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Transition from moderate to excessive wheel running in rats: A function of access length?

by

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Abstract

In rats, long daily wheel access produces an escalation to excessive levels of running while short daily access results in low stable levels of running, paralleling patterns of cocaine-taking (Lattanzio et al., 2000). Experiment 1 explored the effects of previous running experience (Phase I) on future running and feeding (Phase II). Rats with 24 h daily access in Phase I escalated wheel running to a plateau of about 7000 wheel turns per day, while rats with 2 h daily access ran at low levels (about 1000 wheel turns over the final 8 days of Phase I). The 2 h group did not run the maximum possible, but instead ran approximately one half of the maximum two hour running in the 24 h group, replicating recent findings. Rats moved from 24 h to 2 h access dropped immediately to the usual running level seen with short access. Their subsequent gradual decrease over Phase II paralleled Ahmed and Koob's (1999) finding that elevated cocaine use gradually subsides in animals switched from long to short cocaine access. Animals shifted from short to long daily running immediately increased to high levels of running, suggesting that wheel running reward is more salient in these animals. Running plateaus were lower in rats shifted from 0 h to 24 h access. Feeding suppressions were evident in rats increased to long wheel access. Due to the strong circadian pattern of wheel running, Experiment 2 compared running in rats given short wheel access during the light and dark part of the cycle. Running in night-access rats increased steadily over days while the day-access rats' running remained stable and low. Feeding was suppressed in the night access group only. As with cocaine self-administration, the reinforcing properties of wheel running may have a circadian component. Parallels between wheel running and drug-taking behaviours provide support for the use of wheel running as an animal model of addiction.

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Wheel Running as a Natural Reinforcer

Rats will readily begin running when a wheel is introduced. In our laboratory, with continuous access rats begin running at a low level, increasing over a few weeks to a plateau of about 5000-6000 wheel turns per 24 hours, which are run mostly at night (Eikelboom & Mills, 1988; Looy & Eikelboom, 1989; Mueller, Loft, & Eikelboom, 1997). While other experimenters find the same overall circadian pattern of running, the absolute amounts of running vary with strain and general procedures (Richter, 1927). Despite this variability in absolute amounts of running, rats are clearly motivated to run in wheels. Rats will also work by lever-pressing to gain access to a wheel, indicating that wheel-running is a reinforcing or rewarding behaviour (Collier & Hirsch, 1971; Iverson, 1993; Kagan & Berkun, 1954). Examples of other naturally reinforcing behaviours include eating, drinking and mating, behaviours important for organism and species survival.

Natural reinforcers appear to follow similar patterns of consumption and can be defined by at least 3 common characteristics. First, all of these behaviours support operant responding, as rats will work by pressing levers to reach food, drink or a sexual partner (Aberman & Salamone, 1999; Crawford, Holloway, & Domjan, 1993). Second, within a place preference paradigm, animals show a preference for the place associated with these natural reinforcers (Agmo & Berenfeld, 1990; Perks & Clifton, 1997). Third, animals show increased drive for and consumption of these reinforcers after a short period of

deprivation. For example, if deprived of food for a period of less than 12 hours, a rat will compensate by increasing the size of subsequent meals (Bare, 1959).

Exercise, and more specifically wheel running in rats, demonstrates each of these characteristics. As mentioned above, rats' willingness to press levers to gain access to running wheels (Collier & Hirsch, 1971; Iverson, 1993) illustrates the reinforcing nature of wheel running. A recent study indicated that wheel running produces a positive affect, since animals preferred a place associated with the aftereffect of wheel running (Lett, Grant, Byrne, & Koh, 2000). In addition, rats show a similar response to short-term deprivation of wheel access as they do to short-term deprivation of food: they compensate with an elevation in the behaviour (in this case wheel running) when access is restored (Mueller, Herman, & Eikelboom, 1999). These results point to the conclusion that wheel running is an appetitive behaviour similar to other natural reinforcers.

Further to these externally observable similarities between wheel running and other natural reinforcers, running also shares neural reward pathways in the brain, such as the dopamine and opioid systems, with other reinforced behaviours. Dopamine has been implicated as a critical neurotransmitter modulating the pleasure or positive affect that is experienced with access to a reward. Feeding, drinking and sexual interaction all increase dopamine release in the nucleus accumbens and ventral tegmental area in the rat (Agmo & Berenfeld, 1990; Agmo, Federman, Navarro, Padua & Velazquez, 1993; Yoshida et al., 1992). These are two of the critical brain regions composing the mesolimbic dopamine reward pathway. Physical exercise is also known to activate this dopamine pathway (Chaouloff, 1989).

Neurochemically, artificial models of reward such as drug self-administration appear to employ the same reward pathways as natural reinforcers (Leshner & Koob, 1999). For example, addictive opiates such as morphine are known to activate dopaminergic neurons in the mesolimbic system. Because wheel running appears to induce a release of endogenous opioids, the same mechanism responsible for increased drug consumption and drug addiction may be causing the elevation seen in wheel running.

Wheel Running and Drug Self-administration

As wheel running is a novel behaviour that is only experienced in a laboratory environment, it is interesting to compare wheel running to an artificial model of reward, a classic example being drug self-administration. Wheel running and self-administration share reward pathways (Werme, Thorén, Olson, & Brené, 1999; Leshner & Koob, 1999), and because they are novel, their patterns of acquisition from the time of first exposure can be examined and compared. One of the difficulties in measuring the initiation and ensuing increase in reinforcing behaviours is attempting to determine how much of the increase can be attributed to learning, and how much is due to changing motivation in the animal. For example, before wheel running motivation can be measured, the rat must first learn running technique, temporal wheel availability and any affective consequences of running. Similarly, one of the problems with measuring responses to artificially rewarding stimuli is that responding requires the rat to learn the association between the rewarding effect of the stimulus through an operant which is not itself rewarding. Therefore, it is not clear to what degree changes in these behaviours reflect learning, and what portion can be attributed to changes in the incentive to perform the behaviour. An added complication in

drug studies is that response behaviours may be mediated by side effects of the drug. For example, drug-induced sedation or stereotypic behaviour may reduce an operant response rate compared to the rate possible if based purely upon the drug's rewarding effects. These particular problems do not occur in the study of wheel running, although fitness is an example of a physical limitation that may cloud the rat's true motivation for running. A low fitness level may prevent an animal from running at excessively high levels when first given running wheel access. Unfortunately, it is difficult to increase a rat's fitness without introducing possible rewarding effects of the fitness-inducing exercise. The link between wheel running and reward is not likely a coincidence: the process of becoming and remaining physically fit may be adaptive.

Long term stable low levels of drug self-administration seem difficult to achieve. When first given the opportunity to self-administer rewarding drugs such as cocaine, rats begin to administer the drug at a relatively low rate. Over time however, the behaviour increases in frequency, becomes more erratic (possibly due to secondary drug effects), and if unrestricted, will continue to increase until toxicity and overdose occur (Bozarth & Wise, 1985; Fitch & Roberts, 1993; Johanson, Balster & Bonese, 1976; Tornatzky & Miczek, 2000). The increasing expression of this behaviour is considered an important defining factor of potentially disruptive or addictive behaviours.

Like self-administration, wheel running behaviour also increases when rats are given ad lib access, reaching a plateau of about 5000 - 6000 wheel turns or 5 - 6 km per day after three weeks of wheel access (Eikelboom & Mills, 1988; Mueller et al., 1997; Richter, 1927). Although older, inactive rats introduced to running wheels may not show

an elevation (Looy & Eikelboom, 1989) and research suggests a gradual decline in running as a function of age (Peng, Jiang, & Hsu, 1980, Peng & Kang, 1984), an escalating pattern of running is well documented in young adult rats with continuous access to wheels. The reason rats voluntarily expend energy on a behaviour that has no immediate benefit is unclear. The transition from a low, steady running level to high and arguably excessive levels provides an interesting parallel between wheel running and other behaviours that are considered addictive.

This parallel between wheel running and drug self-administration is being explored, and further links between the two behaviours are being discovered. For example, there is suggestion that rats with a tendency toward addiction are also prone to high levels of wheel running, as Werme et al. (1999) noted in their comparison between Lewis and Fischer rats. Fischer and Lewis rats are genetically differing inbred strains of the Sprague-Dawley rat. Lewis rats are less sensitive to stress than Fischer rats, as evidenced by their lower levels of corticosterone both normally and when stressed (Ortiz, DeCaprio, Kosten & Nestler, 1995), and display a greater place preference to addictive substances such as cocaine (Kosten, Miserendino, Chi, & Nestler, 1994). With ad lib access to a wheel, Lewis rats run at high levels, while Fischer rats run at a lower steady rate. The observation that these strains naturally differ in running levels possibly suggests that a genetic factor predisposing Lewis rats to addiction also increases their likelihood to develop excessive running. This appears to manifest itself in the down-regulation of receptors specific to the dopamine system (Werme et al., 1999), although details of this process are as yet unknown. As mentioned above, the Fischer and Lewis strains are

inbred; the outbred Sprague-Dawley rats typically used in wheel running experiments are more genetically diverse, and show a larger variability in running.

As the number of parallels found between wheel running and drug-taking increases, wheel running gains potential as a possible animal model of addictive behaviours.

Short vs. Long Access : Drug and Wheel

In studies of the acquisition of drug addictions, the length of daily access has been suggested as a factor influencing whether drug use escalates or whether it remains stable over time (Fitch & Roberts, 1993; Tornatzky & Miczek, 2000). Ahmed and Koob (1998) found a difference in the pattern of cocaine self-administration when rats were given a series of relatively long (6 h) self-administration sessions, compared to short (1 h) daily self-administration sessions. The escalation in cocaine use that they suggest is characteristic of the onset of addiction occurred only with long sessions. The pattern of cocaine-taking in the rats with 6 h access changed so that both the overall consumption of cocaine increased over sessions, and drug loading became evident during the first hour of access. Drug loading occurs when rats administer a high number of infusions at the beginning of a self-administration session followed by a slower, steady rate of self-administration. It is thought that the rat uses drug loading to raise its blood level of the drug to a certain optimal point, and then attempts to maintain this level, administering new doses only as the drug's concentration drops. With intermittent wheel access, the running pattern parallels this drug-loading behaviour, as rats will "load up" on running when a wheel is reintroduced after brief periods of deprivation (Mueller et al., 1999) and when the

wheel is provided on alternate days (Mueller et al., 1997). It is possible that rats in this situation are similarly attempting to titrate their endogenous neurotransmitter levels to produce a relatively consistent "high".

Recent work in our lab (Lattanzio, Eikelboom, & Wainwright, 2000) provided a direct comparison of long and short daily access to running wheels, with rats given either 24 h per day, 2 h per day during the light cycle, or no wheel access to for 24 days. While ad lib access in this experiment produced an elevation in running to a plateau of approximately 3000 wheel turns per day (an unusually low plateau), 2 h daily access resulted in stable low levels of running closer to 500 wheel turns per day, showing no increase over the course of the experiment. In order to determine if rats were running the maximum amount possible during their two hour access, running in the 2 h group was compared to the maximum running in a two hour span of the rats in the 24 h group during the running plateau. The maximum two hour running in the 24 h rats was higher than the 2 h group's total running, indicating that the 2 h animals did not run the maximum possible in two hours. It appears from these data that rats do not show the same escalation to high levels of running when given shorter access to a wheel as they do when given longer access. Thus, as in cocaine self-administration (Ahmed & Koob, 1998), length of access appears to influence the onset of an escalation in wheel running.

Ahmed and Koob (1999) furthered their study of differential cocaine access by examining the long-term effects of escalation in cocaine use. They found what appears to be a long-lasting change in the animals provided with long (6 h) daily access to cocaine, compared to animals given short (1 h) daily access. Specifically, when rats given long

sessions of cocaine access were subsequently restricted to one hour cocaine access sessions, their cocaine intake was elevated compared to animals chronically in the 1 h condition. Their intake gradually decreased to pre-escalation levels, but over an extended period of time (> 2 months). Ahmed and Koob (1999) argue that the change in preferred level of cocaine in animals with longer sessions of cocaine access produces residual effects even after these animals are moved to short, 1 h sessions.

