2008). The illness usually begins in adolescence or early adulthood, the behaviour is continued despite harmful consequences, the individual experiences a subjective sense of craving and reward while partaking in the behaviour, experiences withdrawal when the behaviour is discontinued and has a tendency to relapse. These individuals neglect other areas of life and often show high comorbidity between the drug and non-drug addictions. The common clinical features linking substance abuse with behavioural addictions may represent an overlap in neurobiological substrate. Wheel running may be a useful tool for teasing apart the similarities and differences between types of addictions.

Addiction is a term that defies simple definition. Simple consumption of a drug is not sufficient evidence of abuse, dependence, or addiction. Abuse and dependence usually refer to particular patterns of drug seeking, drug taking and relapse that are performed without regard to other activities and are distinctive from controlled regulated intake (Robinson, 2004). Many people have taken a potentially addictive drug but very few become 'addicted' (Koob, 2004; Robinson, 2004). *The Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV-1994) defines substance abuse and dependence but not addiction. The criteria the *DSM-IV* uses are multi-faceted and it is not clear which aspect are, or are not, important in defining substance dependence. The substance dependence criteria as defined by *DSM-IV* are:

> A maladaptive pattern of substance use, leading to clinically significant impairment or distress, as manifested by three (or more) of the following, occurring at any time in the same 12-month period:
>
> 1. Tolerance, as defined by either of the following:
> a. A need for markedly increased amounts of the substance to achieve intoxication or desired effect.
> b. Markedly diminished effect with continued use of the same amount of the substance.
>
> 2. Withdrawal, as manifested by either of the following:
> a. The characteristic withdrawal syndrome for the substance (refer to Criteria A and B of the criteria sets for Withdrawal from the specific substances).
> b. The same (or a closely related) substance is taken to relieve or avoid withdrawal symptoms.
> c. The substance is often taken in large amounts or over a longer period than was intended.
>
> 3. There is a persistent desire or unsuccessful efforts to cut down or control substance use.
>
> 4. A great deal of time is spent in activities necessary to obtain the substance (e.g., visiting multiple doctors or driving long distances), use the substance (e.g., chain-smoking), or recover from its effects.
>
> 5. Important social, occupational, or recreational activities are given up or reduced because of substance use.
>
> 6. The substance use is continued despite knowledge of having persistent or recurrent physical or psychological problem that is likely to have been caused or exacerbated by the substance (e.g., current cocaine use despite recognition of cocaine-induced depression, or continued drinking despite recognition that an ulcer was made worse by alcohol consumption). (page 181).

These criteria can be distilled down to three core features that define drug abuse and will be used in this thesis (Deroche-Gamonent, Belin and Piazza, 2004):

1. The subject has difficulty stopping or limiting drug use.
2. The subject has high motivation to acquire the drug and will focus on activities directed at attaining the drug.
3. Drug use is continued despite adverse consequences to the user.

Although the above *DSM-IV* criteria have been traditionally used by clinicians to diagnose and treat human drug abusers, these core criteria have also been demonstrated to occur in laboratory animals. In a number of experiments using the self-administration procedure freely moving rats implanted with a drug cannula, self-administered cocaine by poking their nose into a hole. To determine if the *DSM-IV* criteria could be applied to these rats, self-administration was followed for about 3 months, during which the three behaviours resembling those in the core features of *DSM-IV* were repeatedly evaluated. Daily self-administration sessions consisted of three drug periods (40 minutes each) separated by two 15 minute drug free periods. Initially during the drug periods, each time the rat inserted its nose into the hole it received an infusion of cocaine. During drug free periods nose pokes were without consequence. In the first 5 days of the experiment an FR1 (Fixed Ratio) schedule of reinforcement was used in which every nose poke resulted in a 0.8 mg/kg cocaine infusion. The FR schedule was subsequently increased to FR3 and finally FR5 for the rest of the experiment. The criterion for cocaine acquisition was defined by a stable number of self-infusions over the three last consecutive self-administration sessions (± 10%). Following the 3 months of drug self-administration, animals received 5 days of extinction. During this time, animals received 1 hour of sessions during which they were placed in operant boxes and could nose poke, but their behaviour was without consequence. Following extinction training sessions all animals were challenged with a non-contingent 0.2 mg/kg cocaine infusion, placed into operant boxes, and their nose poke behavior was measured for 10 minutes. Animals were divided into groups based on their responses after this cocaine challenge. The two groups consisted of the 40% lowest (LoRein) and 40% highest (HiRein) responders following the cocaine challenge. These two groups of animals differed strikingly in regards to the core features based on the *DSM-IV* criteria:

1. The subject has difficulty limiting or stopping drug use. The persistence of cocaine seeking was measured during the non-drug periods. The daily self-administration sessions each had two 15 minutes of non-drug periods separating drug periods. The animals' responses during the non-drug period served as an index of the persistence to cocaine seek. HiRein rats progressively increased their drug seeking behaviour relative to the LoRein rats in these non-drug periods over the 30 days of self-administration.

2. The subject has a high motivation to take the drug, with activities directed toward consumption of the drug. Motivation was assessed using a progressive-ratio (PR) schedule. The number of responses needed for one cocaine infusion was progressively increased (10, 20, 30, 45, 65, 85...). Progressive ratio sessions were terminated when 1 hour elapsed since a previous infusion, the point at which this occurred was termed the 'breakpoint'. HiRein rats had higher 'breakpoints' than LoRein rats.

3. Drug use is continued despite adverse consequences to the user. In these sessions, animals were placed in operant chambers for 40 minutes. An FR 5 schedule was used. After five nose-pokes the animal received both an electric shock (0.8 mA, 2 seconds) and a cocaine infusion (0.8 mg/kg) for the entire 40 minute session. HiRein rats increased their drug-seeking behaviour even though it was paired with an adverse consequence, while LoRein rats did not increase their responding.

These results suggest that important fundamental features of a human pathology can be observed in laboratory rodents. They argue animal models have great face validity in studying both human drug abuse, and the transition from controlled to excessive consumption.

Not only do these studies suggest that human pathology can be modeled in rats, they also highlight the differences in drug vulnerability seen in humans. Only HiRein, and not LoRein rats

appeared to be 'addicted'. These differences between rats suggest individual differences play a role in abuse liability. Piazza, Deminiere, Le Moal, and Simon (1989) have demonstrated that when rats have access to low doses of cocaine, only some animals start to self-administer. Piazza et al. (1989) call these rats High vs. Low drug responders. The qualitative differences between High and Low responder observed in animal drug self-administration may serve to identify factors that determine which individuals have a susceptibility to addiction. In line with this argument, non-drug addictions clearly have a strong phenotypic component in that only some individuals appear to be vulnerable. This thesis will also address this issue by examining high and low wheel running levels.

Escalation Model

The escalation of drug intake is an important feature of the first core criterion of drug dependence and has been documented to occur in laboratory animals (Ahmed and Koob, 1998). The transition from a controlled regulated intake to one that is compulsive and arguably 'addictive' involves the interplay of genetic, developmental, and environmental factors (Ahmed, 2005). Among the environmental factors involved, the duration of drug availability in the self-administration rodent model has been implicated as a major factor in the development of compulsive drug use (Ahmed, 2005; Ahmed and Koob, 1998). Rats were allowed to self-administer cocaine (250 μg per infusion) under a continuous schedule of reinforcement (Ahmed and Koob, 1998). Rats had either short (1 hour) or long (6 hours) sessions to self-administer for 12 days. Given short access, cocaine consumption started at 19 infusions/session and remained at that level until day 12, whereas with long access, cocaine intake started at 71 infusions/session and gradually escalated to 110 infusions/session. The escalation of cocaine self-administration was even evident over the first hour for the long access rats; suggesting that it should have been

possible for the short access rats to increase their intake, even though they did not. The escalating intake of cocaine in the long access group and the regulated intake with short access may represent an environmental aspect important in abusive vs. casual drug use in humans.

Animals with a history of short and long drug access differ in terms of the core criteria defined by *DSM-IV*. To assess the *DSM-IV* criterion of difficulty in stopping or limiting drug intake, rats were given daily short or long access to heroin self-administration until a significant escalation occurred in the long access group (Ahmed, 2000). Two weeks after the escalation, all rats were given 25 extinction sessions in which lever presses did not result in heroin delivery, but were recorded. Responding for heroin decreased in both groups during extinction, but it was much slower to extinguish in rats given long access relative to those given short access. To assess the motivation for drug consumption, after long and short access sessions using intravenous heroin self-administration as described above (Lenoir and Ahmed, 2007), the 'price' for heroin was increased by implementation of a PR schedule in which each heroin infusion required an increasing number of responses (e.g., PR 1, 4, 7, 10...). The breakpoint was set at 30 minutes without a response. Increasing heroin price reduced drug consumption in both groups. Despite the increase in the 'price' however, long access rats continued to self-administer more heroin than short access rats; that is they had higher breakpoints. Lastly, to assess if drug seeking occurred despite adverse consequences, short and long access rats were given heroin reinstatement training with foot-shock (Ahmed, Walker, and Koob, 2000). Again escalation of heroin self-administration occurred in long but not short access rats. Then drug availability was terminated and each lever press resulted only in foot-shock. Results showed that long access rats were much more likely than short access rats to lever press even though their behaviour had only an aversive consequence. That the consumption of and dependence on drugs as defined by core

DSM-IV criteria are changed by access duration suggests that animals with long drug access develop the major signs of drug abuse. Drug access duration may therefore represent an important factor in the transition from controlled to excessive drug use in humans.

Wheel Running

As mentioned, 'addiction' may not be limited to drug use. For instance, a pathological gambler has difficulty curtailing his/her gambling and has a high motivation to engage in the behaviour. The gambler appears unable to resist gambling impulses and continues to gamble regardless of aversive consequences, such as being arrested for forgery and fraud (Orford, 2001; Petry, 2006). Currently, there are no generally accepted animal models for the study of non-drug 'addictions' such as pathological gambling. An animal model for these types of 'addictions' would be useful given the prevalence of these problems in humans, and may help in understanding the neurobiology of non-drug 'addictions'. Such work might also help in determining factors critical to the development of all types of abuse.

Our laboratory has been studying wheel running in rats as a potential pre-clinical model of non-drug 'addiction' (Eikelboom and Lattanzio, 2003; Inkster, 2005 unpublished masters thesis; Lattanzio and Eikelboom, 2003; Weiss, 2003 unpublished masters thesis). Young adult male rats initially voluntarily run about 1000 wheel turns per day when given *ad lib* access to freely moving wheels with minimal friction. Over days running gradually increases until it levels off after about 4 weeks of access at 5000-6000 wheel turns which equates to a distance of 5-6 km (Afonso and Eikelboom, 2003; Lattanzio and Eikelboom, 2003; Mueller, Loft and Eikelboom, 1997). Wheel running is not correlated with general locomotor activity (Koh et al. 2000) nor is it an accurate index of any other locomotor activity (Sherwin, 1998). It is not clear why rats run in wheels and why their running increases over time (Sherwin, 1998). Uncovering

the mechanisms responsible for this increase may help in understanding the change from low and controlled to high and abusive levels of behaviour.

A number of behavioural, neurochemical and genetic lines of evidence suggest that wheel running mimics, at least to some extent, the processes evident in drug abuse (Eikelboom & Lattanzio, 2003; Eikelboom & Mills, 1988; Iversen, 1993; Lett et al., 2000; Lett, Grant, Koh, & Smith, 2001; Mathes and Kanarek, 2006; Serwatkiewicz, Limebeer, & Eikelboom 2000; Silva & Heyman, 2001; Sisti & Lewis, 2001; Werme et al. 2002; Werme, Thoren, Olsen, & Brene, 1999). These similarities are described in more detail in the subsequent sections.

Behavioural Evidence for the Rewarding Nature of Running

As an index of reward, the conditioned place preference (CPP) paradigm has been used extensively to study the affective effects of drugs of abuse. A typical CPP experiment involves differentially pairing the drug under study with a particular environment. This requires two environments differing on a number of dimensions. They may vary in flooring, size, shape, colour, pattern, or olfactory cues. Conditioning involves repeatedly pairing the drug (unconditioned stimulus, or US) with one environment (conditioned stimulus, or CS), and pairing injection of the drug's vehicle with the other environment. Following repeated conditioning trials there is an un-drugged choice test in which the animal receives unrestricted access to both environments. If more time is spent in the drug-paired chamber, it is understood that the drug is rewarding. But if more time is spent in the alternative chamber, the drug is said to be aversive (Bardo and Bevins, 2000). Using the CPP paradigm drugs such as cocaine (Hernandez-Rabaza, 2008), amphetamine (Cruz, Martin, and Planeta, 2008), morphine (Solecki, Krowka, Kubik, Kaczmarek and Przewlocki, 2008), and ethanol (Groblewski, Bax and Cunningham, 2008) have been shown to be rewarding to the animal.

The CPP paradigm shows that wheel running produces a rewarding aftereffect (Lett et al. 2001; Lett, Grant, Byren and Koh, 2000). Rats were given six pairings of one environment with the aftereffect of wheel running and six pairings of the aftereffect of confinement in a small cage (that was not a home cage) with an alternate environment (Lett et al, 2000). The rats preferred the environment paired with 2 hours of wheel access over the one that was not paired with wheel access, suggesting that rats find the wheel to be rewarding as indexed by the CPP paradigm.

Wheel running can also be shown to act as a reinforcer in an operant paradigm. For example, rats will lever press to run in wheels (Belke, 1997; Belke & Wagner, 2005; Iversen, 1993). In these experiments animals are usually first given *ad lib* wheel running exposure for several days. Then lever pressing is shaped by giving rats a palatable solution such as sucrose every time they lever press. After lever pressing for sucrose is stabilized, sucrose access is discontinued. Instead, retractable levers are extended during wheel running sessions and the opportunity to run for a designated period of time is made contingent on a lever press (Belke, 1997). It has been demonstrated that if a short period of running (about 60 seconds) is contingent on lever pressing, rats will press under a number of FR (in which a fixed number of responses are reinforced) and VR (in which a random number of responses, usually an average is reinforced) schedules to be permitted to run (Iversen, 1993).