In the first experiment of this thesis, the effects of previous running history on rats' future running were examined. This allowed three specific questions to be answered. First, do rats with previous experience wheel running at a high plateau continue to run at an elevated level compared to typical 2 h running when restricted to 2 h daily wheel access? Second, do rats with previous experience running at a low, steady level show an elevation in running when their access is increased to 24 h per day, and if so, how quickly? Third, if rats are 24 days older at the time of wheel introduction, do they run at a lower plateau than animals of the same age with running experience, suggesting there is a time window during which the propensity to excessive running is high?

Effects on Feeding

Introduction of a running wheel is known to impact feeding, but the interaction between these two behaviours is complex. An increase in food intake might be expected when a wheel is introduced, to compensate for the increased energy expenditure as rats begin running. (The actual cost of running may be difficult to determine as the micro pattern of running and the bearings in the wheels may determine the energy required for each wheel turn.) What has been observed, however, is a pronounced drop in feeding

lasting up to ten days, followed by a slow increase until feeding is slightly higher than in control animals (Mueller et al., 1997; Looy & Eikelboom, 1989; Lattanzio et al., 2000). While the ultimate elevation in feeding seems understandable as normal weight gain returns, the initial suppression is paradoxical. A suggested cause for this drop in feeding is that the reinforcing properties of wheel running are directly competing with those of feeding (Mueller et al., 1997). Specifically, if wheel running elevates dopamine levels compared to basal levels, the rewarding value of feeding may be diminished by comparison. Immediate or direct competition appears to be an insufficient explanation of the wheel-induced feeding suppression, since feeding takes place mostly at night, yet feeding suppressions still occur when wheel access is limited to 2 h in either the day or night (Overduin, 1999; Lattanzio et al., 2000). Daytime access may indirectly suppress feeding by longer term changes in dopamine levels in the dopamine system, with a consequent decrease in the salience of food.

Shorter periods of forced exercise have also produced a drop in feeding. In these cases the animal must continue running on a treadmill to avoid an air puff or shock. It can be argued that this procedure causes stress, which may be a factor inducing the feeding suppression (Larue-Achagiotis, Martin, Verger, Chabert, & Louis-Sylvestre, 1993). Because wheel running is voluntary, stress is not a likely explanation for the feeding suppression seen with wheel access. This suggests that the feeding suppression seen with forced exercise is not simply a stress effect, but is at least partially caused by other exercise-related factors.

Access to other activities commonly causes a suppression in the amount of a given

reinforcing behaviour. For example, social interaction attenuates morphine self-administration (Alexander, Beyerstein, Hadaway, & Coambs, 1981), and access to a wheel can cause a suppression in oral amphetamine intake (Kanarek, Marks-Kaufman, D'Anci, & Przypek, 1995) and oral morphine and methadone consumption (McLachlan, Hay, & Coleman, 1994). These interactions support the suggestion that the rewarding effects of various stimuli depend on the relative reward associated with other stimuli in the environment.

In the absence or restriction of food, wheel running can increase to extremely high levels (Routtenberg & Kuznesof, 1967). Rats are normally able to adapt to restricted feeding schedules, consuming a large percentage of their usual daily intake within the hour when food is provided (Reid & Finger, 1955). However, if rats are introduced simultaneously to restricted feeding and to a running wheel, the usual wheel-induced feeding suppression is still evident and running escalates to extreme levels (Hall & Hanford, 1954; Routtenberg, 1968; Routtenberg & Kuznesof, 1967). The combined effect of this feeding suppression and elevated running is so severe that most rats soon die unless the experiment is terminated. This paradigm, labeled "activity anorexia" (Epling & Pierce, 1992) appears to have a parallel in humans who decrease their food intake while simultaneously beginning a rigorous exercise regimen. This exercise may become compulsive and be accompanied by, or even arguably precipitate, anorexic behaviour in humans, suggesting that the relationship between exercise and feeding suppression is a direct one (Eisler & le Grange, 1990; Epling & Pierce, 1992). The current study allowed us to further examine the interaction between wheel running and feeding, comparing the

feeding effects seen with various changes in the length of daily wheel access. Specifically, the effects of increasing wheel access (2 h - 24 h), reducing wheel access (24 h - 2 h, 24 h - 0 h, 2 h - 0 h), and introducing wheel access after a 24 day delay (0 h - 2 h, 0 h - 24 h) on feeding were determined, using groups experiencing no change in wheel access as controls.

EXPERIMENT 1

This experiment was designed to explore the effects of previous wheel running history (24 h wheel/day, 2 h wheel/day or no wheel) on future running and feeding patterns. In a 3 x 3 design, rats were assigned one of the conditions (24 h wheel/day, 2 h wheel/day or 0 h wheel/day) for 24 days, and then again assigned to one of these same three conditions for an additional 24 days. The first 24 days (Phase I) of this experiment was an attempted to replicate a previous experiment in the lab (Lattanzio et al., 2000) and to confirm results found in the previous study using new equipment. To review, there were three main objectives in Phase II of this study. The first was to see what effect 24 days experience with 24 h per day wheel access would have on running in rats subsequently restricted to 2 h per day for 24 days. These animals were running at a high plateau, and so were adequately fit and possibly sensitized to the effects of wheel running. Having the endurance and arguably the motivation, did these animals, like the cocaine-taking animals in Ahmed and Koob's (1999) study, show a persistent elevation in behaviour? The second objective was to determine whether 24 days of experience with 2 h wheel access changes the subsequent running acquisition when animals are moved to 24 h access. Does experience with the wheel sensitize these animals to the rewarding effects

of wheel running? Third, running in animals first given no wheel access for 24 days and then given 24 h per day wheel access was compared to running in animals having had 24 h per day access throughout the experiment to see if a 24 day delay prior to wheel introduction had an effect on the eventual running plateau. Animals who have been singly housed in their homecages for a period of time are most likely sedentary, and grow heavier than active animals of the same age. It might be argued that these animals become obese. Previous experiments show lower running plateaus in rats introduced to a wheel after a substantial (50 day) delay (Looy & Eikelboom, 1989), therefore when given wheel access for the first time the 0 h to 24 h rats were not expected to run at excessive levels. A lower running plateau in these animals compared to animals of the same age with previous running experience (the 24 h - 24 h group) would suggest that inactivity rather than age was responsible for the lower running.

In a previous experiment comparing 24 h per day and 2 h per day wheel access (Lattanzio et al., 2000), similar feeding suppressions were seen in the two groups. A secondary objective in Experiment 1 was to compare feeding effects at the point of transition in animals moved from one condition to another, using animals remaining in the same wheel condition as controls. The specific questions that may be asked are the following. First, does increasing an animal's wheel access from 2 h to 24 h result in the usual suppression in feeding seen with 24 h wheel access? Second, does reducing an animal's wheel access cause any feeding effect, in the 24 h - 2 h, 24 h - 0 h or 2 h - 0 h groups? And, does a 24 day delay in wheel access (0 h - 24 h group) change the usual feeding suppression seen with 2 h or 24 h per day wheel access?

Method

Subjects

Eighty-one male outbred Sprague-Dawley rats, weighing between 200-225 g (47-49 days old) were obtained from Charles River Canada. They were housed individually in clear plastic shoebox cages (20 X 24 X 45 cm) and kept on a 12:12 light:dark cycle with lights on at 0600 h. Rat diet pellets and tap water were available ad lib. Holding rooms were maintained at a constant temperature (21°C) and controlled humidity. All procedures in this and the subsequent experiment were approved by the Wilfrid Laurier University Animal Care Committee.

Apparatus

Nalgene running wheels (diameter 30 cm, width 11 cm) were placed in shoebox cages similar to the homecages. Vitalview, a Mini-Mitter Co. data collection system, continuously recorded wheel revolutions in 5 s bins using a magnetic contact closure system.

Procedure

Animals were habituated to homecage conditions and handling for one week, after which a four-day baseline of weight was measured. Rats were weighed daily. In Phase I, a third of the animals (n = 27) were placed in cages with running wheels attached, with 24 h access to the wheels (24 h group). Another third (n = 27) were given 2 h access to wheels (2 h group) during the daytime, spending the remaining time in their homecages. The final third (n = 27) remained in their homecages (0 h group). Wheel turns were recorded whenever rats had access to the wheels. After 24 days Phase II began. Each

group of 27 in the original 3 conditions (24 h, 2 h or 0 h) was subdivided into 3 groups of 9 animals such that the new groups were roughly balanced with respect to weight and Phase I running levels. Having divided the groups into thirds, each third of the rats in each of the original conditions was again assigned to one of the original three conditions for an additional 24 days in a completely crossed 3 x 3 design. On the day of the switch the wheels were detached and washed, all cages were cleaned, and groups were placed into the appropriate new clean cages as soon as possible. Bedding was changed twice weekly, and wheels were washed bi-weekly. Food intake was measured for 3 days before the switch, and during Phase II of the experiment. Feeding was measured by weighing rat diet pellets placed in food hopper, weighing the amount remaining after 24 h, and calculating the difference.

This study was divided over three replications due to equipment restrictions. Differences in procedure between the three replications were minimized. Animals in the 2 h group were given wheel access 2 and 4 h into the day cycle in replication 1, 6 and 8 h into the day cycle in replication 2, and 2 and 4 h into the day cycle in replication 3 due to experimenter schedules. These times were kept as consistent as possible within each replication. Analyses including replication as a factor consistently showed no significant differences between the three replications or any interactions involving replication, so replication was not included as a factor in the reported results.

Analysis

Data are presented on a daily basis in all figures. Data analysis for the wheel running data was performed at two levels. First, to simplify the analysis of the overall

pattern of running over days, the 24 days of both Phase I and then Phase II were divided into three blocks of 8 days, and 8-day averages were calculated for each group during the first, middle and last eight days of each phase. Thus 3 block by 2 or 3 group (depending on which groups are being compared) repeated measures ANOVAs were performed across each phase to determine any between-group differences and any changes over time. In all analyses involving repeated factors, results were reported as significant only if significant using a Greenhouse-Geisser correction. In the case of significant interactions, subsequent simple main effects were done as appropriate, in the form of three block repeated measures ANOVAs performed on the individual groups. To directly compare running in the various groups and to determine whether groups were stable or changing at the beginning and the end of each phase, 8 day by 2 or 3 group ANOVAs were performed on the first eight and then on the last eight days of each phase. If there were significant group differences for these 8 days, Tukey HSD tests were carried out to see which groups differed. Where means are reported, the standard error of the mean is used as an indication of variability.

The food data are presented for the 8 critical days following the switch to Phase II to determine the immediate effects of changing wheel access. Eight-day by 3 group repeated measures ANOVAs were performed to determine changes in feeding over days and any between group differences in feeding immediately after the switch. In the case of interactions, feeding was compared on a day to day basis between groups until feeding no longer differed between groups.

Results

Phase I - Wheel Running

As seen in Figure 1, the 27 rats given 24 h per day wheel access for 24 days showed a pronounced increase in wheel running over days, whereas the 27 rats given 2 h per day showed a small increase, continuing to run at a low level throughout Phase I. Changes in running over the 24 days of Phase I (divided into three blocks of 8 days) as well as differences in running between the two groups were tested in a 3 block by 2 group (2 h vs. 24 h group before switch) by 3 groupafter (0 h, 2 h and 24 h groups after switch) mixed ANOVA. Groupafter was included as a factor in order to test whether the groups designated to be moved into each of the different wheel conditions after the switch differed in running before the switch. This analysis revealed a significant effect of block ($F(2, 96) = 54.2$, $p < 0.001$), a significant effect of group ($F(1, 48) = 62.5$, $p < 0.001$) and a significant block by group interaction ($F(2, 96) = 34.0$, $p < 0.001$). There was neither an effect of groupafter ($F < 1$) nor any significant interactions with groupafter ($Fs < 1$). This indicated that Phase I running within each of the two groups (2 h and 24 h) did not differ among animals that were later moved into each of the three conditions in Phase II. As a result, further analyses of Phase I wheel running combined the rats given 24 h and the rats given 2 h access into single groups with 27 animals each. As the block by group interaction was significant, separate three-block repeated measures ANOVAs on the 2 h and 24 h groups were performed. These analyses confirmed a change over blocks in the 24 h group's running ($F(2, 48) = 44.8$, $p < 0.001$), and also revealed a change in the 2 h group's running ($F(2, 48) = 19.8$, $p < 0.001$). As seen in Figure 1, the 24 h group's

running increased to a high level, while the 2 h group elevated slightly over days.

An 8 day by 2 group ANOVA was performed on the first 8 days after wheel introduction in Phase I (Figure 1) to determine whether running patterns in the two groups differed at this early stage. This ANOVA revealed a significant days effect ($F(7, 336) = 29.6$, $p < 0.001$), a significant group effect ($F(1, 48) = 43.1$, $p < 0.001$) and a significant days by group interaction ($F(7, 336) = 18.6$, $p < 0.001$). From Figure 1 it is evident that the 24 h animals showed a more pronounced increase in running over the first 8 days than did the 2 h animals. A similar 8 day by 2 group ANOVA performed on the last eight days of Phase I revealed a strong group effect ($F(1, 48) = 63.1$, $p < 0.001$), but there was no longer a days effect ($F < 1$) nor a days by group interaction ($F(7, 336) = 1.7$, p = ns). As can be seen in Figure 1, both the 24 h group and the 2 h group had reached a running plateau over these last 8 days, with the 24 h group running at a higher plateau (7343 ± 735 WT) than the 2 h group (1165 ± 160 WT).

Because the 2 h group had limited wheel access, it is possible that their low level of running was the result of a ceiling effect: they may have been running as much as possible within the two hours provided. To determine whether this was the case, a comparison between the 2 h group's total daily running and the 24 h group's maximum running in a two hour span (derived by dividing each 24 h day into twelve 2 h bins and choosing the one with the highest running for each rat) was made. It should be noted that the maximum 2 h running in the 24 h group took place during the dark cycle the majority of the time. Figure 2 compares the total daily running in the 2 h group (the same data as in Figure 1) to the maximum running in a 2 h span of the 24 h group. A 3 block by 2

group ANOVA on this data showed that overall there was a significant increase in maximum running over the blocks ($F(2, 104) = 78.5$, $p < 0.001$), there was a significant difference in maximum running between groups ($F(1, 52) = 11.4$, $p = 0.001$), and a significant block by group interaction ($F(2, 104) = 16.8$, $p < 0.001$). Subsequent repeated measures ANOVAs for each group over the three blocks showed that, as noted above, the 2 h group's running increased over the blocks, and that the 24 h group's maximum running in two hours also increased over time ($F(2, 52) = 57.9$, $p < 0.001$).

Looking more closely at this maximum running comparison, an 8 day by 2 group ANOVA on the first 8 days of Phase I (Figure 2), revealed a significant days effect ($F(7, 364) = 21.8$, $p < 0.001$), and day by group interaction ($F(7, 364) = 5.4$, $p < 0.001$) but no overall effect of group ($F < 1$). An analysis of each group separately using 8 day repeated measures ANOVAs showed that both the 2 h group ($F(7, 182) = 6.2$, $p < 0.001$) and the 24 h group ($F(7, 182) = 17.3$, $p < 0.001$) were increasing over the first eight days. In an examination of the last eight days, an 8 day by 2 group ANOVA highlighted a group difference ($F(1, 52) = 16.4$, $p < 0.001$), but no change over days ($F < 1$) and no days by group interaction ($F(7, 364) = 1.9$, $p =$ ns). As seen in Figure 2, both the 24 h and the 2 h groups showed no change in running over these last eight days, however the average of the 24 h group's maximum running was almost double the average of the 2 h running (2235 ± 210 WT compared to 1165 ± 160 WT) at this plateau. This indicates that, as in an earlier comparison of 2 h vs. 24 h running (Lattanzio et al., 2000), rats with 2 h per day wheel access did not run the maximum possible in 2 hours. It should be noted that 2 h day access was provided during the light cycle, and is here being compared to maximum

running levels that occurred in the dark cycle. A direct comparison of day and night running will be explored in Experiment 2.

Phase II - Wheel Running

Figure 3 compares running in rats with different previous running experience (0, 2, 24 h per day) after their switch to 2 h wheel access (Phase II). A 3 block by 3 group (0 h - 2 h, 2 h - 2 h, 24 h - 2 h) ANOVA shows that there is no main effect of block ($F < 1$), and no main effect of group ($F < 1$), however the block by group interaction was significant ($F(4, 48) = 5.6$, $p < 0.01$). Three block repeated measures ANOVAs performed on the individual groups indicated that the 0 h - 2 h group showed a significant gradual increase in running ($F(2, 16) = 5.1$, $p < 0.05$), the 2 h - 2 h group did not show any change in running over the three blocks of Phase II ($F < 1$), and the 24 h - 2 h group showed a gradual decline in running ($F(2, 15) = 5.7$, $p < 0.05$). The gradual increase seen in the 0 h - 2 h group parallels the running pattern seen in the 2 h group in Phase I. The steady running over the three blocks of Phase II in the 2 h - 2 h group indicates that the stable running level achieved by the 2 h group during the final eight days of Phase I (Figure 1, 2) is indeed a plateau, as rats maintained at 2 h per day access continued to run at this level for 24 additional days without a further change in running. The group of greatest interest here is the 24 h - 2 h group. After restricting their access to 2 h per day, these rats immediately ran at a low level similar to that seen in 2 h per day rats with no previous experience. Therefore, unlike the persistent elevation in cocaine self-administration seen in rats restricted from 6 h to 1 h sessions (Ahmed and Koob, 1999), rats did not show a persistent elevation in wheel running following the switch from 24 h to 2 h daily access. However, this group did show a gradual decrease in running over the

three blocks of Phase II, after their switch to 2 h access. In this way, the 24 h - 2 h group does parallel Ahmed and Koob's (1999) study, where animals restricted from 6 h sessions to 1 h sessions showed a gradual decrease in cocaine self-administration over sessions. The following analyses examine the switch to 2 h access more closely in the first 8 and the last 8 days.

The first eight days after the switch to 2 h running (Figure 3) were examined in an 8 day by 3 group mixed ANOVA. The days effect was significant ($F(7, 168) = 2.35$, $p < 0.05$). Although the 0 h - 2 h group appears to run less during the first 8 days, this group effect only approached significance ($F(2, 24) = 3.1$, $p = 0.06$), and the days by group interaction was not significant ($F < 1$). During the last 8 days of Phase II, an 8 day by 3 group mixed ANOVA revealed no significant effects. Thus by the end of the experiment, all groups moved to 2 h access were running similar daily amounts that were stable over days.

Figure 4 displays the total wheel running in rats moved from the three original conditions (0 h, 2 h, and 24 h) to 24 h access in Phase II. A 3 block by 3 group mixed ANOVA revealed a significant main effect of block ($F(2, 48) = 7.8$, $p < 0.01$), a significant main effect of group ($F(2, 24) = 4.9$, $p < 0.05$) and a significant blocks by group interaction ($F(4, 48) = 2.9$, $p < 0.05$). In an examination of the individual groups over the 3 blocks, the 24 h - 24 h group did not show a significant change over the three blocks ($F < 1$), nor did the 2 h - 24 h group ($F(2, 16) = 2.8$, $p > 0.05$). In fact, the running in the 2 h - 24 h group closely resembled that in the 24 h - 24 h group (Figure 4). As is clear in Figure 4, the 0 h to 24 h group did show an increase in running over the three blocks ($F(2,$

16) = 9.2, $p < 0.002$). An analysis of the first 8 days and the last 8 days compared these groups more explicitly.

An 8 day by 3 group ANOVA looking at the first eight days after the switch to 24 h running (Figure 4), showed a days effect ($F(7, 168) = 7.3$, $p < 0.001$), and a significant group effect ($F(2, 24) = 9.4$, $p < 0.01$), but no days by group interaction ($F = 1.0$). A post-hoc Tukey HSD test indicated that the 0 - 24 h group is running less (1696 ± 299 WT) than both the 24 h - 24 h group (7242 ± 1201 WT) ($p < 0.01$) and the 2 h - 24 h group (5775 ± 1054 WT) ($p = 0.01$) during these eight days. Interestingly, the rats moved from 2 h - 24 h show an immediate increase in running from their earlier 2 h running levels (Figure 1) such that their running does not differ from that of the 24 h group within the first 8 days after the switch ($p > 0.05$). This acquisition of excessive running is very rapid compared to the running seen in the 0 h - 24 h group (Figure 4) as well as in the 24 h group in Phase I (Figure 1).

A similar 8 day by 3 group ANOVA run on the last 8 days indicated no significant effects or interactions. Because the 0 h - 24 h group was expected a priori to plateau at a lower level than the 24 - 24 h group (Looy & Eikelboom, 1989), this planned comparison was tested in an 8 day by 2 group ANOVA of the final 8 days of Phase II, and the running plateau seen in the last 8 days in the 0 h - 24 h group (4614 ± 778 WT) was found to be significantly lower than the 24 h - 24 h (7314 ± 938 WT) running plateau ($F(1, 24) = 4.9$, $p < 0.05$). This supports previous experiments (Looy & Eikelboom, 1989) suggesting that restricting rats to their homecages for a period of time prior to wheel introduction may lower their eventual running plateau.

In a comparison of maximum 2 h running in all groups moved to 24 h access for Phase II (Figure 5), a pattern similar to that seen in the overall 24 h running in the same groups emerges. A 3 block by 3 group repeated measures ANOVA revealed a significant blocks effect ($F(2, 48) = 12.6$, $p < 0.001$), group effect ($F(2, 24) = 6.0$, $p < 0.01$) and block by group interaction ($F(4, 48) = 3.5$, $p < 0.05$). Subsequent repeated measures ANOVAs for each group over the three blocks showed that the maximum 2 h running after the switch to 24 h access was stable over the blocks for both the 2 h - 24 h group ($F(2, 16) = 2.5$, $p > 0.05$), and the 24 h - 24 h group ($F(2, 16) = 1.1$, $p > 0.05$). The maximum running in the 0 h - 24 h group increased over blocks ($F(2, 16) = 14.9$, $p < 0.001$).

Upon closer examination of the maximum running across the first 8 days following the switch to 24 h access, an 8 day by 3 group ANOVA showed a significant days effect ($F(7, 168) = 6.7$, $p < 0.001$) and group difference ($F(2, 24) = 11.8$, $p < 0.001$), but no days by group interaction ($F < 1$). A post-hoc Tukey HSD test revealed that during the first eight days, the maximum 2 h running in the 0 h - 24 h group is lower than the maximums for both the 2 h - 24 h and the 24 h - 24 h groups ($p < 0.005$ for both). During the last eight days, an 8 day by 3 group ANOVA yielded no significant effects or interactions. As above, the 0 h - 24 h group was expected a priori to plateau at a lower level than the 24 - 24 h group, therefore a specific 8 day by 2 group ANOVA was performed on the maximum running in these two groups over the final 8 days of Phase II. The maximum running was significantly lower in the 0 h - 24 h group than in the 24 h - 24 h group ($F(1, 24) = 5.1$, $p = 0.04$), indicating that if wheel running is delayed by 24 days,

rats with continuous access do not show the maximum running of rats with 48 days of wheel access.