Neurochemistry of Wheel Running

It has been suggested that the rewarding properties of wheel running are mediated by endorphins (Eisenstein and Holmes, 2007; Lett et al., 2001; Lett et al., 2002; Smith and Yancey, 2003). Chronic administration of morphine can decrease (Baker and Tiffany, 1985) the sensitivity of relevant brain systems to the drug. For example morphine-induced CPP is difficult to induce in rats with extensive morphine experience (Shippenberg, Emmett-Oglesby, Ayesta

and Herz, 1988). Repeated activation of brain systems by wheel running may also reduce the sensitivity of the animals to morphine. Rats either received 2 hours a day of wheel exposure for 8 consecutive days or were placed in a small metal cage for the same period of time (Lett et al., 2002). The next day, conditioning trials started. Morphine was paired with one environment and saline with the other. Morphine induced a place preference for the cage-morphine group but not in the wheel-morphine group. The eight sessions of wheel running that were given prior to CPP training diminished the ability of morphine to induce CPP, suggesting the reward system was less sensitive to morphine. Thus, rats given a history of wheel running exposure are tolerant to morphine's rewarding effects, suggesting that endorphins were repeatedly activated by wheel running.

In another study (Lett et al., 2001), two groups of rats with 2 hours of wheel exposure for 7 days were either given naloxone (a μ opioid receptor antagonist) or saline and CPP was found in the wheel-saline group while it was diminished in the wheel-naloxone group. Thus, blocking μ opioid receptors attenuates the rewarding effect of wheel running.

Naloxone also has been shown to decrease wheel running at doses too low to decrease general locomotor activity (Sisti and Lewis, 2001). Rats were given wheel access for 1 hour per day. Once running had reached a plateau, naloxone injections were administered immediately before animals were given wheel access. Naloxone produced a dose-dependant decrease in the number of wheel turns. Thus, the rewarding efficacy of wheel running while animals are running may also be mediated by endorphins. If endorphins are blocked by the antagonist, running decreases.

Wheel running has also been suggested to alter the endorphin system controlling pain sensitivity (Mathes and Kanarek, 2001; Mathes and Kanarek, 2006; Smith and Yancey, 2003).

The antinociceptive effects of morphine were compared in active and inactive rats (Mathes and Kanarek, 2006). One group of rats was given 3 weeks of *ad lib* wheel access, while the other group remained in their home cages. Then wheels were locked and both groups received morphine infusions. Pain sensitivity was tested using the radiant heat tail flick test. Rats were placed in the tail flick apparatus with their tail placed into a groove containing a photocell. A light source then was turned on and the amount of time before the rat moved its tail was taken as an index of pain sensitivity. Tail flick latencies were much lower in rats with a previous wheel history than home cage rats. Tail flick latencies were also lower in wheel rats than home cage rats after morphine infusions. This suggests that rats with a previous wheel history have a lower pain threshold and show a decreased morphine analgesic effect as measured by the tail flick test.

Unpublished work from our laboratory has shown that rats with a history of *ad lib* wheel access when injected with morphine show a locomotor pattern in an open field similar to rats with a previous history of morphine injections. Initially when given a morphine injection a rat's locomotor activity in an open field is biphasic; first there is a decrease (sedation) in activity followed by an increase (hyperactivity) in activity. If over days morphine is repeatedly administered there is a reduction in the initial sedation (tolerance) followed by an enhancement in the hyperactivity (sensitization). In our study, rats were either given *ad lib* wheel access for 24 days or left in their home cages. On day 25 all rats received 10 mg/kg i.p. morphine and locomotor activity was quantified for 2 hours in an activity chamber. Rats naïve to the wheel displayed the classic decrease and increase in locomotor activity when given morphine, while rats with a wheel history showed tolerance to the initial sedative effects and sensitization to the later enhancement effect. Rats with previous wheel history showed the same locomotor profile as rats with previous morphine exposure.

The above experiments detailing the neurochemistry of wheel running provide some critical data which argue against the simple interpretation that the escalation of running observed following prolonged access is due simply to enhancement of fitness. Rather repeated activation of certain brain systems by wheel running seem to effect the animal's sense of reward, pain, and opiate induced locomotor activity.

The endorphin neurons synapses on dopamine neurons on the reward system (Spanagel and Weiss, 1999), and opiate drugs are capable of modulating mesolimbic dopamine activity for example, the systemic administrating of a μ opioid receptor agonist like other reinforcers increases nucleus accumbens dopamine levels. Wheel running can also affect cocaine self-administration (Cosgrove, Hunter and Carrol, 2002; Smith, Schmidt, Iordanou and Mustroph, 2008).

Prior wheel exposure decreases the positive-reinforcing effects of cocaine in rats responding on a progressive ratio (PR) schedule of reinforcement (Smith et al, 2008). Rats were left in their home cages or lived in their home cages with wheel access for 6 weeks. Then both groups were given self-administration training with cocaine (1.0 mg/kg/infusion) and later trained on a PR schedule. Compared to sedentary rats breakpoints were lower in rats that lived with wheels in their home cages. Thus, long term wheel exposure decreases the sensitivity to the positive-reinforcing effect of cocaine.

Genetic Factors in Wheel Running

Werme et al. (1999) have speculated that there may be a common mechanism in drug addictive behaviours and compulsive running. Similar neurobiological circuits may be activated by both these behaviours since rats that are more sensitive to a number of different drugs of abuse (Lewis rats) also run more than their less drug-sensitive counterparts (Fischer rats).

Lewis and Fischer strains are both inbred and originally derived from the Sprague-Dawley rat. Lewis rats are known to be more vulnerable to drug addiction, as they are more sensitive to the locomotor stimulant effects of cocaine, and orally consume more cocaine solution (George & Goldberg, 1989). When Fischer and Lewis rats were given a choice between regular food and food containing morphine, Lewis rats developed a strong preference for the morphine food while Fischer rats remained indifferent (Suzuki, Otani, Koike, and Misava, 1988). Lewis rats also developed a CPP for nicotine after five pairings, while Fischer rats showed no preference (Horan, Smith, Gardner, Lepore, and Ashby, 1997). After ten pairings, Lewis rats still showed a place preference, while Fischer rats had developed a place aversion.

Werme et al. (1999) have shown marked differences in running patterns between addiction prone Lewis and non-addictive Fischer rats. When first given wheel access, Lewis rats begin running around 2 km and increase this distance to about 10 km per 24 hours over 35 days. Fischer rats start running at around 0.5 km per night and increased this distance to 1.5 km over 35 days.

Research Rationale

When naïve rats are given *ad lib* wheel access, running is initially low. Continuous access over 24 days however results in running levels that are quite high (Afonso and Eikelboom, 2003). The transition from the initial low levels (1000 wheel turns) to higher (5000-6000 wheel turns, 5-6 km) and arguably excessive levels may be the result of a number of different factors. Similar to effects seen with drug self-administration, one of the factors involved in the transition to excessive running has been found to be the duration of wheel access (Eikelboom and Lattanzio, 2003).

Eikelboom and Lattanzio (2003) gave wheel naïve rats either 1 or 4 hours of nighttime wheel access. This procedure was continued for 24 days. Rats given 1 hour of access showed a small, but significant increase over the 24 days while those given 4 hours showed a marked escalation in running. The increase in running for the 4 hour group may have been merely because rats had more time to run. When given the opportunity to run for 1 hour vs. 4 hours it is not surprising that rats would run more if given a longer duration. To explore this issue, Eikelboom and Lattanzio (2003) (and drug SA studies) have looked at the access time that was common to both groups, in this case the first hour. It was found that when comparing the first hour of wheel access, running of the first hour in the 4 hour group increased more than that in the 1 hour group. This difference became evident after 16 days of wheel access (Eikelboom & Lattanzio, 2003). Eikelboom and Lattanzio (2003) suggested that the dramatic increase seen with long wheel running duration (4 hours) and stable running with short wheel running duration (1 hour) may parallel similar differences seen with short and long drug self-administration.

Research Question

This thesis extends the work of Eikelboom and Lattanzio (2003) by examining more closely running escalation as a function of nightly access duration, but the present study also considers the individual differences in running. This thesis was designed to answer a number of questions. First, what is the minimum duration of time needed for an increase to occur in wheel running? Does time of wheel introduction affect running escalation? What is the role of individual differences in running escalation? In other words, can the escalation in running be predicted from initial running levels?

Experiment 1

Experiment 1 was designed to examine the effect of duration of nightly wheel access and time of wheel introduction on running escalation. Rats received different durations of wheel access but the time wheels became available was also manipulated.

Methods

Subjects

32 male Sprague-Dawley rats (200-225 g, 47-50 days upon arrival) were obtained from Charles River Canada, housed in individual cages (48 X 27 X 20 cm) and kept on a 12:12 dark: light cycle. Lights went out at 12:00 and came on at 24:00. The temperature in the animal holding room was 21 ± 1 or 2 ◦C. Rats were weighted daily at 10:00, the animal room was cleaned by the staff between 8:30 and 9:00, with cages and bedding changed once a week. All procedures for this and following experiments were approved by the Wilfrid Laurier University Animal Care Committee, which follows the policies and guidelines of the Canadian Council of Animal Care.

Apparatus

Rats were housed in polycarbonate shoebox cages equipped with Nalgene™ running wheels (11 cm wide, 30 cm diameter). The number of wheel turns was recorded by Vital View™ computer software (Mini Miter Corp) at one second resolution. Binder clips were used to lock wheels when rats were not permitted to run.

Procedure

Upon arrival, rats were given a one week habituation to the facility. They were then given an initial 1 day (24 hours) of *ad lib* wheel access (from 12:00 to 12:00 the next day) to familiarize them to the wheel. Wheels were locked at 12:00 the next day. Restricted wheel

access started two days later at 13:00 1 hour after lights went out in the animal room. Rats were assigned to groups based on the running distance for the previous 24 hour access (Although rats were allocated to groups equally, they were not allocated to the early and late condition equally. This issue is discussed more in the results and discussion sections). Each day of the experiment 8 rats (Group 90) were given 90 minutes of wheel access from 13:00 to 14:30. Eight rats were given 60 minutes of wheel access; 4 of these rats received wheel access for 60 minutes starting at 13:00 to 14:00 (Group 60A) and 4 received wheel access from 13:30 to 14:30 (Group 60B). Eight of the rats were given 45 minutes of wheel access; 4 rats from 13:00 to 13:45 (Group 45A), and 4 from 13:45 to 14:30 (Group 45B). The last eight rats received 30 minutes of wheel access; 4 from 13:00 to 13:30 (Group 30A), and 4 from 14:00 to 14:30 (Group 30B). Thus for the 60, 45 and 30 minute groups half the rats started at the same time as the 90 minute group (at 13:00, A conditions), and half of the rats finished at the same time as the 90 minute group (14:30, B conditions). This procedure was continued for 24 days.

Results

Analysis of Initial 12 Hour Night Running

For the initial 24 hours of *ad lib* wheel access only the 12 hours of nighttime wheel data was analyzed. Rats run very little during the day (Eikelboom and Mills, 1988), so the daytime running data were not analyzed. Figure 1 shows the mean number of wheel turns for all groups in the initial 12 hour night before rats were given restricted access. A 4 group one-way ANOVA was run for the initial night running to see if there were any prerestriction running differences. The groups did not differ significantly ($F < 1$).

Analysis of Absolute Wheel Turns

Figure 2 shows the absolute number wheel turns for the 24 days of restricted access for all groups. Absolute wheel turns were combined for the A and B condition, ignoring whether the rats started running at 13:00 or finished at 14:30. For analysis, daily running for the first and last 4 days of running was compared as two blocks for initial (first 4 days) and end (last 4 days) running, with groups (90, 60, 45 and 30 minute access) in a 4 (Day) X 2 (Block) X 4 (Group) mixed analysis of variance ANOVA. There was a main effect of Block $F(1,28) = 11.39, p < 0.05$, a Block X Group interaction $F(3,28) = 3.71, p < 0.05$ and Block X Day interaction $F(3, 84) = 4.22, p < 0.05$. Running increased over the 24 days of the experiment with groups increasing their running differently. To determine which group's running changed, separate ANOVA's were run using a 4 (Day) X 2 (Block) design for each group of animals. Results showed a main effect of Block $F(1,7) = 6.03, p < 0.05$ only for the 90 minute group. Rats in the 90 minute group increased their running over the 24 days of restricted access, while those in the 60, 45 and 30 minute groups were running similar amounts at the beginning and end of the experiment.

Analysis of First 30 Minutes of Wheel Access

The above results show absolute wheel turns for groups with differing access times. In order to equate the running across groups, analyses were run examining the first 30 minutes of restricted wheel access for each group, and running per minute.

Figure 3 shows running for the first 30 minutes of wheel introduction averaged for the first and last four days of restricted running. For the first 30 minutes, the individual mean of the first and last 4 days of 24 days of restricted access was treated as a block of early and late running with groups (90, 60, 45 and 30 minute) in a 2 (Block) X 4 (Group) mixed analysis of variance ANOVA. Results showed only a main effect of Block $F(1, 28) = 25.19, p < 0.01$. This

suggests that for the first 30 minutes of access running was the same in all groups and running increased equally in all groups from the first to the last block.

Analysis of Running per Minute

In order to calculate running per minute, each animal's absolute number of wheel turns was divided by the minutes of wheel access. Figure 4 shows running per minute for each group, again averaged over the first and last 4 days. The mean number of wheel turns per minute for the first and last 4 days of 24 days of restricted access was explored in a 2 (Block) X 4 (Group) mixed analysis of variance ANOVA. Results showed only a main effect of Block $F(1, 28) = 14.72$, $p < 0.05$. This suggests that running per minute was the same for all groups and showed a similar increase over the experiment.