Phase II - Feeding

Daily feeding is displayed for the three groups from 0 h access (Figure 6), from 2 h access (Figure 7), and from 24 h access (Figure 8) during the 8 days after they are moved into the three different wheel conditions in Phase II. This organization of the data highlights any differences in feeding between groups with the same previous running experience after the switch to the three wheel conditions, allowing feeding in groups experiencing a transition to be compared to feeding in groups not experiencing a transition.

Figure 6 illustrates feeding in the three groups moved from 0 h wheel access to 0 h , 2 h or 24 h wheel access in Phase II. (An analysis of feeding in the baseline immediately preceding the shift found no differences among animals previously in the same wheel condition ($\underline{F}$s < 1)). An overall 8 day by 3 group ANOVA indicates that there is a days effect ($\underline{F}(7, 175) = 5.3$, $\underline{p} < 0.001$), and an effect of group ($\underline{F}(2, 25) = 6.9$, $\underline{p} < 0.004$) but no interaction ($\underline{F} = 1.0$). A post-hoc Tukey HSD test revealed that the 0 h - 24 h group showed a suppression in feeding relative to the 0 h - 0 h group ($\underline{p} < 0.003$) over the 8 days following the switch, while the 0 h - 2 h group was not significantly different from either of these groups ($\underline{p}$ = ns). This suggests rats given ad lib wheel access after a 24 day delay show a feeding suppression similar to the effect seen in rats immediately given ad lib access (Lattanzio et al., 2000), however rats given 2 h per day access after a 24 day delay do not show a significant suppression in feeding.

Figure 7 displays total daily feeding of animals moved to 0, 2 or 24 h access from 2 h access. An 8 day by 3 group repeated measures ANOVA revealed a significant effect of days ($F(7, 168) = 12.7$, $p < 0.001$), a group effect that approached significance ($F(2, 24) = 3.0$, $p = 0.07$), and a significant day by group interaction ($F(14, 168) = 3.9$, $p < 0.001$). Due to the significant interaction, one-way ANOVAs were performed on each of the 8 days. Where differences were found, post-hoc Tukey HSD tests were performed to find specific group differences. On the first day following the switch, a significant group effect was found ($F(1, 24) = 8.6$, $p < 0.01$), and a Tukey test revealed that the 2 h - 24 hour group showed a feeding suppression compared to both the 2 h - 2 h ($p < 0.05$) and the 2 h - 0 h ($p < 0.01$) groups. On the second day post-switch, again a group difference was found ($F(2, 24) = 4.3$, $p < 0.05$). Here the Tukey test revealed that the 2 h - 24 h group was still suppressed compared to the 2 h - 0 h group ($p = 0.02$), with no other significant differences. On day 3, the overall difference between groups persisted, ($F(2, 24) = 4.7$, $p < 0.05$), and the Tukey test revealed that again, the 2 h - 24 h group showed suppressed feeding compared to the 2 h - 0 h group ($p < 0.05$). Analyses of days 4 - 8 post switch found no further significant differences between groups. These data indicate that previous experience with 2 h per wheel access does not prevent the feeding suppression seen when animals are subsequently provided with 24 h per day wheel access.

Figure 8 depicts total daily feeding in groups moved from 24 h wheel access to 0 h, 2 h or 24 h access. An overall 8 day by 3 group ANOVA shows a significant days effect ($F(7, 168) = 10.1$, $p < 0.001$), but no significant group difference ($F(2, 24) = 2.9$, $p = 0.08$) and no interaction ($F < 1$). These results indicate that there are no detectable

feeding effects in animals removed from 24 h access.

In the above 3 analyses, the main effect of days was significant in each case. As seen in Figures 6, 7 and 8, feeding on day 1 after the switch is low for most groups, including those remaining in the same wheel condition. Because this difference occurs in groups not changing conditions, it is possible that the brief, one day drop in feeding was caused by a combination of unusual noise levels, changes in routine, and cage changing which all animals experienced.

Discussion

Phase I - Wheel Running

Phase I of Experiment 1 was effectively a replication of an experiment previously performed in the lab, with animals receiving either 0, 2, or 24 h access to a wheel for 24 days (Lattanzio et al., 2000). The overall pattern of running seen in the 24 h and 2 h groups in Phase I was similar to the results seen in the previous experiment: rats given 24 h per day access showed a large escalation in running over days reaching a high plateau during the final 8 days, while the 2 h per day rats ran at a low level throughout Phase I (Figure 1). Unlike the results seen previously, the rats given 2 h per day did show a slight elevation over the course of the 24 days. In addition, the plateau seen in the 24 h group was unusually high (7343 ± 735 WT) in comparison to previous work.

These differences may have been caused by the new equipment used: Nalgene wheels replaced the 12 wheel rack used previously. This difference in the wheels may account for the unusually high plateau seen in the 24 h group. A recent compilation of data from 22 rats given 24 h daily wheel access in the rack reports an average running

plateau of 6481 WT per day (Eikelboom, 2001), suggesting that running is systematically higher in the Nalgene wheels. One possible explanation for this is that access to these two types of wheel differs; the Nalgene wheels are mounted within the cage, allowing rats to jump out of the wheel into the cage while the wheel continues to spin. With the rack, wheels are attached to the outside of the cages, so the rat must exit the wheel via a small hole, ensuring that the wheel has stopped turning before the rat is able to climb out. It is possible that the numbers in the Nalgene wheels are unusually high because of these additional free spinning counts, but more explicit observational studies would be required to confirm this. A second possible explanation is that the bearings and weight of the Nalgene wheels differ from the rack wheels. This may mean that less work is required to turn the Nalgene wheels than the wheels in the rack.

Because the higher ad lib running seen in Phase I in the 24 h group may be equipment related, it is possible that the difference in equipment could also be responsible for the unexpected elevation in the 2 h running. For example, it is not clear whether these rats were actually increasing their running, or whether as they grew larger, heavier, and began to run faster, they could be accumulating higher spinning counts after leaving the wheel. There are two possible tests of this hypothesis. The first would involve close observation of the animals during their two hour session of wheel running to determine the actual number of wheel turns accounted for by free spinning, and the second would mean a modification to the wheel/cage apparatus such that rats must stop running to exit the wheel through a smaller door.

Although the animals given only 2 h per day to run in the wheel did show a slight

elevation in running over Phase I, a more important test was whether they increased to the excessive levels seen in the animals given ad lib access. An analysis comparing their running to the maximum 2 h running of animals with 24 h access showed that they do not run the maximum amount it is possible to run in 2 h. In fact they only run approximately half of the 24 h group's maximum amount (see Figure 2). The low level of running seen with 2 h access raises the question: is it a simple difference in fitness level achieved in the two conditions? It is safe to say no, first, because animals moved from 2 to 24 h access in Phase II quickly run at high levels, (see Figure 4) and second, since animals running at high maximums in the 24 h condition in Phase I immediately dropped to low levels of running when restricted to 2 h per day running (see Figure 3). It is possible that the 24 h - 2 h group drops to a low level of running because they are given wheel access during a normally inactive period. Future studies could examine this transition to 2 h access during the night period to see if a similar result occurs. (See Experiment 2 and General Discussion for further discussion of night vs. day running).

Because rats given 2 h daily access do not run at the high maximum level possible in two hours, the argument may still be mounted that short (2 h) daily access to a wheel is not sufficient to trigger the escalation to excessive levels characteristic of addictive behaviors. Like a previous experiment in the lab (Lattanzio et al., 2000), this result parallels Ahmed and Koob's (1998) cocaine study in which short (1 h) periods of access to cocaine self-administration did not trigger escalated cocaine use.

Phase II - Wheel Running

Animals with three different levels of previous wheel experience - 0, 2 and 24 h

per day wheel access - were given 2 h wheel access in Phase II. Rats moved to 2 h access from the 0 h per day condition show a similar running pattern to animals given 2 h access in Phase I. Running increased gradually over the 24 days, reaching a plateau equivalent to the 2 h - 2h group's running (Figure 3). This gradual increase confirms the reliability of the results found for the 2 h group in Phase I (Figure 1). A 24 day delay in 2 h wheel access did not appear to influence 2 h running. Of particular interest was the running seen in the group moved from 24 h to 2 h per day access. This group, previously running at a maximum higher than the 2 h animals in Phase I (Figure 2), dropped immediately to a low level of running indistinguishable from the 2 h - 2 h group's running (Figure 3) either in the first 8 days or over the 3 blocks. However they also showed a gradual decline in running over the three 8 day blocks which parallels aspects of Ahmed and Koob's (1999) finding that rats restricted from 6 h to 1 h per day cocaine access showed long lasting elevations in consumption during their 1 h sessions. While there was a within group decline over the 24 days, this effect was not strong enough to be evident as a between group difference. Many procedural differences between this study and Ahmed and Koob's study (1999) could be responsible for the weak nature of this running decline in the 24 h - 2 h rats. In Ahmed and Koob's experiment, rats were restricted to one hour self-administration sessions instead of six hour sessions. In the first of their shortened sessions, these animals would not expect to be removed from the session after 1 h (rather than after 6 h), so it is not surprising that their self-administration rate is similar to what it would be if it were the first hour of their usual sessions. In contrast, rats moved from ad lib access to restricted 2 h per day access in Experiment 1 had already been deprived of their usual access to the wheel for half of a day by the time they received their first 2 h

exposure, so they may have learned that the wheel was no longer available. In addition, in Experiment 1, all the 2 h groups were given wheel access during the day, while the 24 h animals chose to run during the dark cycle in the days before the switch. It is possible that the 24 h - 2 h group would run differently if given their 2 h access during the nighttime. Despite the weak nature of this decline in running, the fact that running declines in this group suggests that there may be changes induced by the 24 h wheel access that last for an extended period, causing this group to run differently than other 2 h groups without previous 24 h wheel access experience.

Switching animals from the 0, 2 and 24 h wheel conditions to 24 h access led to a few interesting findings. The first was the rapid transition seen in the 2 h to 24 h group. Their running increased rapidly to plateau levels seen in the 24 h group - both their total daily running and their maximum running in 2 h. A slower transition would suggest that the rats needed to gradually increase their fitness to run at the high level seen in the 24 h group. Instead, their rapid acquisition suggests that their fitness was high after having 2 h running, and the switch to 24 h access somehow triggered the high levels of running. Previous experience with low levels of running seems to offer no protection from the excessive running seen in animals given 24 h access. The response of the 2 h - 24 h rats suggests a heightened motivation to run as they acquire the excessively high running levels within a few days of the switch. This is not likely a simple practice effect: these animals have experienced a total of 48 hours wheel access during Phase I, so the 0 - 24 h rats have had as much experience within the first 2 days of Phase II. As seen in Figure 4, it takes much longer than 2 days for the 0 h - 24 h group to reach the high levels of running seen in the 2 h - 24 h group immediately after the switch.

As expected, the rats given free access to the wheel after 24 days of inactivity showed a lower running plateau than animals given free access in Phase I. It is not the case that animals of this age are not able to run at a higher plateau - in this experiment, animals of the same age in the 24 h - 24 h condition are running at a higher plateau (see Figure 4) than the plateau seen in the 0 to 24 h group. Rather it suggests that some combination of 24 days of inactivity, individual housing and possible overeating causes the subsequently lower running level. Perhaps these animals are less inclined to become addicted to the wheel because they are introduced to it when they are 24 days older: it is possible that the propensity for addiction decreases with age.

Feeding results will be addressed in the General Discussion.