Time of Wheel Introduction

The above analysis did not compare rats that were introduced to the wheel early (starting at 13:00, 1 hour after lights went out A Groups) versus later (finishing at 14:30, B Groups). Figure 5 A and B show rats that started running at 13:00 and those who ended at 14:30 respectively for groups 30, 45 and 60. The 90 minute group was not included as it did not have early and late sub-groups. Rats that were introduced to the wheel at 13:00 started running at 405 (±219) (Groups 30A, 45A, and 60A combined) wheel turns and increased to 654 (±311) wheel turns by day 24. Rats that finished running at 14:30 (Groups 30B, 45B, and 60B combined) started running at only 191 (±121) wheel turns on day 1 and increased their running to only 311 (±263) wheel turns by day 24. Thus, it appears there were differences in running between these two conditions with 'early' rats running more than 'later' rats.

A 3 (Group) X 2 (Condition) X 2 (Block) ANOVA was conducted to analyze this early and late data over the mean of the first and last four days of the experiment. Results showed the

expected main effect of Block $F(1, 18) = 8.88, p < 0.05$ but Condition was also significant $F(1,18) = 13.36, p < 0.05$. Rats that were given wheel access at 13:00 (1 hour after lights went out) (Figure 5A) ran more and increased their running more than rats given wheel access ending at 14:30.

To determine if the differences observed in the early and late running were due to pre-existing differences an analysis was carried out looking at group differences in the initial night of running, before the period of restricted access. Figure 6 shows running for rats in each group and condition for the initial 12 hour nighttime running. A 2 (Condition) X 3 (Group) ANOVA was carried out again excluding the 90 minute group. Results showed a main effect of condition $F(1, 23) = 21.11, p < 0.01$. Rats in the A condition were running more than those in the B condition even before restricted access.

Discussion

The aim of the first study was to determine at what access duration wheel running increases. It was found that rats given 90 minutes of access increased their running over 24 days while those with shorter durations did not. These results are consistent with Lattanzio and Eikelboom (2003) who showed that with long access (4 hours) running escalated over days while with 1 hour (short access) it did not.

When looking at the absolute number of wheel turns, rats with a longer duration (e.g, 90) have more time to run than rats in the 30 minute group. Thus, the escalation seen in the 90 minute group may represent only the fact that there is more time to run, while the rats in the 30 minute group are running the most they can given that time. A similar question has been considered in drug self-administration studies. To explore this issue both drug self-administration studies (e.g., Ahmed and Koob, 1998) and previous wheel running experiments

looking at duration (Eikelboom and Lattanzio, 2003) have looked at the time common to all rats, measuring the same running duration. Thus, for this experiment the first 30 minutes were explored. Another way to equalize the running data which has not been used before is to look at running per minute. This gives an index of the amount of running per rat and equalizes the groups so access time differences are corrected. In Experiment 1 no group differences in the first 30 minutes of wheel access or running per minute were found. Rats in all groups increased their running in the first 30 minutes and showed per minute increases, but the increase was equal across groups. These findings will be discussed more thoroughly in the general discussion.

Experiment 1 was also designed to control for differences in running that may have occurred because of time of wheel introduction. Rats introduced to the wheel at 13:00 (1 hour after lights went out) increased their running over days but rats that finished running at 14:30 ran less and did not increase their running nearly as much over days. Groups in the early condition had higher baseline running at the initial 12 hour night of wheel exposure than those in the later condition. This initial difference may have affected running over the entire restricted access period. An alternative possibility however is that time of wheel introduction may have made the wheel more or less 'salient'. Starting to run at 13:00 may have been more salient than starting later. It has been reported that differences in drug intake (Baird and Gauvin, 2000; Roberts, Brebner, Vincler and Lynch, 2002) and sensitization (Sleipnes, Sorg and Jansen, 2005) occur depending on time of drug access. Whether time of wheel introduction is an important factor in running was explored in Experiment 2.

Experiment 2

The more pronounced running observed in the A condition of the previous experiment was most likely due to preexisting baseline differences. However, it is also logical to think that

the time rats were given wheel exposure affected their running. Starting to run at 13:00 (1 hour after lights went out) may have been more salient than starting to run after 13:00. Experiment 2 was designed to address time of wheel introduction as a possibility for results obtained in the early and late conditions of Experiment 1. In Experiment 2 all rats were given 45 minutes of wheel access spread out through the first 6 hours of the night. A duration of 45 minutes was chosen because rats in the 45 minute A were running more than rats in 45 minute B, relative to other groups and their respective conditions.

Methods

Subjects

32 Sprague-Dawley rats (200-225 g, 47-50 days upon arrival) obtained from Charles River Canada were housed in individual cages and kept on a 12:12 dark: light cycle. Lights went out at 10:00 and came on at 22:00. Rats were weighted daily at 9:00. The animal room was cleaned by the staff between 8:30 and 9:00. All other conditions were similar to Experiment 1.

Apparatus

Same as Experiment 1

Procedure

The procedure for first initial night of *ad lib* was the same as experiment 1. Rats were then allocated to restricted groups based on their running in the 24 hour initial night of *ad lib access*. Eight rats were given 45 minutes of wheel access from 10:00 to 10:45 (when lights went off); 8 from 10:45 to 11:30; 8 from 11:30 to 12:15; 8 from 13:00 to 13:45 and lastly 8 from 16:00 to 16:45. Thus the groups started, 0, 45, 90, 180, and 360 minutes after lights went off. This procedure was followed for 15 days.

Results

Figure 7 shows running for the initial night of wheel exposure. A one way 5 group ANOVA was run for the initial night running. Groups did not differ in this pre-restricted phase ($F < 1$).

Figure 8 shows mean running for all five groups of rats over the 15 days of the experiment. The mean of the first day of running for all groups indicated that rats started running 115 (±164) wheel turns and increased their running to 212 (±299) wheel turns by day 15. The first, second and third 5 days of the experiment were divided into three blocks and analyzed in a 5 (Day) X 3 (Block) X 5 (Group) ANOVA. There were main effects of Day, $F(4, 140) = 7.78, p < 0.01$, and Block, $F(2, 70) = 5.50, p < 0.05$, but no interactions were significant. Groups introduced to the wheel at different times of the night but with equal duration increased their running slightly over the course of the experiment but the increase was the same for all groups.

Figure 8 suggests that rats introduced earlier to the wheel (10:10:45, 10:45-11:30) appeared to be running less than rats introduced to the wheel later (13:00-13:45, 16:00-16:45) on the last 5 days of wheel access. To see if these differences were significant, the last five days were averaged in a one way ANOVA with groups (time of wheel introduction) used as a between groups factor. Groups were not different ($F < 1$).

Discussion

Wheel running did not differ across groups as a function of time of introduction. All groups were running a similar distance over the 15 days of wheel access. Although running increased slightly, it increased equally in all groups. Thus, time of wheel introduction does not appear to be a factor in wheel running escalation (at least over the night) and this suggests the results Experiment 1 were due to initial animal running differences. In Experiment 2, rats were allocated to the groups based on their 12 hours of initial night running (Figure 7). In Experiment

1 the A and B conditions were initially different (a difference that was noticed after the study was finished). At this point the simplest explanation for the difference of the 'early' and 'late' condition in Experiment 1 may be individual differences evident prior to restricted access. Thus, Experiment 3 was designed to address a broader duration of wheel access periods with a single time of wheel introduction. Time of wheel introduction at night did not seem to affect running in the second experiment, so all animals were introduced to the wheel at the same time, one hour after lights went out in Experiment 3.

Experiment 3

Experiment 2 suggested that the differences in the earlier and later conditions observed in Experiment 1 was most likely due to preexisting baseline differences. The longest duration in Experiment 1 was the 90 minute group but this group ran less than 1000 wheel turns by the end of restricted access. This low level of running indicates that durations longer than 90 minutes may be needed to see a more pronounced increase in running. Thus, Experiment 3 was designed to include a duration longer than 90 minutes. In addition to looking at mean group running, individual animal running was also analyzed and was one of the reasons for the large number of rats for Experiment 3. Individual differences in drug self-administration has been suggested as an important factor in addiction (Piazza et al. 1989). Additionally, wheel running may show a strong phenotypic component (Afonso and Lattanzio, 2003). Lattanzio and Eikelboom (2003) only looked at mean running in their duration of access study so in this experiment individual animal correlations between the initial night, first four days, and last four days of running in all groups individually and combined were examined.

Methods

Subjects

Seventy-two Sprague-Dawley rats (200-225 g, 47-50 days upon arrival) ordered from Charles River Canada were housed in individual cages and kept on a 12:12 dark: light cycle. Lights went out at 12:00 and came on at 24:00. All other conditions were similar to Experiment 1.

Apparatus

Same as Experiment 1

Procedure

Experiment 3 was conducted in three replications because of resource limitations. The procedure for the initial night and restricted group designation was the same as that in Experiment 2. Then at 13:00 one day later rats were given either 30, 60, 90, or 180 minutes of wheel access (starting one hour after lights out). Replication one had 8 rats in the 30 minute group, 8 in the 90, and 8 in the 180 minute group. Replication two had 4 rats in the 30, 12 in the 60, 4 in the 90, and 4 in the 180 minute group. Replication three had 8 rats in the 30 minute group, 4 in the 60, 4 in the 90 and 8 in the 180 minute groups. In total, groups 30 and 180 minutes each had 20 rats, while groups 60 and 90 minutes had 16 rats each. This restricted phase of the experiment lasted for 24 days. On day 25 all rats were given 180 minutes of wheel access.

Results

In Experiment 3 an attempt was made to allocate rats to their groups based on the initial 12 hours night of access. Since Experiment 3 was conducted in three replications this was difficult to do systematically. Given the importance of initial running on the results in Experiment 1 the first 4 days running (wheel turns per minute) for this third experiment were analyzed more closely. This was done to make sure that any initial group differences were minimized. Figure 9 shows the group means for the four groups with individual animal running

levels for these four days. Looking at the distribution of running levels in the groups it appears there were a disproportionate number of low runners in the 180 minute group. Using a 2.5 wheel turn per minute criteria for low running, 9 rats in the 180 minute group, 2 rats in the 90 minute group, 0 in the 60 minute group and 1 in the 30 minute group fell below this cutoff. The attempt that was made to balance groups used the initial 12 hour nighttime access. This procedure may not be the ideal way to allocate rats since running in the initial 12 hour night may not be a good predictor of later running (Afonso and Eikelboom, 2003). This issue is explored more thoroughly in the discussion. Previous wheel running experiments have removed animals that do not run very much (Epling, Pierce, Amy and Boer, 1987; Holloszy, Smith, Vining, and Adams, 1985; Mondon, Dolkas, Sims and Reaven, 1985). Thus, in Experiment 3 two sets of analyses were conducted, one analysis including all rats, and one excluding rats in each group that ran less than 2.5 wheel turns per minute over the first 4 days.

Analysis of Initial 12 hour Nighttime Running

Figure 10A shows running for the initial 12 hours of nighttime access. A 4 group one way ANOVA did not reveal any initial running differences ($F < 1$). Figure 10B shows running for the initial 12 hours of nighttime access, with animals running less than 2.5 wheel turns/minute removed. The 4 group one way ANOVA did not reveal any initial running differences ($p > 0.05$).

Analysis of Absolute Wheel Turns

Figure 11A shows running for all groups for the 24 days of restricted access. The first 4 days and last 4 days of absolute wheel running were analyzed in a 2 (Block) X 4 (Day) X 4 (Group) ANOVA. Results showed main effects of Day $F(3, 204) = 10.46$, $p < 0.01$, Block $F(1, 68) = 33.78$, $p < 0.01$, and Group $F(3, 68) = 6.86$, $p < 0.01$, and a Group X Block interaction $F(3,$

68) = 5.74, $p < 0.05$. The Block effect and Group X Block interaction suggest running changed over the course of restricted access, with groups increasing their running by different amounts.

To determine if the groups had changed their absolute levels of running over the restricted phase, separate ANOVAs were carried out for each group. A 4 (Day) X 2 (Block) ANOVA revealed a main effect of Block for the 30 $F(1, 19) = 9.50$, $p < 0.05$, 60 $F(1, 15) = 20.00$, $p < 0.01$, 90 $F(1, 15) = 18.12$, $p < 0.01$ and 180 $F(1, 19) = 12.48$, $p < 0.05$ minute groups; and a Day effect for the 60 $F(3, 45) = 5.94$, $p < 0.05$ minute group. All groups increased their absolute level of running over the 24 days but it must be pointed out that for the 30 minute group it was only increased from 223 (±27) to 343 (±51) wheel turns. Thus, although significant this increase was relatively small.

Figure 11B shows running for all groups for the 24 days of restricted access, with low running animals removed. The first 4 days and last 4 days of absolute wheel running were analyzed in a 2 (Block) X 4 (Day) X 4 (Group) ANOVA. Results showed main effects of Day $F(3, 168) = 11.89$, $p < 0.01$, Block $F(1, 56) = 79.48$, $p < 0.01$, and Group $F(3, 56) = 22.84$, $p < 0.01$, and significant Group X Block interaction $F(3, 56) = 16.87$, $p < 0.01$, and Day X Group interactions $F(9, 168) = 2.71$, $p < 0.05$. The Block main effect and Group X Block interaction suggests running changed over the course of restricted access, with groups increasing their running by different amounts.

To determine how the groups had changed their absolute levels of running over the restricted phase, separate ANOVAs were carried out for each group. A 4 (Day) X 2 (Block) ANOVA revealed a main effect of Block for the 30 $F(1, 18) = 9.44$, $p < 0.05$, 60 $F(1, 16) = 20.00$, $p < 0.01$, 90 $F(1, 13) = 34.12$, $p < 0.01$ and 180 $F(1, 10) = 18.07$, $p < 0.01$ minute

groups; and a Day effect for the 60 $F(3, 45) = 5.94, p < 0.01$ and 180 $F(3, 30) = 3.91, p < 0.01$ minute groups. All groups increased their absolute level of running over the 24 days.