EXPERIMENT 2

Another factor known to influence the rats' motivation for wheel running is the time of day the wheel is provided. Rats are nocturnal animals, so under ad lib conditions, activities like feeding and wheel running occur mostly at night (Eikelboom & Mills, 1988; Mueller et al., 1997). If rats are provided with food or water only during the day, however, their drives to eat and drink are strong enough that they will adapt to this schedule, consuming these essential substances during the day. Similarly, if a wheel is provided only during the day, rats will run when it is available (Boakes & Dwyer, 1997; Lattanzio et al., 2000). It is clear from the amount of running seen during day access, that rats are motivated to adjust their natural circadian rhythm in order to run. However, because rats are naturally active during the night, it is possible that their motivation to run when the wheel is provided during daylight is lower than it would be if the wheel was provided at night.

As mentioned above, it is a well-established fact that with ad lib access, rats choose to run mostly during the dark part of the light cycle. In addition, examination of data from Experiment 1 indicated that the maximum 2 h block of running consistently occurred during the dark part of the light cycle (98.6% of maximum running blocks occurred in dark cycle during the final 8 days of Phase II). An explicit examination of 2 h daytime running vs. 2 h nighttime running was clearly required to see whether the low level of running seen with 2 h day access would be greater if the restricted access was provided during the night. Pilot studies had suggested that 2 h night runners do not run more than 2 h day runners (Overduin, 1999), but a more controlled study was needed to confirm this finding. In these pilot studies, the rats provided wheel access during the night were placed in the wheel just prior to the onset of the night. Overhead lights were then turned on when the rats were removed from the wheels 2 hours later. It is not clear whether the proximity of wheel access to the day/night transition combined with the lighting changes caused the rats to behave as though there was an elongation of the light cycle. If so, this may not have provided a clear reflection of motivation to run during the night cycle.

In the current experiment, effects on feeding were also examined. A similar feeding suppression had been observed upon introduction to 2 h per day wheel access and 24 h per day wheel access (Lattanzio et al., 2000), so the feeding suppression does not appear to be an effect of direct competition for time. Instead it may be related to a temporary imbalance in the reinforcement of feeding due to the introduction of a strong new reinforcer. Similar feeding suppressions in both the 2 h per day and the 2 h per night wheel running conditions were therefore expected.

Method

Subjects

Twenty-four male Sprague-Dawley rats, weighing between 200-225g were obtained from Charles River Canada. They were housed individually in clear plastic shoebox cages (20 X 24 X 45 cm) and kept on a 12:12 light:dark cycle with lights on at 0000 h. Rat diet pellets and tap water were available ad lib. Holding rooms were maintained at a constant temperature (21°C) and controlled humidity.

Apparatus

A three-tiered rack was set up to hold 12 wire cages (25 x 17 x 20 cm) each with a running wheel (diameter 30 cm, width 11 cm) attached. Dataquest III, a Mini-Mitter Co. data collection system, recorded wheel revolutions in 5 s bins using a magnetic contact closure system.

Procedure

Animals were habituated to homecage conditions and handling for circa one week, after which a four-day baseline of weight and food was measured. Rats were weighed daily between 1030 and 1130 h (1.5 - 0.5 h before the lights went out). Feeding was measured by weighing rat diet pellets placed in food hopper, weighing the amount remaining after 24 h, and calculating the difference. A third of the animals (n = 8) were placed in the wire cages with running wheels attached from 0830 h until 1030 h giving them 2 h access to the wheels during the last third of the day cycle. Another third (n = 8) were placed in the wheel cages from 1230 h until 1430 h, giving them 2 h access to wheels during the early part of the night cycle. The final third (n = 8) remained in their homecages. Care was taken to ensure that rats were not weighed or disturbed within at

least 1 h before their wheel access, so that running was not affected by immediately prior handling. Wheel turns were recorded whenever rats had access to the wheels.

Results

Wheel Running

Figure 9 displays the total 2 h running in two groups of rats, given either daytime or nighttime 2 h access. Running patterns in the two groups differed strikingly: rats given 2 h access during the day maintained a low, steady level of running over days, while rats given 2 h access during the night showed a marked elevation in running over days. A 3 block by 2 group mixed ANOVA revealed a significant effect of blocks ($F(2, 28) = 16.5$, $p < 0.001$), and of group ($F(1, 14) = 16.3$, $p < 0.01$) and a significant group by block interaction ($F(2, 28) = 15.5$, $p < 0.001$). Simple effects of each group over the three blocks indicated that the daytime running rats showed no increase in running over the three blocks ($F < 1$), while the nighttime running rats did increase their running over blocks ($F(2, 14) = 16.6$, $p < 0.001$).

An 8 day by 2 group ANOVA was performed to allow a closer examination of the first 8 days of the experiment. Main effects of days ($F(7, 98) = 4.2$, $p < 0.001$), and group ($F(1, 14) = 8.6$, $p < 0.05$) were evident. The day by group interaction was also significant ($F(7, 98) = 3.7$, $p < 0.01$) prompting individual examination of each group over the first 8 days. The 8 day repeated measures ANOVA performed on the day running indicated that rats given access during the day ran at a steady level with no increase over the 8 days ($F < 1$). In contrast, a similar ANOVA performed on the night running revealed an increase in running over the first 8 days ($F(7, 49) = 4.1$, $p < 0.01$). This suggests that as early as the first 8 days of 2 h access, rats given night access showed an increasing pattern of running

contrasting with the low steady running seen in rats given day access (Figure 9).

An 8 day by 2 group ANOVA performed on the last 8 days showed similar results. There was a main effect of days ($F(7, 98) = 4.9$, $p < 0.001$), and of group ($F(1, 14) = 17.3$, $p < 0.01$), and a significant interaction ($F(7, 98) = 4.2$, $p < 0.001$). Separating the analysis over the two groups, the day running group showed no change over the last 8 days ($F(7, 49) = 1.2$, p = ns) whereas the night running group ran an increasing amount even over the last 8 days ($F(7, 49) = 4.8$, $p < 0.001$).

Feeding

Figure 10 illustrates the daily feeding in both the 2 h day and 2 h night wheel running groups and the no wheel control. An overall 8 day by 3 group ANOVA shows a significant effect of days ($F(7, 154) = 19.8$, $p < 0.001$), a significant effect of group ($F(2, 22) = 4.0$, $p < 0.05$) and a significant day by group interaction ($F(14, 154) = 2.0$, $p < 0.05$).

Separate ANOVAs for each of the eight days were performed to explore the day by group interaction. Beginning with the first day after the switch, a one-way ANOVA showed a significant group difference ($F(2, 22) = 4.6$, $p < 0.05$). A subsequent Tukey test attributed this difference to a significant suppression of the day running group's feeding compared to the no wheel group's feeding ($p < 0.05$). Although the night running group appeared to eat less than the no wheel group, this difference is not significant ($p > 0.05$). On day 2, there is also a significant group difference ($F(2, 22) = 4.1$, $p < 0.05$) which a Tukey test shows is the result of a suppression in the night group's feeding compared to the day group's feeding ($p < 0.05$). The night running group was not significantly lower than the no wheel group on day 2 ($p = 0.07$). On the third day, the group difference

persisted ($F(2, 22) = 5.5$, $p < 0.05$). By this day, the night running group's feeding was suppressed compared to both the day running group ($p < 0.05$) and the no wheel group ($p < 0.05$). On day 4, the overall group difference remained significant ($F(2, 22) = 7.3$, $p < 0.01$), with the subsequent Tukey test again revealing that the night running group was suppressed compared to both the day running group ($p < 0.05$) and the no wheel group ($p < 0.01$). Day 5 also showed an overall group difference ($F(2, 22) = 5.9$, $p < 0.01$). On this day, a Tukey test revealed that the night runners were again showing a feeding suppression, this time compared to the no wheel group ($p < 0.01$). Analyses of days 6 to 8 following the switch no longer showed any significant differences between the groups. Overall, these results suggest that the 2 h day wheel access condition caused only a mild, 1 day feeding suppression, whereas 2 h night wheel access caused a longer feeding suppression lasting 5 days.

Discussion

Experiment 2 highlighted an important finding: in a direct comparison of two groups of rats given 2 h of wheel access during the day and during the night, night running rats ran at a much higher level in 2 h than day running rats ran in the same amount of time. Pilot data had suggested that there was no difference (Overduin, 1999), but this experiment has shown that rats given access during the dark cycle run much more than rats given access during the day. The night-running rats escalate running to arguably excessive levels, comparable to 24 h rats' 2 h maximums (Experiment 1, Figure 2). This suggests that two hours is a sufficient time to develop excessive running, provided that wheel access is given during the night cycle. It is interesting that rats are able to develop excessive running when given only two hours of running during the night. It confirms that

the lower running seen in the daytime is a function of differing motivation, and not an endurance plateau that cannot be surpassed with short access. This difference in motivation is consistent with rats' nocturnal nature.

The rats given 2 h access during the day in Experiment 2 showed a different pattern of running than the rats given 2 h day access in Experiment 1. Instead of the slight, gradual elevation in running seen with 2 h access in Experiment 1, the rats with 2 h per day access in this experiment show consistent low running that did not increase over the 24 days. This experiment, performed in the wheel rack, is consistent with previous data obtained in the rack (Lattanzio et al., 2000) and inconsistent with data from Nalgene wheels (Experiment 1), supporting the suggestion that running patterns may be affected by equipment differences.

Feeding in Experiment 2 will be addressed in the General Discussion in conjunction with Experiment 1 feeding results.

General Discussion

This thesis aimed to further the parallel between wheel running, which appears to be a rat form of exercise addiction, and drug addiction. As in Ahmed and Koob's (1998) study of differential daily access to cocaine, animals in Phase I of Experiment 1 given a "long" daily access to a running wheel showed the rapid escalation to excessive levels characteristic of addiction, whereas with "short" or 2 h daily access, the behaviour remained at a low level (Figure 1). Within these parameters, this finding was consistent with previous data collected in the lab (Lattanzio et al., 2000).

In these two experiments, however, it became evident that in certain conditions, two hour wheel access is sufficient to allow a minor elevation in running (Experiment 1,

Figure 1) and that if access is provided at night, two hour wheel access may be sufficient to allow the increase to excessive running levels (Experiment 2, Figure 9). This, as well as the feeding suppression sometimes observed with 2 h wheel access, suggests that 2 h per day access may be on the borderline between a "low" dose of exercise and a "high" dose. This issue will be addressed further at a later point in this discussion.

Three interesting findings in Experiment 1, Phase II, lend support to the suggestion that wheel running may parallel drug self-administration. The first was the gradual decrease in running in Phase II seen in animals switched from 24 h access to 2 h access (Figure 3). This decrease is reminiscent of the gradually decreasing cocaine self-administration pattern in animals switched from long (6 h) to short (1) daily sessions, (Ahmed & Koob, 1999) and suggests that similar changes have occurred in animals with a long history of wheel running as in animals with a long history of cocaine exposure. The surprising result that their running immediately following the switch to 2 h access was not elevated compared to the usual 2 h running level seen in the 2 h - 2 h group may have been related to the time of day the wheel was provided, since these rats were running maximum amounts during the dark cycle prior to the switch, but were subsequently provided restricted (2 h) access only during the light cycle. Because 2 h night running differs from 2 h day running (Experiment 2, Figure 9), it would be interesting to test the 2 h running level of rats given 24 h per day access and subsequently restricted to 2 h night access. Perhaps results would resemble those seen in Ahmed & Koob's (1999) experiment more exactly.

The second interesting finding was that rats switched from 2 h - 24 h showed an immediate elevation to excessive running, a much more rapid escalation than was seen in

the 0 h - 24 h group. Although they did not run at the maximum level possible while given restricted (2 h) access in Phase I, they were sufficiently fit that they were able to run high amounts immediately after the switch to 24 h access. This rapid transition suggests an increase in the salience of the rewarding effects of wheel running in the 2 h - 24 h group. As previously mentioned, this difference is not simply an effect of practice, since the 2 h - 24 h group has only 48 hours of experience by the time of the switch, and the 0 h - 24h group does not increase to high levels of running after the same amount of experience, specifically their first 2 days of wheel access in Phase II.

The third finding of interest in Experiment 1 was the lower running plateau seen in the 0 h - 24 h group. These rats ran less than rats of the same age with previous running experience (24 h - 24 h) suggesting that it is not simply age, but some consequence of the delay in wheel access that is responsible for the low plateau. Perhaps rats introduced to the wheel at a later time have a lower propensity for addiction.

Further experiments should test 1 h per day access in various conditions, an access level that may be too low to induce an elevation in running. It would be interesting to compare the effects of short 1 h sessions of wheel access to longer 6 h sessions, the session lengths used in Ahmed and Koob's (1999) cocaine study, to see if persistent elevation of behaviour will be seen with wheel running as with cocaine self-administration when procedures are paralleled more closely. With respect to the chosen session length, the question arises: is the daily session of exposure required to produce "addiction" similar across various reinforcers that are potentially addictive?

In Experiment 2, rats given wheel access during the early part of the night cycle ran at higher levels than the rats given wheel access during the latter part of the day cycle.

Although rats are nocturnal and prefer to run during the dark part of the cycle when given continuous access, this difference between day and night running was surprising based on previous data collected in the lab. Previous experiments suggested that rats overcome their circadian cycle in order to take advantage of the wheel access provided (Lattanzio et al., 2000), and that rats given 2 h access during the day run similar amounts to rats given 2 h access during the night (Overduin, 1999). In the current experiment rats were provided wheel access at times clearly dissociated from the transition from light to dark, so that any difference in running can clearly be attributed day vs. night access.

In light of the paralliels between drug self-administration and voluntary wheel running, it is interesting to examine the effects of time of day on drug self-administration paradigms. Many studies have documented differences in drug action depending upon the time during the 24 h cycle the drug is administered (Moore-Ede, 1973). More specific to the current experiment, studies have shown that the reinforcing properties of drugs are also affected by the circadian cycle of the animal, as demonstrated by patterns of ethanol self-administration in rats (Gauvin et al., 1997). Surprisingly little research has been conducted on the chronopharmacology of cocaine, and the studies that exist do not show strong or conclusive differences in intake depending on time-of-day. A recent experiment (Baird & Gauvin, 2000) examining rates and patterns of cocaine self-administration at four times of day (early day, mid day, early night and mid night) suggested that rats may have a higher sensitivity to low dose cocaine during early day and early night, as compared to mid day and mid night. There was no evidence to suggest that pure day vs. night differences occurred, however, only established self-administration behaviour was explored. In light of the current results, it might be interesting to look at the chronopharmacology of the

acquisition of self-administration.

The results of Experiment 2 do temper the conclusions we may draw from Experiment 1. If day and night running were similar, which is what may have been expected, the low level of running seen in the 2 h groups of Experiment 1 would clearly be a result of limited access. Because night running in Experiment 2 was elevated compared to day running, and even approached the maximum 2 h running seen in the 24 h group of Experiment 1, the low level of running in the 2 h groups of Experiment 1 appears to be influenced by the time of day wheel access was provided. In order to more clearly dissociate the effects of time of day and length of daily access, further studies must be performed wherein rats are provided short and long access during both the light and the dark cycles.

Recent research has focused on the physiological changes marking the switch to addiction, and the long-lasting changes producing tolerance and sensitization. Studies show structural changes in the neurons of the nucleus accumbens and prefrontal cortex following administration of addictive substances such as morphine, cocaine and amphetamine. Neurons of the ventral tegmental area (VTA), are known to project to other areas of the mesolimbic dopamine system, part of the important pathway signaling opiate reward. Sklair-Tavron et al. (1996) provided evidence that chronic administration of the opiate morphine induced permanent changes in dopamine neurons found in the VTA. Specifically, the area and perimeter of dopamine neurons was reduced by 25% in chronic morphine treated animals, although the number of dopamine neurons remained constant. Nondopaminergic neurons within the same brain region appeared unchanged.

Other structural changes resulting from repeated morphine exposure have also

been reported. Robinson and Kolb (1999b) found a decrease in the branching of dendrites and a reduced number of spines on medium spiny neurons of the nucleus accumbens and on pyramidal cells in the neocortex in rats after repeated morphine injections. These researchers went on to test for changes in neurons in these brain areas after repeated cocaine and amphetamine treatment (Robinson & Kolb, 1999a) and found quite the opposite effect: amphetamine and cocaine experience resulted in an increase in the number of dendritic branches, as well as increased density of dendritic spines. This difference between the effects of morphine and cocaine are surprising, but a control group in this experiment was of special interest. As a control for increased activity levels in the animals treated with stimulant drugs, a wheel running group was included in their analysis. The rats in this group were given 4 weeks with free (24 h per day) access to wheels, and reportedly ran approximately 5.43 km per 24 hours. Upon analysis 24 days after wheel access ended, rather than showing the same increases in dendritic branching and spines as the amphetamine and cocaine treated groups, rats actually showed a significant decrease in dendritic branching. Interestingly, this decrease in neuron size more closely parallels the effects seen above with morphine administration (Robinson & Kolb, 1999b). As wheel running is thought to activate endogenous opioid systems, it may not be surprising that the neuronal effects from an opiate such as morphine are similar to the effects of wheel running.

Care must be taken not to overextend the similarity seen in morphine (Robinson & Kolb, 1999a, 1999b) and wheel running effects. These morphological changes were found in two different studies, so a separate study would have to be performed in order to

compare the results directly. In addition, the drug studies mentioned above all include experimenter-administered injections of the drugs. Wheel running is a voluntary behaviour, and therefore is more appropriately compared to the self-administration of rewarding drugs. Self-administration studies performed with cocaine have shown similar neural effects to the experimenter-administered drug studies (Robinson, Gorny, Mitton & Kolb, 2001), but self-administration studies using morphine have not yet been performed. Despite these issues, the suggestion that wheel running may cause long-lasting effects in the neurons of the mesolimbic dopamine system similar to morphine is very intriguing and demands further exploration.

The feeding data presents an interesting story. Almost all animals in both Experiment 1 and Experiment 2 (including the control animals which were in the same wheel condition before and after the switch) ate less than the normal amount on the first day after the switch. A feeding disruption of a single day is not characteristic of the feeding suppressions usually seen with wheel introduction. Instead, it mirrors closely the effects on feeding seen when animals experience environmental disruptions such as unusual loud noise, changes of cages, movement from individual to paired housing conditions, or an experience with a procedure unfamiliar to them (Lopak & Eikelboom, 2001). Thus the one day of low feeding seen in many of the groups is likely due to the disruption in their routine: they were moved into clean cages, some with a wheel, some were moved to different rooms and all experienced more noise than usual. The long-lasting feeding effects that are observed in the 0 h to 2 h, the 0 h to 24 h and the 2 h to 24 h groups of Experiment 1 and the night group of Experiment 2 are more characteristic of

wheel running induced effects.

In Experiment 1, Phase II, the shift from 0 h - 2 h wheel exposure during the day appears to be sufficient to induce feeding disruptions. This suggests that the effects of the running on feeding are long lasting and that the drop in feeding is not due to direct competition for the time needed to run. In Experiment 2, however, rats given 2 h wheel access during the day did not show a feeding suppression beyond the first day following wheel introduction. This was unusual, as previous studies (Lattanzio et al., 2000) and Experiment 1 show distinct feeding suppressions with 2 h wheel access. Comparing the running (see Figure 2, 2 h group and Figure 9, day group), the rats in the day group in Experiment 2 are running at a low level compared to that usually seen in 2 h rats. Perhaps the low levels of running in this group were insufficient to activate the dopamine system, and so did not elicit the feeding suppression.

It is also interesting that the group moved from 2 h to 24 h in Experiment 1 shows a feeding suppression. Even though this group is familiar with the wheel, the rats show a suppression in feeding that corresponds to their acquisition of excessive running.

Studies have shown that a high dose of amphetamine inhibits food intake (Dobrzanski & Doggett, 1976), and that this effect is suppressed by the prior administration of dopamine antagonists such as haloperidol (Evans & Vaccarino, 1990). These data are consistent with the suggestion that activation of the dopamine system is mediating the feeding suppression. Others have speculated that the wheel-induced feeding suppression is also caused by dopaminergic activation (Lett, Grant & Gaborko, 1996). Our data would suggest that increased dopaminergic activation is taking place in animals

given a wheel for 2 h as well as for 24 h per day, and also for rats given increased access from 2 h to 24 h.

In contrast to the feeding suppressions seen with high doses of amphetamine, low doses can cause an elevation in feeding (Evans & Vaccarino, 1987; Evans & Eikelboom, 1987). Recent research has determined that low "doses" of wheel running, specifically 30 minutes in an activity wheel, may also cause an immediate elevation in feeding (Lett et al., 1996). Rats in the 2 h wheel running condition show a suppression in daily feeding rather than an elevation, but food measurements were taken daily instead of in the hour after wheel access. It is possible that this group did show an immediate elevation in feeding that was lost within daily food measurements.

There is a suggestion of a feeding elevation in the group moved from 2 h to 0 h compared to the 2 h - 2 h group (Figure 7), although it is not significant in the post-hoc tests used. Previous studies have noted feeding elevations which occur when wheel access is denied to animals accustomed to wheel running (Premack & Premack, 1963). In these studies animals were given continuous access before deprivation. Strangely, we did not see a feeding elevation in animals moved from 24 h to 0 h. A future experiment could directly test for this feeding elevation in animals deprived of wheel access.

Overall, it appears that wheel running may potentially be used to model drug-taking behaviour in rats. If further research shows that wheel running differs from drug self-administration, then wheel running may provide important insights into how artificial stimulation of the reward system differs from the stimulation provided by natural behaviours. If after further experiments wheel running continues to prove an effective

model of addictive behaviours, the implications are numerous. Wheel running provides a relatively simple, easy to measure and non-invasive method of manipulating a rewarding behaviour. Because it is simple, the behaviour itself is not complicated by side effects, and therefore gives a quite accurate index of a rat's motivation. Simple animal models become powerful tools in the effort to understand the complexities of addiction.

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Figure Captions

Figure 1: Daily mean total number of wheel turns (± SEM) per day in the six groups given either 2 h or 24 h wheel access over the 24 days of Phase I, Experiment 1.

Figure 2: Mean number of wheel turns in two hours (± SEM) for all animals given 2 h wheel access compared to the mean maximum number of wheel turns in two hours (± SEM) for all animals given 24 h wheel access over the 24 days of Phase I, Experiment 1. For 2 h animals, these values are the same as their daily totals. The values for the 24 h group were obtained by extracting the single 2 h block with the highest amount of running per day for each rat, and calculating the average.

Figure 3: Daily mean total running in three groups moved to 2 h wheel access (± SEM) over the 24 days of Phase II, Experiment 1.

Figure 4: Daily mean total running in three groups moved to 24 h wheel access (± SEM) over the 24 days of Phase II, Experiment 1.

Figure 5: Mean maximum 2 h running in the three groups moved to 24 h access (± SEM) over the 24 days of Phase II, Experiment 1. Maximum determined as in Phase 1 of the experiment, (Figure 2).

Figure 6: Eight days post-switch of mean daily food (in grams) consumed by the three groups moved out of the 0 h wheel condition (± SEM) in Phase II, Experiment 1.

Figure 7: Eight days post-switch of mean daily food (in grams) consumed by the three groups moved out of the 2 h wheel condition (± SEM) Phase II, Experiment 1.

Figure 8: Eight days post-switch of mean daily food (in grams) consumed by the three groups moved out of the 24 h wheel condition (± SEM) in Phase II, Experiment 1.

Figure 9: Daily mean number of wheel turns (± SEM) per day in the daytime 2 h access group compared to the nighttime 2 h access group over 24 days, Experiment 2.

Figure 10: Eight days of mean daily food (in grams) consumed by the 2 h day group, the 2 h night group and the no wheel group (± SEM), Experiment 2.