Analysis of First 30 Minutes of Wheel Access

Figure 12A shows the first 30 minutes of access for each group averaged over the first and last four days of restricted access. The first and last 4 days of restricted access and groups (30, 60, 90, and 180 minute) were analyzed in a 2 (Block) X 4 (Group) ANOVA. Results showed only main a effect of Block $F(1, 68) = 51.00, p < 0.01$. Running in the first 30 minutes increased for all groups but this increase did not differ for the various access conditions.

Figure 12B shows the first 30 minutes of access for each group averaged over the first and last 4 days of restricted access with low running animals removed. The first and last 4 days of restricted access and groups (30, 60, 90, and 180 minute) were analyzed in a 2 (Block) X 4 (Group) ANOVA. Results showed only a main effect of Block $F(1, 56) = 56.56, p < 0.01$. Running in the first 30 minutes increased for all groups.

The first 30 minutes was chosen for analysis because it was the time duration common to all groups. However, the first 30 minutes may not be the ideal time window to equalize the data since all groups may view this period of time as most salient and thus run a larger distance. An analysis was also conducted on the 1st and 2nd 15 minutes of the first 30 minutes. The mean of the first and last 4 days of the first 15 minutes of wheel access with groups (30, 60, 90, and 180) were analyzed in a 2 (Block) X 4 (Group) ANOVA. Results only showed an effect of Block $F(1, 68) = 18.87, p < 0.01$. On average all groups increased their running over the first 15 minutes of wheel access. The same analysis for the 2nd 15 minutes of wheel access resulted only in a similar Block effect $F(1, 68) = 53.29, p < 0.01$.

Low running animals were removed and the same analyses were conducted for the first and second 15 minutes of wheel access. Results showed an effect of Block for the first 15 minutes $F(1, 56) = 19.49, p < 0.01$, but no interaction. For the second 15 minutes only the Block effect was significant $F(1, 56) = 59.30, p < 0.01$.

Analysis of Running per Minute

Figure 13A shows mean running per minute for the first and last 4 days of restricted access. For analysis, the mean running per minute for the first and last 4 days of 24 days of restricted access and groups (30, 60, 90, and 180) was analyzed as a 2 (Block) X 4 (Group) ANOVA. Results showed a main effect of Block $F(1, 68) = 61.81, p < 0.01$. No effect of group was evident in this analysis. Running per minute had changed over the course of the 24 days of restricted access, but equally for all groups.

Figure 13B shows mean running per minute for the first and last 4 days of restricted access with low running animals removed. For analysis, the mean running per minute for the first and last 4 days of 24 days of restricted access and groups (30, 60, 90, and 180) was analyzed as a 2 (Block) X 4 (Group) ANOVA. Results showed a main effect of Block $F(1, 56) = 83.49, p < 0.01$ and Block X Group interaction $F(3, 56) = 3.82, p < 0.01$. Running over the 24 days increased for all groups with the 180 minute group showing a higher increase than the 30, 60 and 90 minute groups.

Analysis for the First 3 Hours of Unrestricted Access

Figure 14A shows mean running per group when all rats were given 3 hours of wheel access. A 4 group one way ANOVA did not reveal any running differences in the first 3 hours of unrestricted wheel access ($F > 1$).

Figure 14B shows mean running per group when all rats were given 3 hours of wheel access. Low running animals were removed. A 4 group one way ANOVA revealed a marginal significant effect of block $F(3, 56) = 2.67, p = 0.056$, with a follow up Tukey post-hoc revealing a significant difference only between rats in the 30 and 180 minute groups ($p < 0.05$).

Analysis of Individual Animal Data

Piazza et al. (1998) address individual vulnerability to amphetamine self-administration by dividing their animals using a median split. That is one way to split animals into dichotomous categories. However, another way to address the same issue of high and low responding, or high and low wheel running is to run correlational analysis between the initial running levels (when animals are naïve to the wheel) with final running levels. High correlations in this case would suggest that a low runner (low responder in drug studies) remains a low runner throughout the experiment and vise versa with high runners (high responders in drug studies). A low correlation would suggest that low or high runners at the beginning of the experiment are not necessarily low or high runner at the end of wheel access.

Figure 15A shows the scatter plot comparing the mean of the first 4 and last 4 days of running per minute. The sloped line on the right indicates no change in running over the experiment; rats below the line decreased their running, while those above the line increased their running over the experiment. Rats with values on the line did not change. The left line shows a doubling in running. Rats on or above that line have doubled their running over the experiment. Six rats in the 30 minute group (30 %), 11 in the 60 minute group (69 %), 12 in the 90 minute group (75 %), and 9 in the 180 minute group (45 %) doubled their running by the end of restricted access. A chi-square test was conducted to assess whether doubling was more frequent in the higher duration groups. The results were significant $\chi^2 (3, N = 72) = 9.46, p$

<0.05. Rats were more likely to double their running by the end of restricted access in the higher duration groups.

Figure 15B shows the scatter plot comparing the mean of the first 4 and last 4 days of running per minute with low running animals removed. The sloped line on the right indicates no change in running over the experiment; rats below the line decreased their running, while those above the line increased their running over the experiment. Rats with values on the line did not change. The left line shows a doubling in running. Rats on or above that line have doubled their running over the experiment. Five rats doubled their running in the 30 (26 %) minute group, 8 rats in the 60 (50 %), 11 rats in the 90 (78 %) and 8 rats in the 180 minute group (72 %). Thus with longer duration of access more rats doubled their running level. A chi-square test was conducted to assess whether doubling was more frequent in the higher duration groups. The results were significant χ^2 (3, $N = 72$) = 10.89, p <0.05. Rats were more likely to double their running by the end of restricted access in the higher duration groups.

Table 1A shows correlations between the initial night, the mean of the first 4 days and last 4 days of running for the four groups (30, 60, 90, 180) in Experiment 3 using all animals in the study. For these correlations absolute wheel turns were used. Running in the first 30 minutes and running per minute for all groups combined was also analyzed using the same three variables (initial night, mean of first, and last 4 days). For all groups except the 60, initial night's running predicted the first 4 days of night's running, the initial night also predicted the last four days for the 90 and 180 minute groups. The first 4 days predicted the last 4 days in all groups. The same correlations were conducted for groups combined (using the first 30 minutes and running per minute) and all correlations were found to be significant. When considering the overall data, variance in the first 30 minutes running mean over night one to four accounted for

over 50% of the variance in final running over the same 30 minutes for days 21 to 24. Looking at the per minute running levels, it was found that about 36% of the final variance in running level was due to initial days 1 to 4 of running.

To determine how the mean of the first 4 days per minute and duration of nightly wheel access combined predicted final running levels, a multiple regression analysis was conducted. The predictors for the design were duration of wheel access in minutes (30, 60, 90, and 180) and the mean running per minute for the first 4 days of running per minute. The criterion variable was the mean for the last 4 days. The linear combination of the first 4 days and duration was significantly related to final running levels, $F(2, 69) = 32.80$, $p < 0.01$. The sample multiple regression coefficient was 0.70 indicating that approximately 49 percent of the variance in final wheel running can be accounted for by the linear combination of first 4 days and group (duration). This information led to the following equation:

Final Running (per minute) = -3.79 + 2.18i + 0.048d

(Where i is the mean wheel turns per minute for first 4 days, and d is duration of access in minutes)

Table 2A presents the indices to indicate the relative strength of the individual predictors. All the bivariate correlations with the strength variables and last 4 days of running were positive, and both predictors were significant ($p < 0.01$). Both partial correlations were significant ($p < 0.01$). The first 4 days accounted for 41 % (0.64** = 0.41) of the variance in final running while duration accounted for 8% (49% - 41%) of the variance in final running. The standardized betas also suggest that the first 4 days have a stronger association with final running than does the duration of access.

Table 1B shows correlations for the initial night, the mean of the first 4 days and last 4 days of running for the four groups (30, 60, 90, 180) in Experiment 3 with low running animals

removed. For all groups except the 60, initial night's running predicted the first 4 days of running. The initial night of running did not predict the final 4 days for any individual group. The first 4 days was correlated with the last 4 days for all groups. The same correlations were conducted for groups combined (using the first 30 minutes and running per minute) and all correlations were significant, except the initial night did not predict the last four days first 30 minutes running. When considering the overall data, variance in the first 30 minutes running averaged over night one to four accounted for 50% of the variance in final running over the same 30 minutes for days 21 to 24. Looking at the per minute running levels, it was found that 27 % of the final variance in running levels was explained by initial days 1 to 4 running.

A multiple regression was also conducted with the low running animals removed. The predictors for the design were duration of wheel access in minutes (30, 60, 90, and 180) and the mean running per minute for the first 4 days of running. The criterion variable was the mean running per minute for the last 4 days. The linear combination of the first 4 days and group was significantly related to final running levels, $F(2, 56) = 22.8$, $p < 0.01$. The sample multiple regression coefficient was 0.67 indicating that approximately 45 percent of the variance in final wheel running can be accounted for by the linear combination of first 4 days and group (duration). This information led to the following equation:

Final Running (per minute) = -3.09 + 0.65i + 0.44d

(Where i is the mean wheel turns per minute of the first four days, and d is duration of access in minutes)

Table 2B presents the indices to indicate the relative strength of the individual predictors with low running animals removed. All the bivariate correlations were positive, and both predictors were significant ($p < 0.01$). Both correlations were significant ($p < 0.01$). The first 4

days accounted for 27 % (0.52** = 0.27) of the variance in final running while duration accounted for 17 % (44% - 27%) of the variance in final running.

Discussion

Experiment 3 manipulated only access duration. Time of wheel introduction was held constant while duration of availability varied from 30 to 180 minutes. Since there was a disproportionate number of low running animals in the 180 minute group two sets of analyses were conducted; one including all rats and one excluding rats that ran less than 2.5 wheel turns/minute over the initial 4 days. For the three experiments, rats were allocated to their restricted group membership based on the initial night of 12 hour running. This may not have been the best way to allocate rats since the best correlations for the final wheel running levels seemed to be the first 4 days. It has also been shown that when rats are given *ad lib* wheel access, day 2 and 3 are good predictors of final running while day 1 of *ad lib* is not (Afonso & Eikelboom, 2003).

In this experiment groups showed similar initial 12 hour running levels before restricted access (Figure 10A and 10B). The low runners did not change significantly the initial 12 hour running levels.

When looking at the absolute number of wheel turns all groups increased their running over the 24 days of restricted access (Figure 11A and 11B). However, with low running animals removed, the increase in the 180 minute group was much more pronounced (Figure 11B) than that seen in other groups.

Similar to the analysis used in Experiment 1, absolute wheel turns for the first 30 minutes (Figure 12A and 12 B) and running per minute (Figure 13A and 13B) were analyzed. When looking at the first 30 minutes of access, all groups increased their running equally, even if low

running animals were removed (Figure 12B). It is not clear at this point why there were no differential increases in running within this time window. Eikelboom and Lattanzio (2003) observed at the end of their initial access experiment that rats in their 4 hour group were running more in the first hour than rats having only 1 hour to run. Since the 30 minutes chosen for analysis may not have been the ideal time window, analysis were also carried out separating for the initial first and second 15 minutes of the of the initial 30 minutes of access but again groups increase their running equally. This issue will be discussed more fully in the general discussion.

Another way to correct for duration differences in absolute wheel running was to look at running per minute. This analysis provides an index of mean running per minute equated for all groups. When all rats were included in the analysis for per minute running, it appeared that all groups increased their running equally. However, when low running animals were removed from the analysis the 180 group showed a much more pronounced increase in running. This result is consistent with patterns seen drug self-administration (Ahmed and Koob, 1989) and wheel running studies (Eikelboom and Lattanzio, 2003).

A final way to compare running in the groups with different durations is to give all rats 3 hours of unrestricted wheel access to determine if the rats in the lowest group (30 minutes) increase their running when given an acute opportunity to run for a longer duration. When animals were given 3 hours of access, running was not different between groups. However, when low running animals were removed, the 180 minute group ran more than the 30 minute group. This again suggests that access duration has an impact on final running.

Correlations conducted for individual and combined groups revealed strong relationships between initial 4 days and final running levels. This finding may parallel the Hi and Lo responder phenotype difference in cocaine studies (e.g., Piazza , 2000). A regression using both

the animal's initial first 4 days of running and their duration of access combined was used to predict final running levels. This analysis yielded a significant effect of both initial running and access duration. Initial running however, was a stronger predictor of final running than access duration. When low running animals were removed duration became a stronger predictor of final running levels, and the first 4 days became weaker predictor. With the low running animals removed, a higher percentage of rats in the 180 minute group showed a doubling in running by the end of 24 days. Thus, with low running animals removed, the results of the third experiment are more consistent with drug self-administration studies (Ahmed and Koob, 1998).

Removing low running rats from the third Experiment had a number of implications for the results. First, absolute wheel turns for the 180 minute showed a more pronounced increase (Figure 11B). Second, running per minute increased more for the 180 minute group relative to other groups (Figure 13B). Third running in the 3 hours of unrestricted access was higher for the 180 minute group relative to the 30 minute group. Finally, duration accounted for more variance in final running levels. When low running animals are removed from Experiment 3, the results were more consistent with drug self-administration studies. Thus, in the general discussion results for the third experiment will focus on those results obtained with low running animals removed.

General Discussion

It is not clear why over days rats increase their running distance to high levels from initially low levels (Sherwin, 1998). The transition from low to high wheel running levels may elucidate general mechanisms involved in the transition from low to high and possibly excessive levels of behaviour, such as occurs in drug addiction. Two factors involved in the running escalation were explored in this thesis: duration of access, and initial running level. As access

duration increased from 30 to 180 minutes per night, absolute wheel turns increased over days. With short duration (30 minutes) there was a small, yet significant, increase while with longer durations running showed a more pronounced increase. The lowest final absolute level of running was observed in the 30 minute group and the highest in the 180 minute group. These results are consistent with Eikelboom and Lattanzio (2003). They gave rats either 1 or 4 hours of nighttime wheel access for 24 days. Running in their 1 hour group showed a small increase, but with 4 hours access the increase was much more pronounced. These results are also consistent with the pattern seen in numerous drug self-administration studies (e.g., Ahmed, 2005; Ahmed and Koob, 1998).