Figure 1 - Total daily running, Phase I

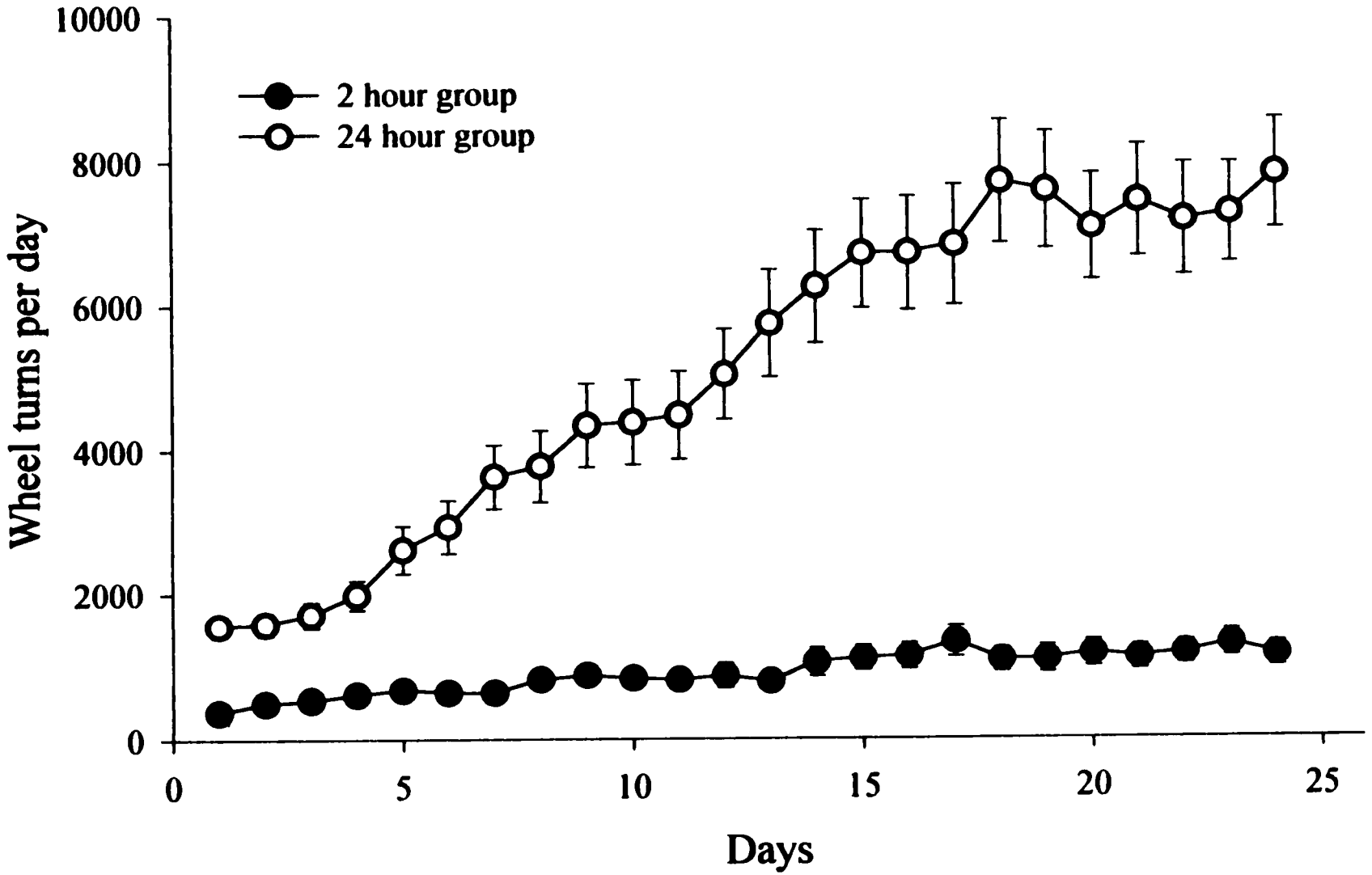

Figure 2 - Maximum wheel turns in two hours, Phase I

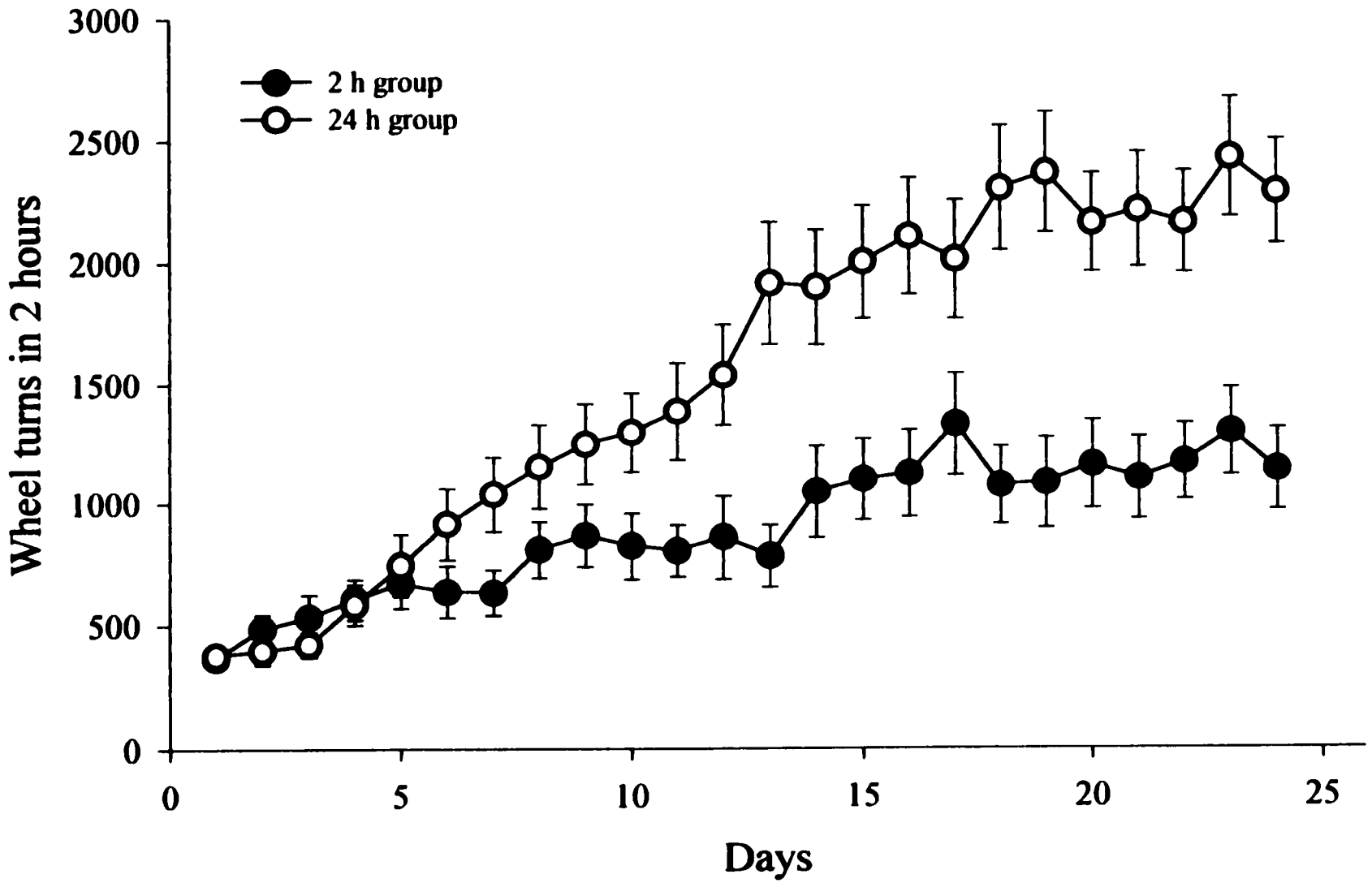

Figure 3 - 3 groups moved to 2 h wheel access, Phase II

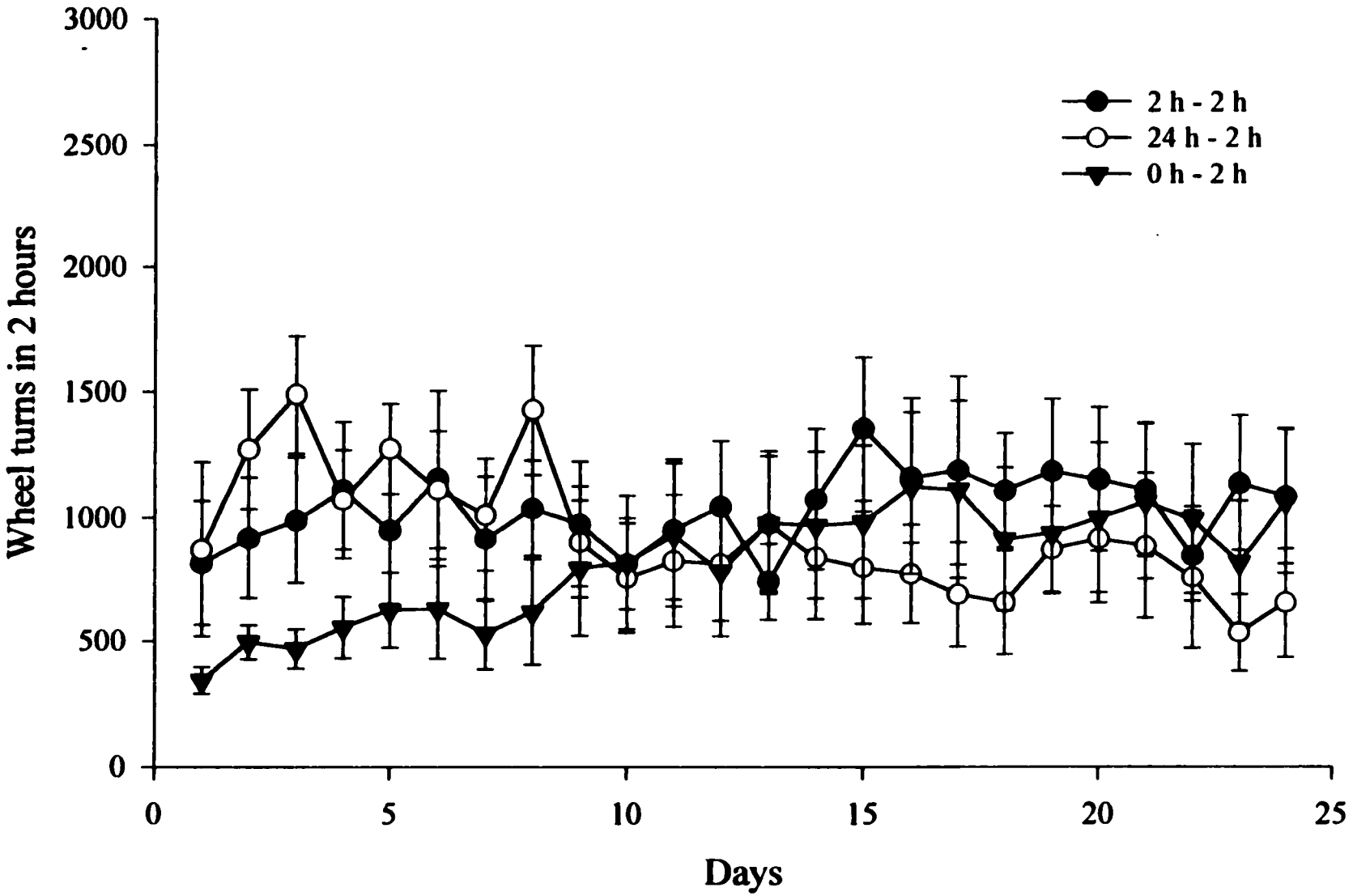

Figure 4 - 3 groups moved to 24 h wheel access, Phase II