In order to equalize the absolute wheel running data given the different access duration, the first 30 minutes of running data and running per minute were analyzed. In both Experiments 1 and 3 the running increase in the first 30 minutes was equal for all access groups. These results are not consistent with previous work. Eikelboom and Lattanzio (2003) observed differences in running for the first hour of access; rats with daily 4 hours of total wheel access came to run more within the first hour than rats with only 1 hour access. Cocaine self-administration studies have observed similar findings (Ahmed and Koob, 1998). Self-administration rates are higher over the first hour in long (6 hours) compared to short (1 hour) access conditions. In Experiment 3 even looking at the first and second 15 minutes of the first 30 minutes did not reveal any group differences. It is not clear why the results of this thesis showed no access duration differences for the first 30 minutes of wheel access. One obvious reason may be that the longest access group, 3 hours, may not have been long enough for rats to show an increase in running over the first 30 minutes. Running escalation has been observed with 2 hours of nighttime access, however in this experiment the first period of access was not examined (Eikelboom and

Lattanzio, 2003). Durations longer than 3 hours may be needed to see changes over the initial period. An alternative possibility is that the first 30 minutes of access may not be the ideal time-window on which to equalize the data. It may be that the first hour is a better index because differences emerge after the first half hour. The initial 30 minutes might give a biased account because rats in all groups use this period as a 'wheel loading' phase similar to the 'drug loading' seen in self-administration since the wheel may be very salient at initial introduction. After a 30 minute loading period, differences between 'non-addicted' (short duration) and 'addicted' (long duration) groups could be present, as 'addicted' rats might continue to run at higher levels than 'non-addicted' ones longer into the session. In this thesis however looking at the first hour would exclude rats with 30 minutes of access. Looking at the first hour of access would be helpful in equalizing rats in the 60, 90 and 180 minute groups. Comparing the first hour running for the 60 and 180 minute groups in Experiment 3, however, did not reveal any group differences in the first and last four days of restricted access, suggesting this explanation is unlikely.

A second way to compare groups was to convert absolute wheel turns to wheel turns per minute. For both Experiments 1 and 3 running per minute increased over days, but in Experiment 3 this escalation was higher for the 180 minute group. Eikelboom and Lattanzio (2003) and drug studies did not use a per minute measure, however the more pronounced increase in the 180 minute group's per minute count suggests that longer duration of access made these rats higher wheel runners even when duration differences were corrected.

The third way to explore running changes induced by access differences was to give all rats a long duration exposure after a period of restricted access. When given an equal opportunity to run, rats in the 30 minute group ran less than those in the 180 minute group,

suggesting that the low running levels of the lowest duration group were probably not due to a ceiling effect.

An alternative way to analyze running data was to study individual rat running. When initially given the opportunity to self-administer low doses of amphetamine only some rats develop a high response rate for the drug, while others do not or minimally respond for amphetamine (Piazza et al. 1989). These two groups of animals have been appropriately called Hi and Lo responders. The Hi responding rats may have a predisposition to addiction, whereas the Lo responders are reflective of individuals who are phenotypically less likely to become addicted. It has been previously reported that of the variables of feeding, drinking, body weight and initial wheel running, only initial wheel running predicts final levels of running (Afonso and Eikelboom, 2003).

For Experiment 3, correlations were used to determine if initial running levels were predictive of final levels in a limited access study. Differences in initial running might predict final running levels and reflect a predisposition to wheel run. Each group's initial night, first 4 days and last 4 days were correlated. In addition, for all groups combined individual rat running for the first 30 minutes and running per minute were used to determine the same correlations. The initial night of running may not be the best predictor of final running levels since rats must learn to associate wheel running with its consequences (Afonso & Eikelboom, 2003). Even so correlations between the initial night and last 4 days were significant for all groups except the 60 minute access rats. A better estimate of final running levels may be the first few days when rats have some limited experience with the wheel. Correlations between the first and last 4 days of running yielded strong relationships for all groups. When the same correlations were conducted

for all groups combined using data for the first 30 minutes and the per minute running, the correlations were all significant.

A multiple regression analysis performed in Experiment 3 indicated that 27% of the variance in final running could be attributed to early running, and 17% attributed to duration of wheel access. Thus, escalation in wheel running is related to both the animal's initial running level or phenotype and duration of access or environmental condition. This notion is consistent with previous drug self-administration studies employing the self-administration paradigm to look at access duration (Ahmed, 2005) and phenotype studies using the Hi and Lo responder paradigm (Piazza et al. 1998).

The first experiment was designed to control for access time onset and completion differences that may also influence running. Rats were allowed to run beginning at 13:00 (1 hour after lights off) or to start running at a time to finish at 14:30. This procedure was not expected to influence results and was included as a design control. Unexpectedly, it was observed that rats that started running at 13:00 were running more and increased their distance over days relative to later access groups. Two possible reasons for the results were explored. First, time of wheel introduction may have an effect on running escalation. Lattanzio and Eikelboom (2003) have shown that given equal duration (2 hours) wheel access during the day or night results in no increase in the day group but a pronounced increase in the night group. Drugs of abuse differ in their effect depending on the time of exposure. Baird and Gauvin (2000) have found that cocaine self-administration is increased when rats were given access in the middle of the day or night relative to animals given access at other times during the day and night. Rats also show differing locomotor sensitization to cocaine as a function of time of administration (Sleipness, Sorg and Jansen, 2005). Separate groups of rats were given one cocaine injection per

day for 5 days during different times in the day and night. Fourteen days later they were challenged with a cocaine injection at the same time as before. Rats challenged in the beginning of dark onset showed a higher sensitization relative to other groups.

Secondly, in Experiment 1 there may have been preexisting running differences in rats that started at 13:00 (1 hour after lights out) and those that started later. Attempts were made in all studies to equalize groups based on running for the 12 hours of the initial night. Although, overall groups in the first experiment were equalized, it was found that running per A and B condition varied (A is onset at 13:00, B is later onset of wheel access). Animals in the A conditions all had significantly higher initial running than those in the B conditions. The disproportionate running levels observed between A and B groups was not intentional and probably represents random variance in assignment to the conditions. However, this preexisting difference may have been responsible for the differences in running level at the end of the experiment. Thus, the second experiment was designed to explicitly test the effect of time of wheel introduction.

In Experiment 2 rats were given wheel access for 45 minutes at different times over the night. No difference in the running pattern for the five groups were found. However, it may be that longer durations of wheel access (more than 45 minutes) over the 12 hours of nighttime are needed to see if time of wheel introduction influences escalation of running. In light of the results from Experiment 2, the simplest explanation for the pattern of running observed in the first experiment is that it was probably due to pre- existing running differences.

In all three experiments rats were allocated to their groups based on the initial 12 hour nighttime running. This may not have been the ideal way to assign rats since in Experiment 3 since a disproportionate number of rats in the 180 minute group turned out to be very low

runners. The mean of the first 4 days of restricted access was a strong predictor of the last 4 days in Experiment 3. Future studies may decide on giving animals 2 or 3 days of *ad lib* wheel access, before a restriction period starts. A problem with this procedure however, is that giving animals unrestricted access for a number of days may washout initial effects, since rats would not be naïve to the wheel. It might also predispose rats to show increases even with shorter access periods. An alternative approach is to initially give all rats a few days of short access before varying access duration. Ideally, it would be advantageous to have some other source of behavioural task predict which rats will become high or low runners before they are given wheel access. Recently work from Robbins and co workers (Belin, Dalley, Robbins, and Everitt, 2008; Dalley et al., 2007) have shown that high impulsive rats self-administer more cocaine and have lower D2 like receptor numbers in their nucleus accumbens. These so called "high impulsive' rats also show the three behavioural signs of drug abuse as described by the core *DSM-IV* criteria. As of yet, no one has examined the role of impulsivity and wheel running. It would be interesting to determine if high impulsive rats are also high runners.

Future experiments should explore a period of extinction following varying degrees of escalation precipitated by different durations of wheel access. Ahmed and Koob's (1998) cocaine self-administration study found that after 35 days of extinction short access animals maintain their low level of cocaine self-administration, while long access animals, despite a temporary decrease increased their self-administration even more than before the extinction period. The same may be true for wheel running. After 30 days of *ad lib* wheel access, if the wheels are reintroduced after a period of 10 day break, running, like cocaine self-administration, is initially low but escalates to levels evident before the 10 day break (Looy and Eikelboom,

1988). It is not clear, at this point, whether having previous short or long duration wheel access would influence running after a break.

A common use of the self-administration paradigm has been to look at causes of relapse (Shaham, Shalev, Lu, De Wit and Stewart, 2003). In such studies, after animals have learned to self-administer they are typically given a period of extinction. Following this extinction a number of different classes of stimuli can precipitate reinstatement of drug seeking. These stimuli include a single non-contingent drug exposure known as a 'challenge', exposure to an environment which has been associated with previous drug consumption, and stress, usually delivered by foot shock. It has been known that each of these classes of stimuli can precipitate reinstatement of drug seeking after a period of extinction (Epestein, Preston, Stewart and Shaham, 2006). Rats that have escalated their wheel running may also be susceptible to reinstatement of running precipitated by some of the same three classes of reinstatement procedures.

A number of advantages potentially make wheel running a good tool to use in this context. First, it is an inexpensive form of behaviour that can be adapted for by laboratory animals. As such, it does not require any type of invasive surgery or expensive drugs. Animals readily learn to run in wheels with minimal training and as such the complication induced by learning for these motivated behaviours is greatly reduced. Wheel running can be conducted without any drugs being administered and results are not confounded by peripheral side effects of the drugs. Thus, it becomes easier to separate motivational from other drug effects. Third, there appears to be a human analog to the behavior, namely excessive exercise. Although, it is not clear at this point if the underlying mechanisms of excessive wheel running are shared by human pathological exercisers, the behaviour seems similar on the surface. Lastly, wheel

running introduces a novel entry point into studies of motivation. Work looking at commonalities and differences between wheel running and self-administration may result in a better understanding of motivated behaviour that is not limited to drug use.

There are a number of disadvantages in using wheel running as a model of non-drug addictions. It is not clear what aspect of the wheel animals are regulating. A number of different variables could be measured such as distance, running speed, and work required to turn the wheel. In an effort to detangle distance from work, results from our lab have shown that rats probably use both distance and work to regulate running (Gheidi & Eikelboom, unpublished results). It is also possible to make wheel running contingent on lever pressing and look at variables such as the number of lever presses, post reinforcement pauses and progressive ratio schedules. However, studies making wheel running contingent on a response often require animals to have extensive free wheel access and as such cannot be used accurately to look at the transition from low naïve initial wheel access to high experienced running. Nor is it clear which aspect of wheel running is important from an addiction point of view. The current thesis dealt with the change from initial exposure to excessive consumption and how this change is influenced by the duration of access and individual vulnerability. Indeed, what may be a critical issue in wheel running is not the distance, speed, or work per se but rather how these variables change over time and the mechanisms responsible for the change. The increase in distance, speed, or work over a period of time may highlight changes occurring that are important in addiction.

Some clinicians argue that individuals who suffer from substance abuse, bulimia, pathological gambling or sexual addiction share a number of clinical features (Goodman, 2008). It has been reported that both duration of access (Ahmed, 2008) and a person's predisposition

(Piazza et al. 1998) are both involved in drug addiction development. The results from this thesis suggest that these two variables may be important in the development of a non-drug addiction and strengthen the arguments some clinicians make from a pre clinical level. As more similarities and differences are discovered between wheel running and drug self-administration, the relationship between drug and non-drug addictions may be better illuminated.

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Figure Captions

Table 1. Correlations between the initial night and first four days, initial night and last four days, and first four days and last for days.

Table 2. Correlations between the initial night and first four days, initial night and last four days, and first four days and last for days. Low running rats were removed.

Table 3. Individual predictors of the last four days of running per minute.

Table 4. Individual predictors of the last four days of running per minute. Low running rats were removed.

Figure 1. Mean (±SEM) absolute wheel turns for all groups during the 12 hours of initial night wheel running for Experiment 1.

Figure 2. Mean (±SEM) absolute wheel turns for all groups for Experiment 1. Day 15 data was not recorded because of computer problems; however rats still received their normal wheel exposure.

Figure 3. Mean (±SEM) wheel turns for the first 30 minutes of access for all groups for Experiment 1. Data represents the mean of the first and last four days of running in the first 30 minutes of wheel access.

Figure 4. Mean (±SEM) wheel turns for running per minute for all groups for Experiment 1. Data represents the mean of the first and last four days of running. Each groups absolute wheel turns was divided by the duration of time (minutes) they had to run.

Figure 5a & b. Mean (±SEM) absolute wheel turns for rats that started running at 13:00 (A condition) and those that ended running at 14:30 (B condition) for

Experiment 1. On day 15 data was not recorded because of computer problems; however rats still received their normal wheel exposure.

Figure 6. Mean (±SEM) absolute wheel turns for all groups and their respective conditions during the 12 hours of initial night running for Experiment 1.

Figure 7. Mean (±SEM) absolute wheel turns for all groups during the 12 hours of initial wheel running for Experiment 2.

Figure 8. Mean (±SEM) absolute wheel turns for all groups for Experiment 2.

Figure 9. Mean wheel running per minute and individual running data of the first 4 days for Experiment 3.

Figure 10A. Mean (±SEM) absolute wheel turns for all groups during the 12 hours of initial wheel running for Experiment 3.

Figure 10B. Mean (±SEM) absolute wheel turns for all groups during the 12 hours of initial wheel running for Experiment 3. Low running animals removed.

Figure 11A. Mean (±SEM) absolute wheel turns for all groups for Experiment 3.

Figure 11B. Mean (±SEM) absolute wheel turns for all groups for Experiment 3. Low running animals removed.

Figure 12A. Mean (±SEM) wheel turns for the first 30 minutes of access for all groups for Experiment 3. Data represents the mean of the first and last four days of running.

Figure 12B. Mean (±SEM) wheel turns for the first 30 minutes of access for all groups for Experiment 3. Data represents the mean of the first and last four days of running. Low running animals removed.

Figure 13A. Mean (±SEM) wheel turns for running per minute for all groups for Experiment 3. Data represents the mean of the first and last four days of running. Each groups

absolute wheel turns was dividing by the duration of time (minutes) they had to run.

Figure 13B. Mean (±SEM) wheel turns for running per minute for all groups for Experiment 3. Data represents the mean of the first and last four days of running. Each groups absolute wheel turns was dividing by the duration of time (minutes) they had to run. Low running animals removed.

Figure 14A. Mean (±SEM) wheel turns for the 3 hours of unrestricted access for Experiment 3.

Figure 14B. Mean (±SEM) wheel turns for the 3 hours of unrestricted access for Experiment 3. Low running animals removed.

Figure 15A. Correlations between the means of the first four days vs. the last four days of running per minute for Experiment 3. The line on the right indicates no change in running over the experiment, while the line on the left represents a doubling in running over the experiment.

Figure 15B. Correlations between the means of the first four days vs. the last four days of running per minute for Experiment 3. The line on the right indicates no change in running over the experiment, while the line on the left represents a doubling in running over the experiment. Low running animals removed.

Table 1
Correlations between the initial 12 hour nighttime access, the first 4 days and last 4 days

Duration	Initial night vs. first 4 days	Initial night vs. last 4 days	First 4 days vs. last 4 days
30 Minutes	0.86**	0.38	0.67**
60 Minutes	0.34	0.35	0.87**
90 Minutes	0.70*	0.61*	0.81**
180 Minutes	0.83**	0.59*	0.86**
First 30 Minutes	0.61**	0.30*	0.76**
Wheel turns per minute	0.59**	0.42**	0.60**

Pearson correlations between initial 12 hour nighttime and first four days of absolute wheel turns, initial and last four days of absolute wheel turns. Correlations for the first 30 minutes of access for the initial 12 hour nighttime, the first 4 days and last 4 days. Correlations for running per minute for the initial 12 hour nighttime, the first 4 days and last 4 days. *$p<0.05$, **$p<0.01$.

Table 2
Correlations between the initial 12 hour nighttime access, the first 4 days and last 4 days with low running animals removed

Duration	Initial night vs. first 4 days	Initial night vs. last 4 days	First 4 days vs. last 4 days
30 Minutes	0.85**	0.32	0.63**
60 Minutes	0.34	0.35	0.86**
90 Minutes	0.65*	0.50	0.76**
180 Minutes	0.72*	0.23	0.74**
First 30 Minutes	0.58**	0.20	0.70**
Wheel turns per minute	0.62**	0.32*	0.52**

Pearson correlations between initial 12 hour nighttime and first four days of absolute wheel turns, initial and last four days of absolute wheel turns. Correlations for the first 30 minutes of access for the initial 12 hour nighttime, the first 4 days and last 4 days. Correlations for running per minute for the initial 12 hour nighttime, the first 4 days and last 4 days. *$p<0.05$, **$p<0.01$.

Table 3
Indices to indicate relevant strength of the individual predictors

Predictors	Correlations between each predictor and last four days	Partial Correlations	Standardized Betas	Colinearity Tolerance	Colinearity VIF
Mean of First 4 Days Per Minute	0.64**	0.69	0.78	0.79	1.27
Duration (30, 60, 90 and 180 Minutes)	0.52**	0.27	0.31	0.79	1.27

Bivariate correlations between strength measure and the last four days of running. Partial correlations between strength measures and last four days. Tolerance and VIF values suggest there is no problem with colinearity. Tolerance is larger than 0.1, and VIF is less than 10
**$p<0.01$.

Table 4
Indices to indicate relevant strength of the individual predictors with low running animals removed

Predictors	Correlations between each predictor and last four days	Partial Correlations	Standardized Betas	Colinearity Tolerance	Colinearity VIF
Mean of First 4 Days Per Minute	0.52**	0.64	0.65	0.91	1.10
Duration (30, 60, 90 and 180 Minutes)	0.23**	0.49	0.44	0.91	1.10

Bivariate correlations between strength measure and the last four days of running. Partial correlations between strength measures and last four days. Tolerance and VIF values suggest there is no problem with colinearity. **$p<0.01$. Tolerance is larger than 0.1, and VIF is less than 10

Experiment 1

Figure 1

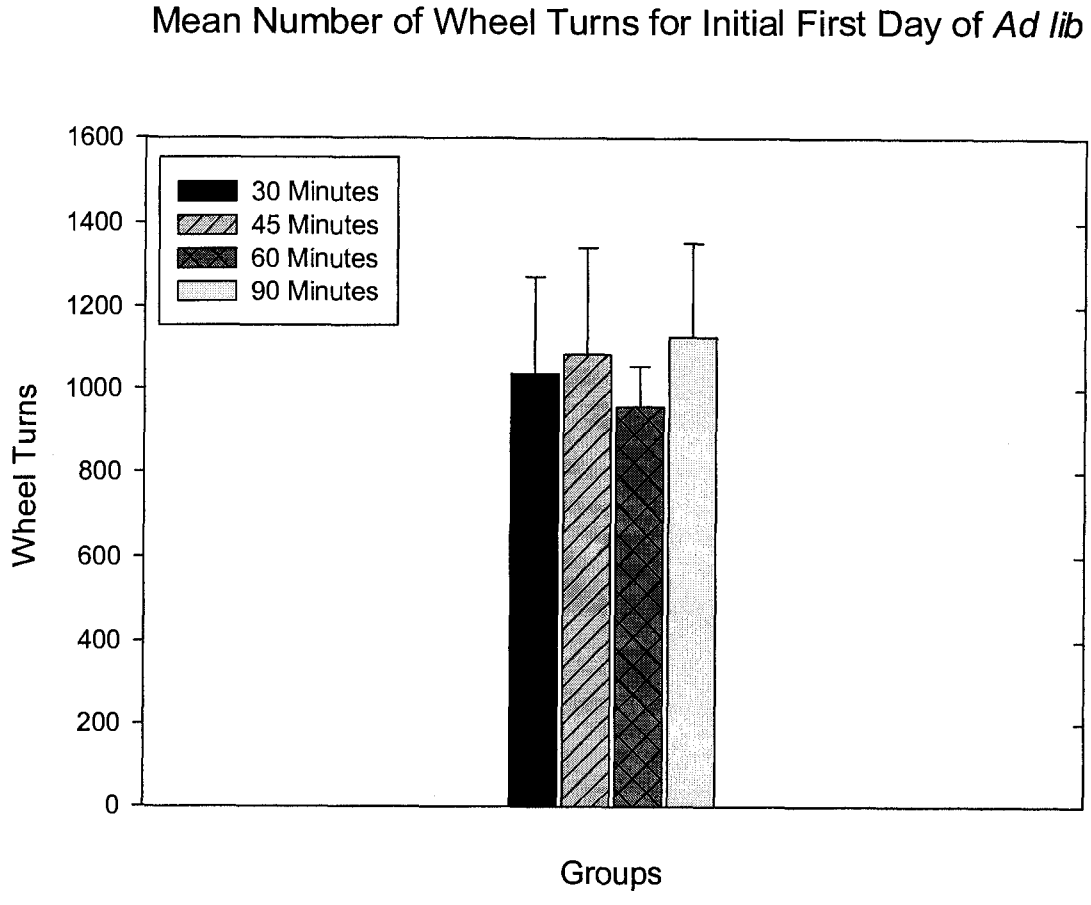


Figure 2

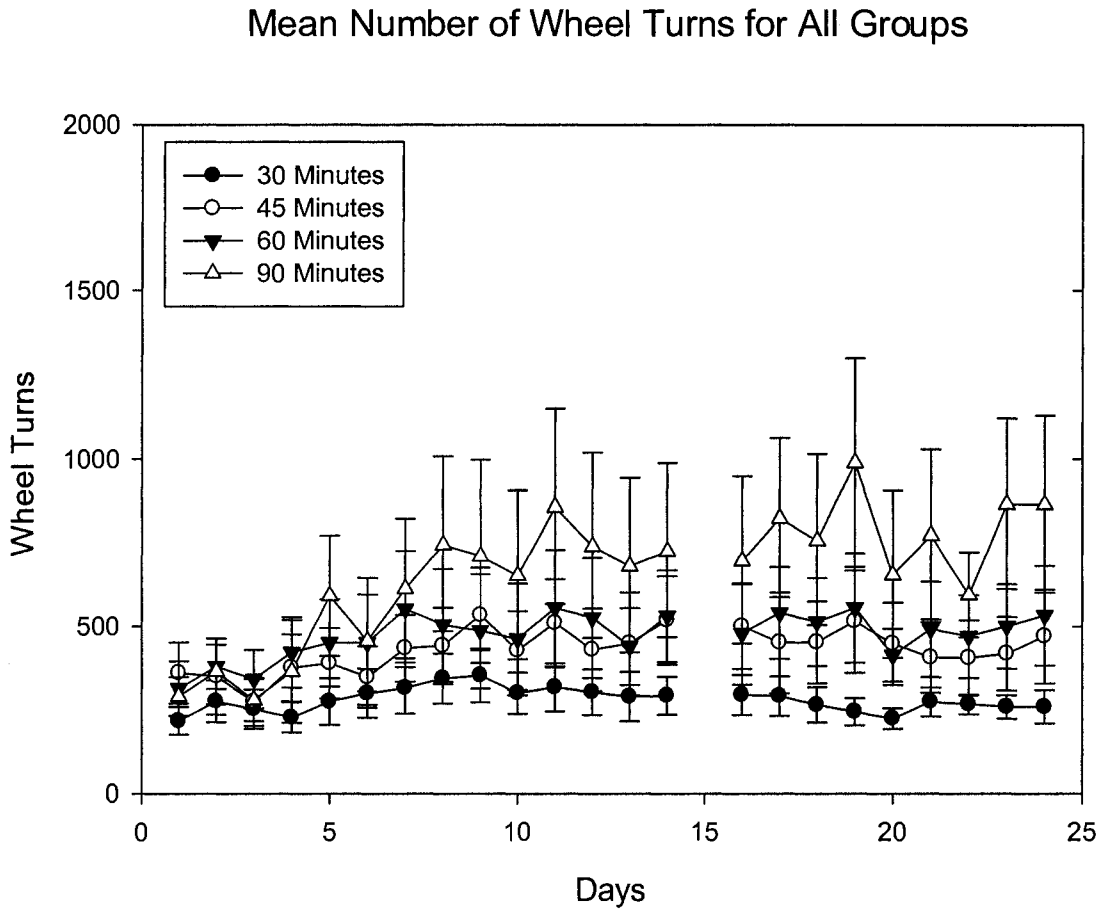

Mean Number of Wheel Turns for All Groups
30 Minutes
45 Minutes
60 Minutes
90 Minutes
Wheel Turns
2000
1500
1000
500
0
0
5
10
15
20
25
Days

Figure 3

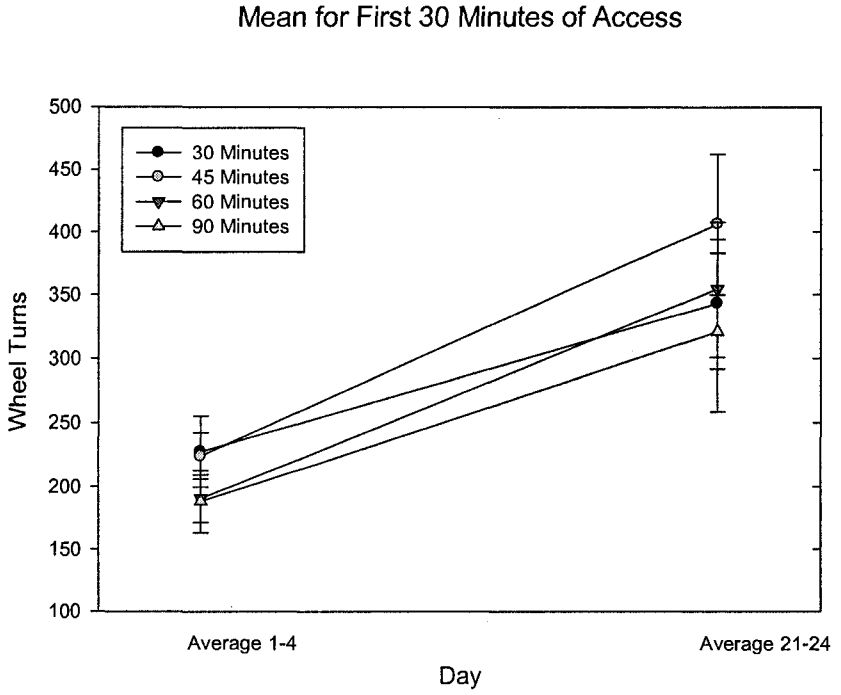

Mean for First 30 Minutes of Access
30 Minutes
45 Minutes
60 Minutes
90 Minutes
Wheel Turns
500
450
400
350
300
250
200
150
100
Average 1-4
Average 21-24
Day

Figure 4

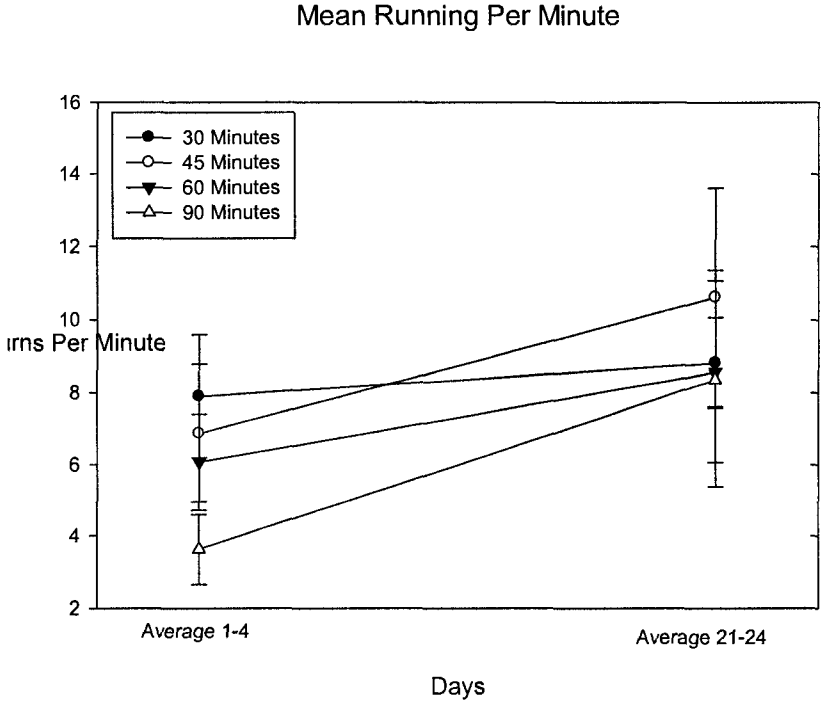

Mean Running Per Minute
30 Minutes
45 Minutes
60 Minutes
90 Minutes
16
14
12
10
8
6
4
2
ırns Per Minute
Average 1-4
Average 21-24
Days

Figure 5A

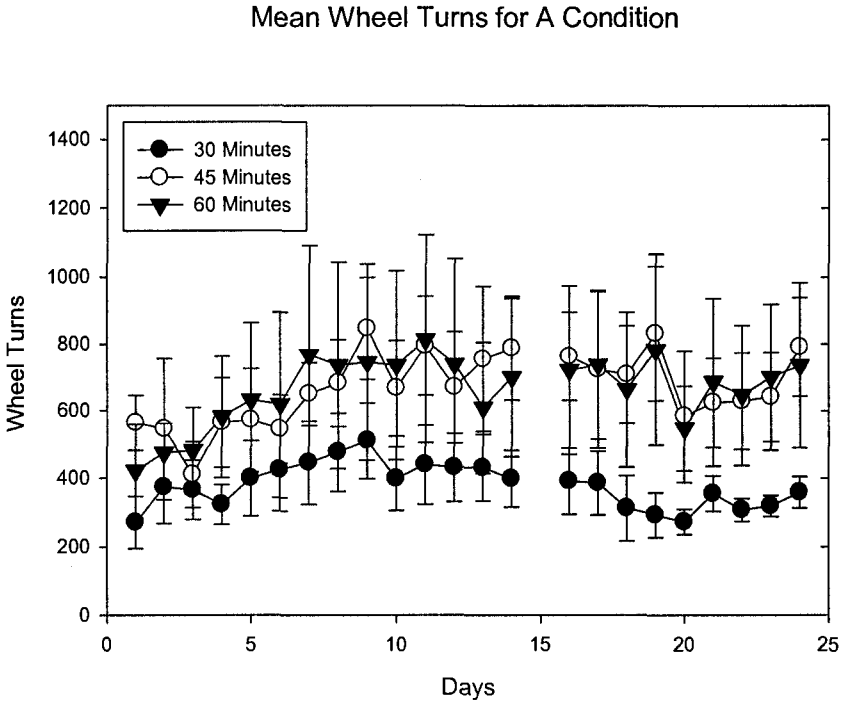


Figure 5B

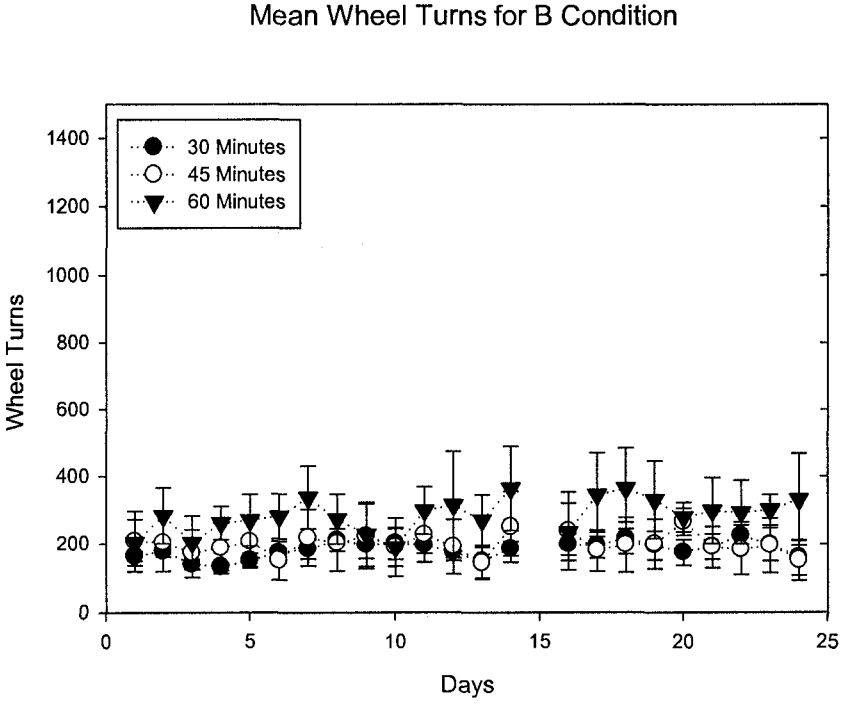
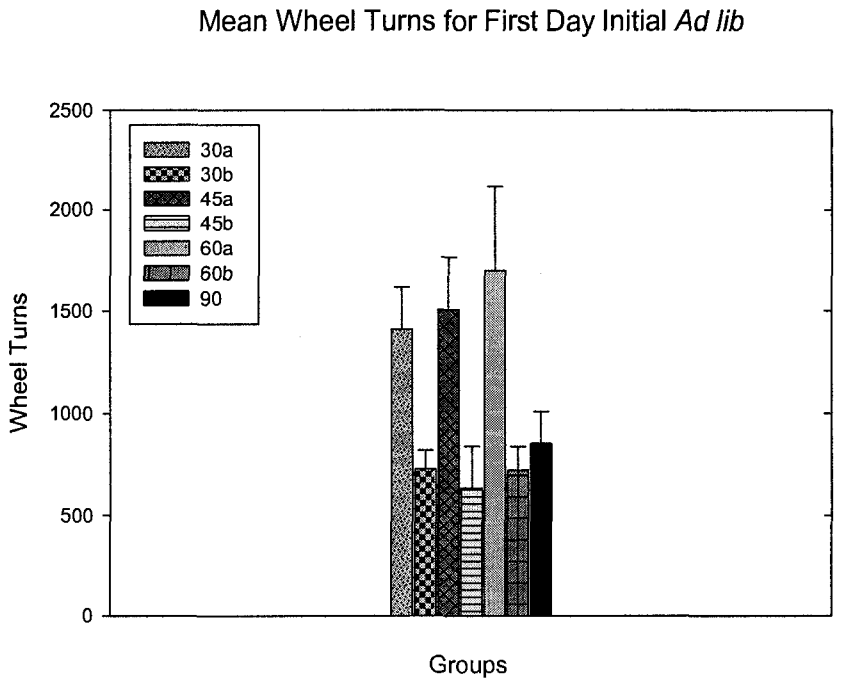


Figure 6


Mean Wheel Turns for First Day Initial *Ad lib*
2500
2000
1500
1000
500
0
Wheel Turns
30a
30b
45a
45b
60a
60b
90
Groups

Experiment 2

Figure 7

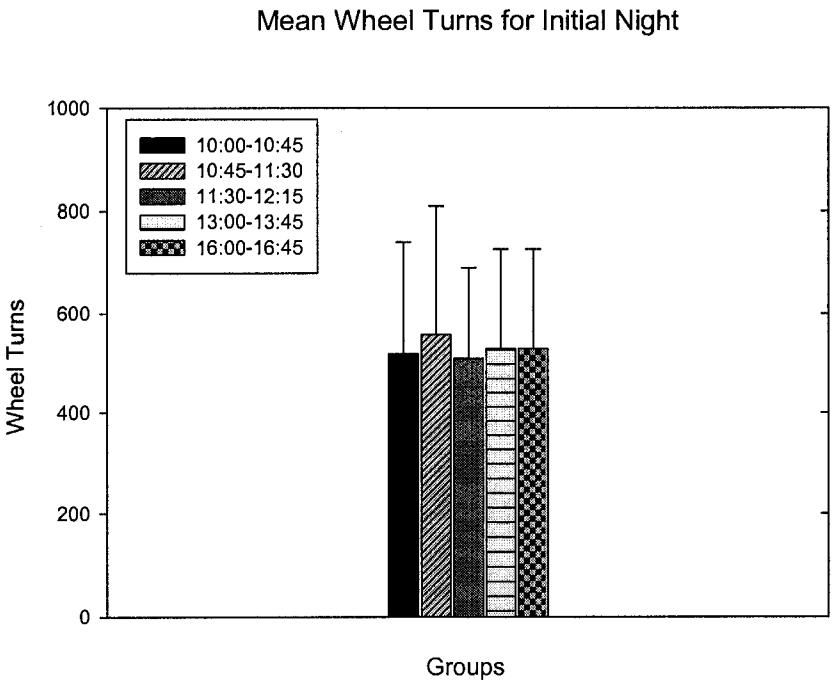


Figure 8

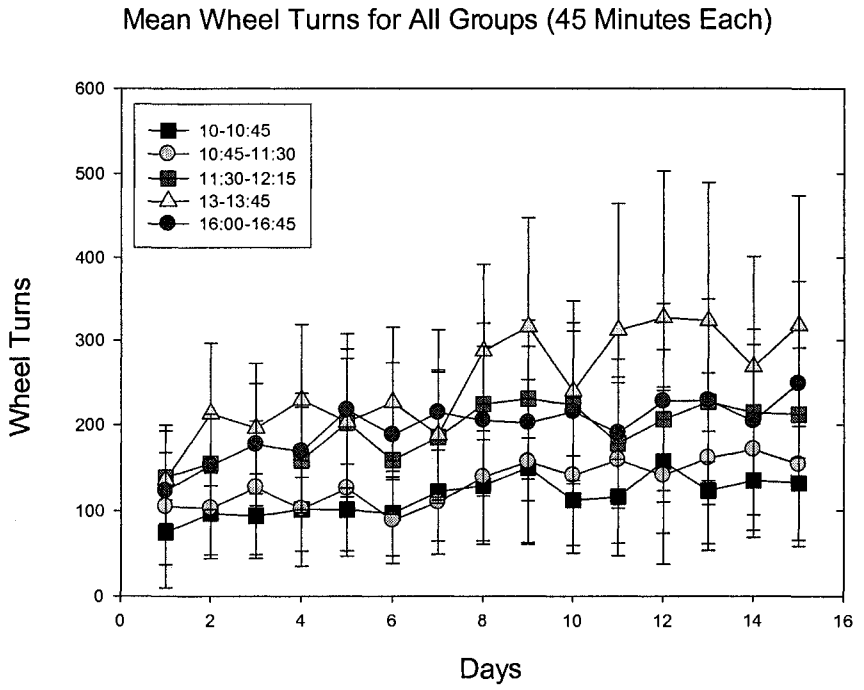

Mean Wheel Turns for All Groups (45 Minutes Each)
Wheel Turns
Days
10-10:45
10:45-11:30
11:30-12:15
13-13:45
16:00-16:45
0
100
200
300
400
500
600
0
2
4
6
8
10
12
14
16

Figure 9

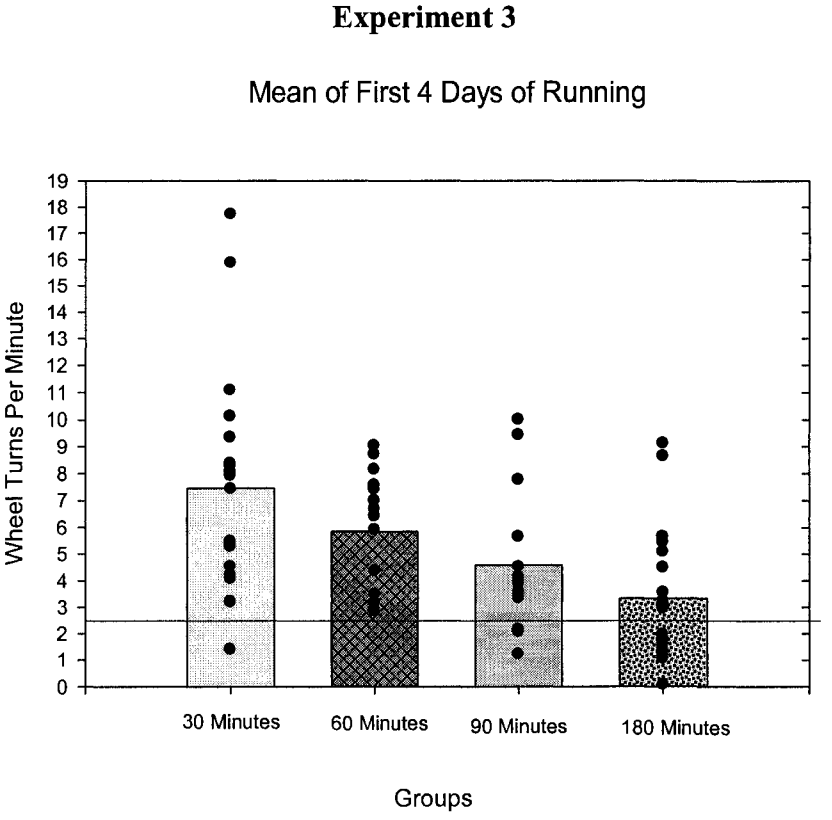

Experiment 3
Mean of First 4 Days of Running
Wheel Turns Per Minute
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
30 Minutes
60 Minutes
90 Minutes
180 Minutes
Groups

Figure 10A

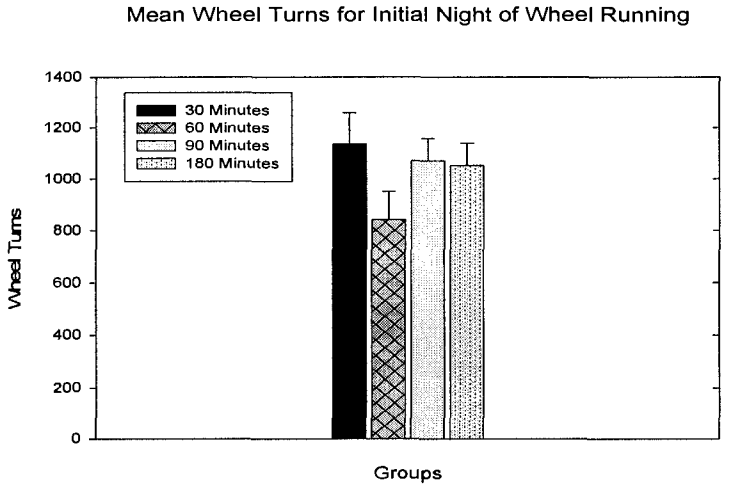


Figure 10B

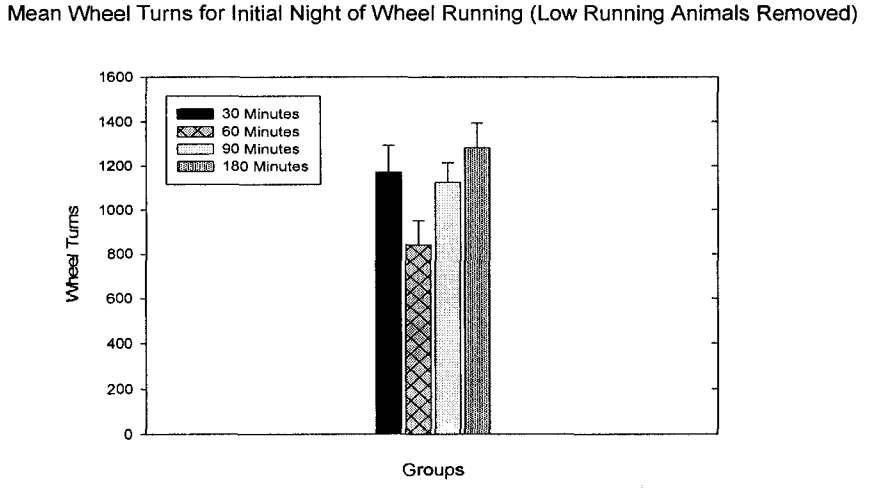


Figure 11A

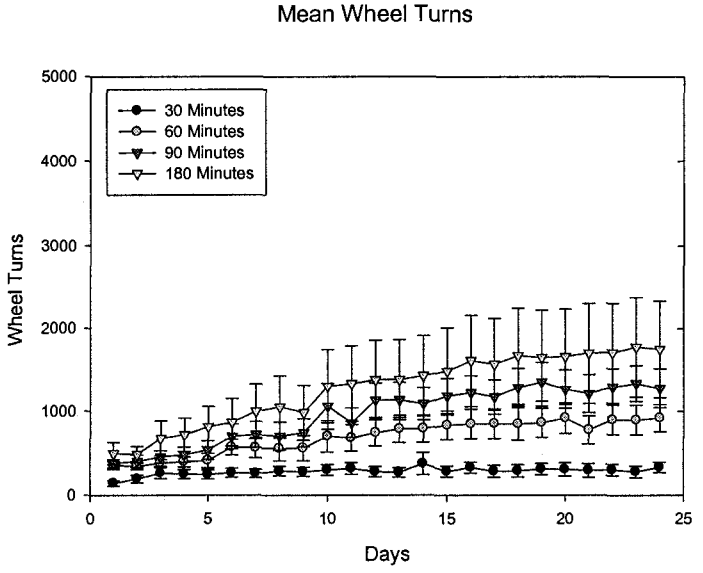


Figure 11B

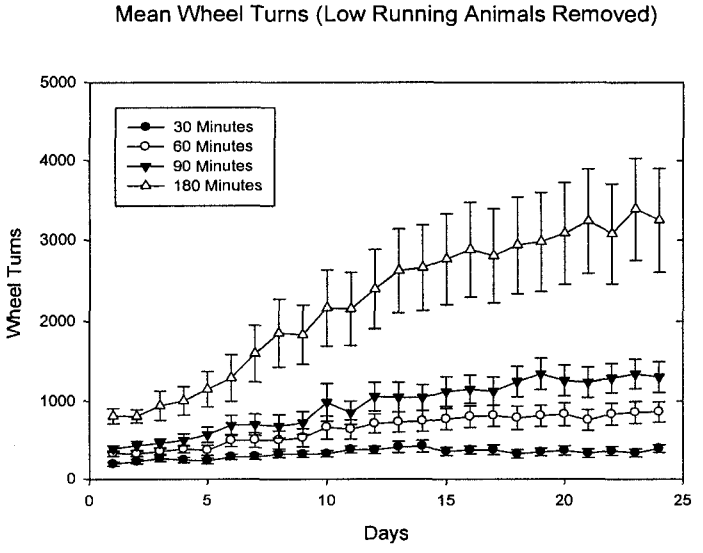


Figure 12A

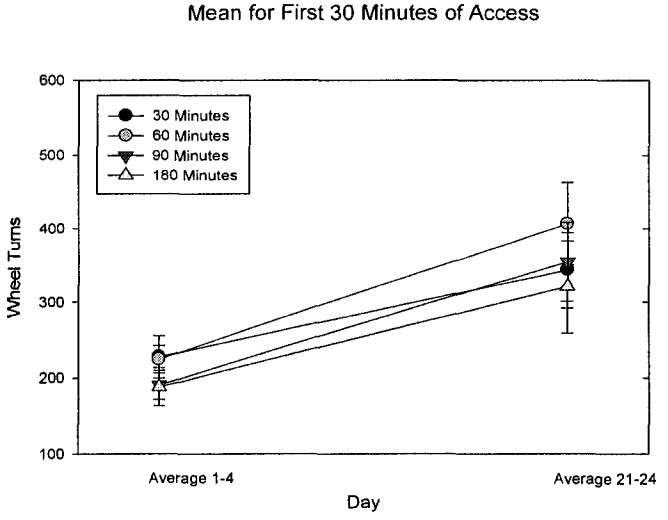


Figure 12B

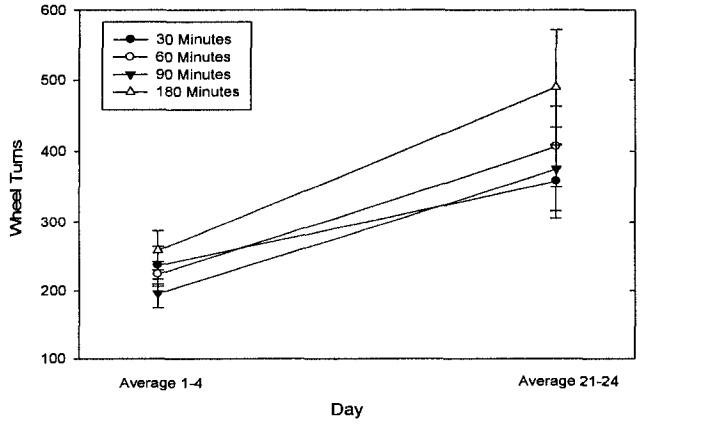


Figure 13A

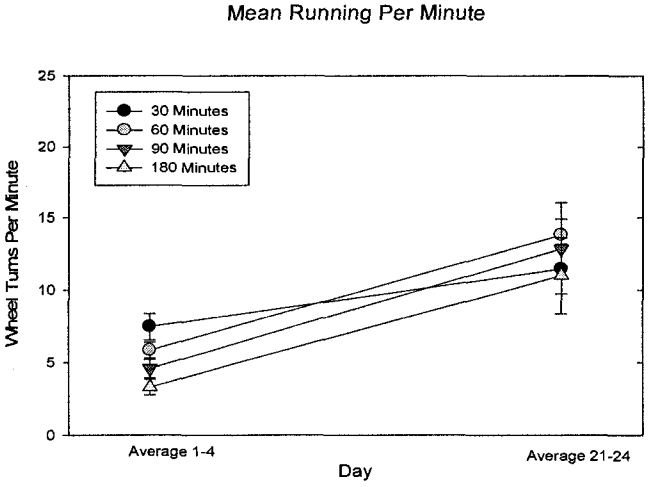


Figure 13B

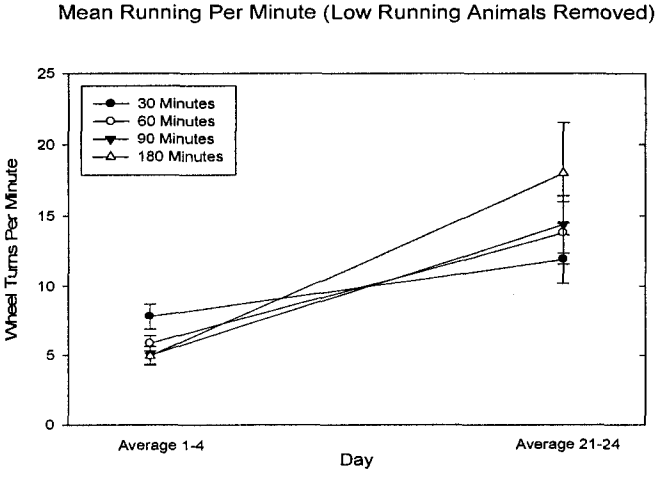


Figure 14A

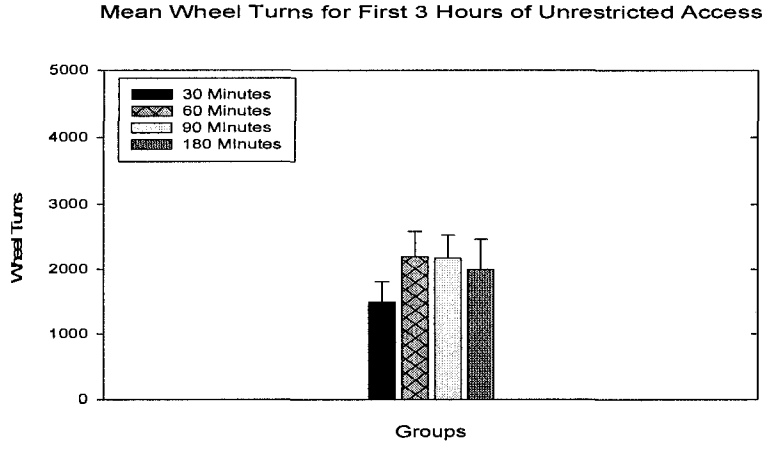


Figure 14B

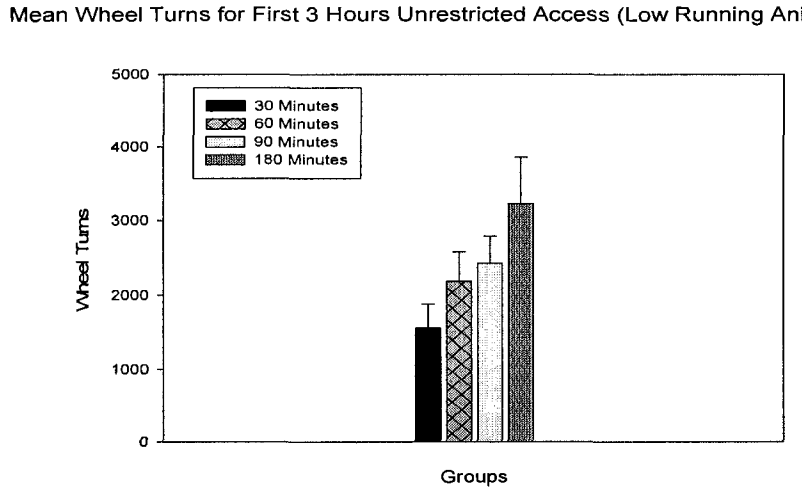


Figure 15A

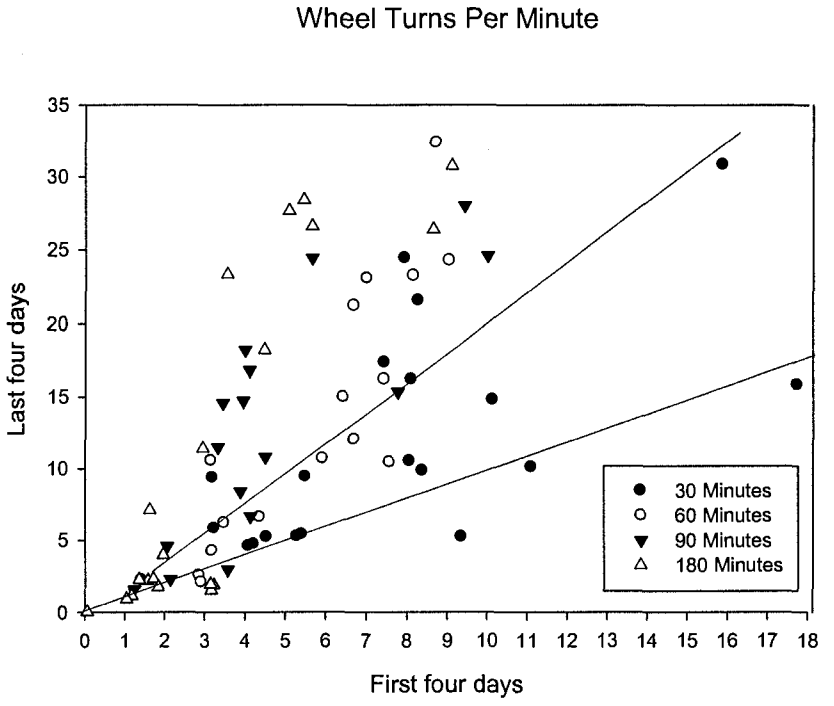


Figure 15B