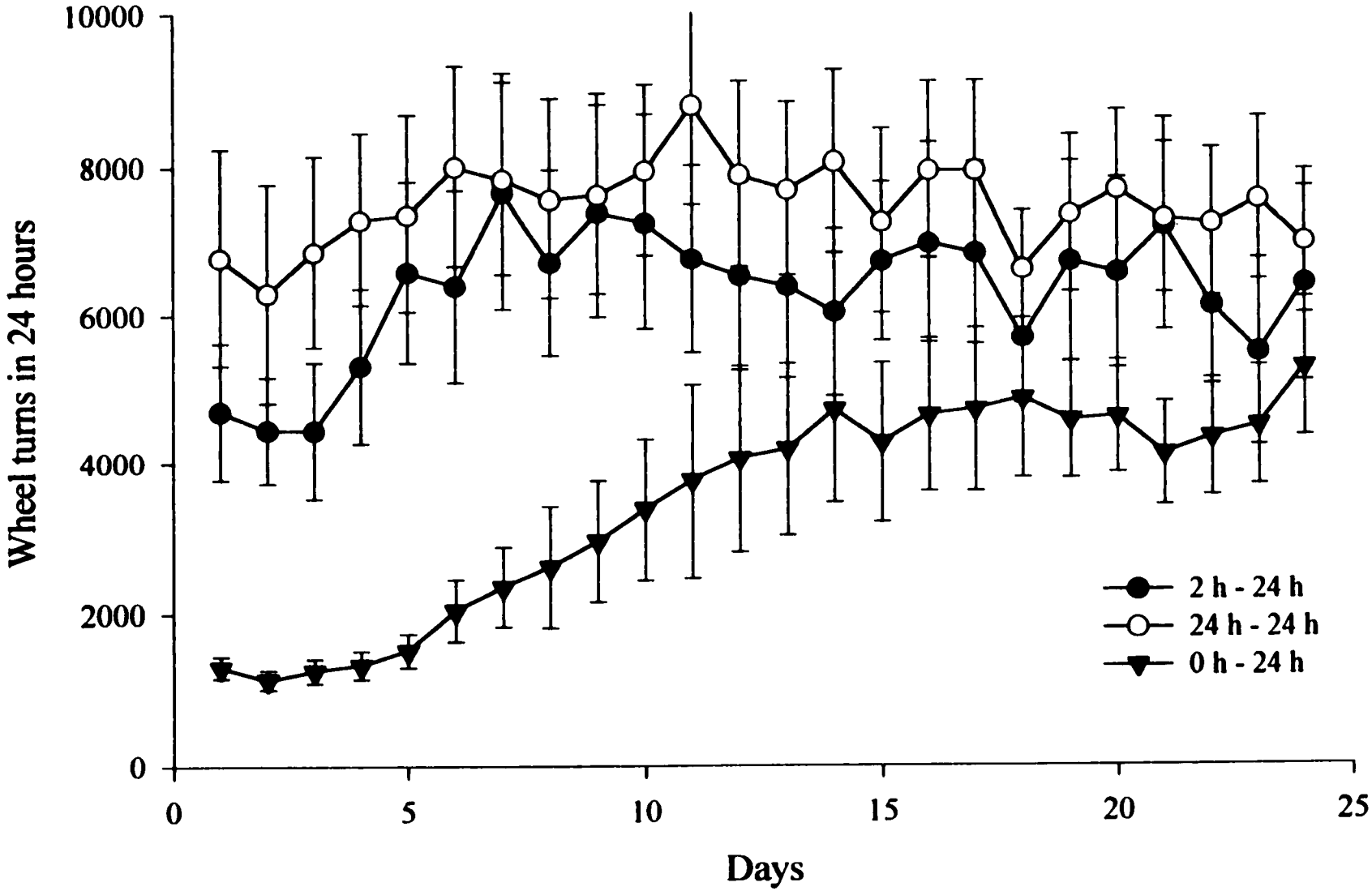

Figure 5 - Maximum running in groups moved to 24 h access, Phase II

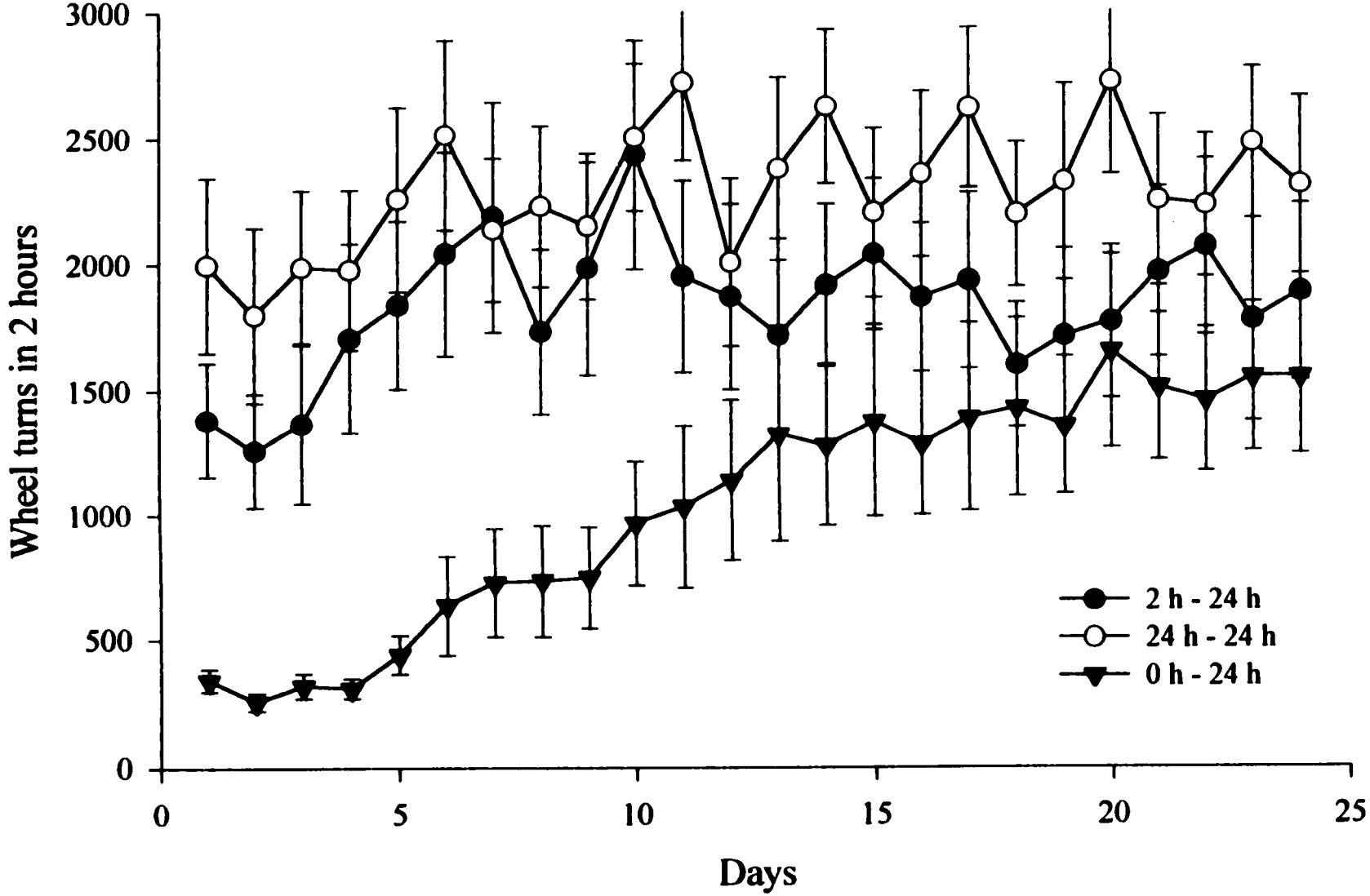

Figure 6 - Feeding in groups moved from 0 h access, Phase II

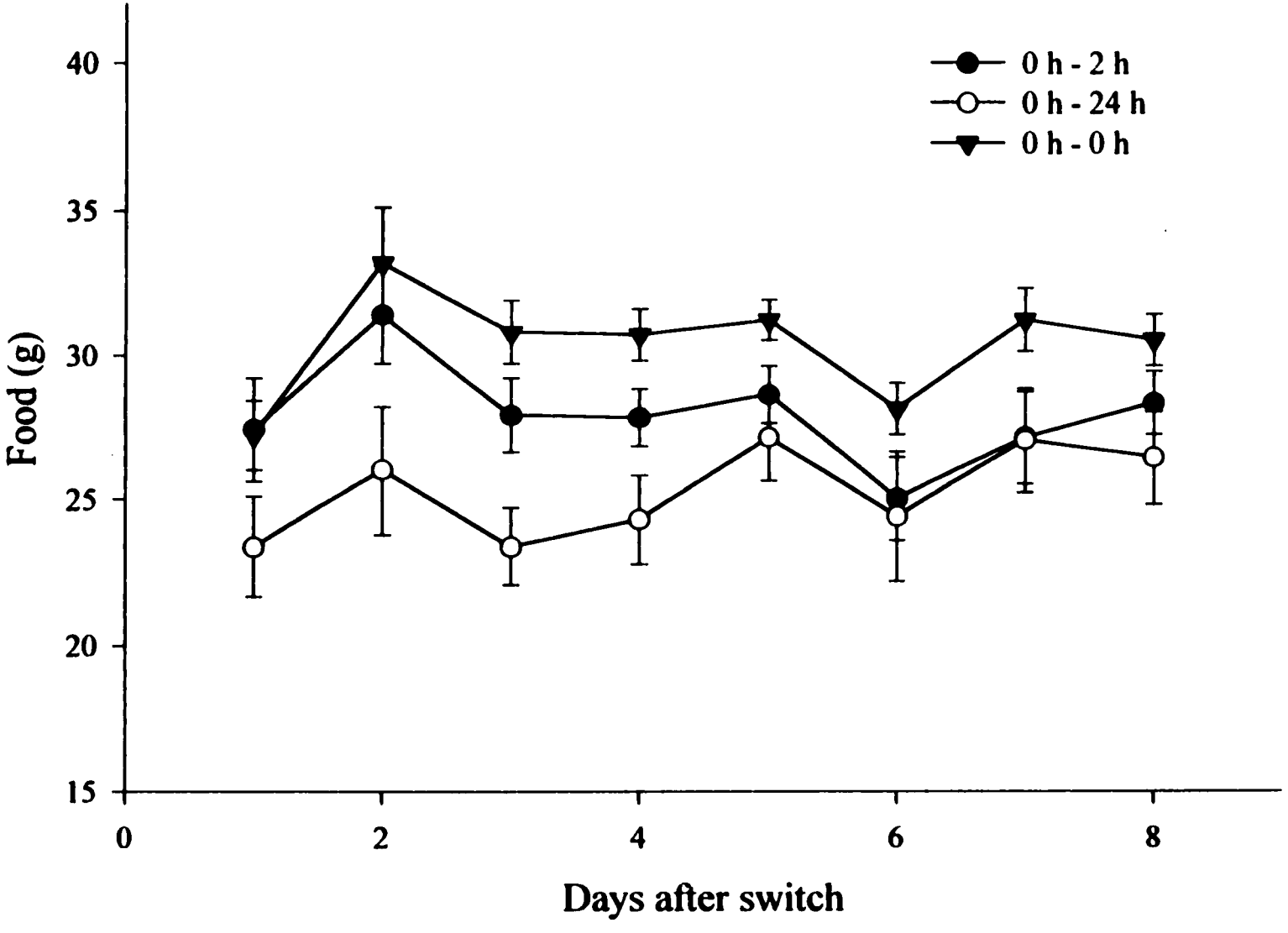

Figure 7 - Feeding in groups moved from 2 h access, Phase II

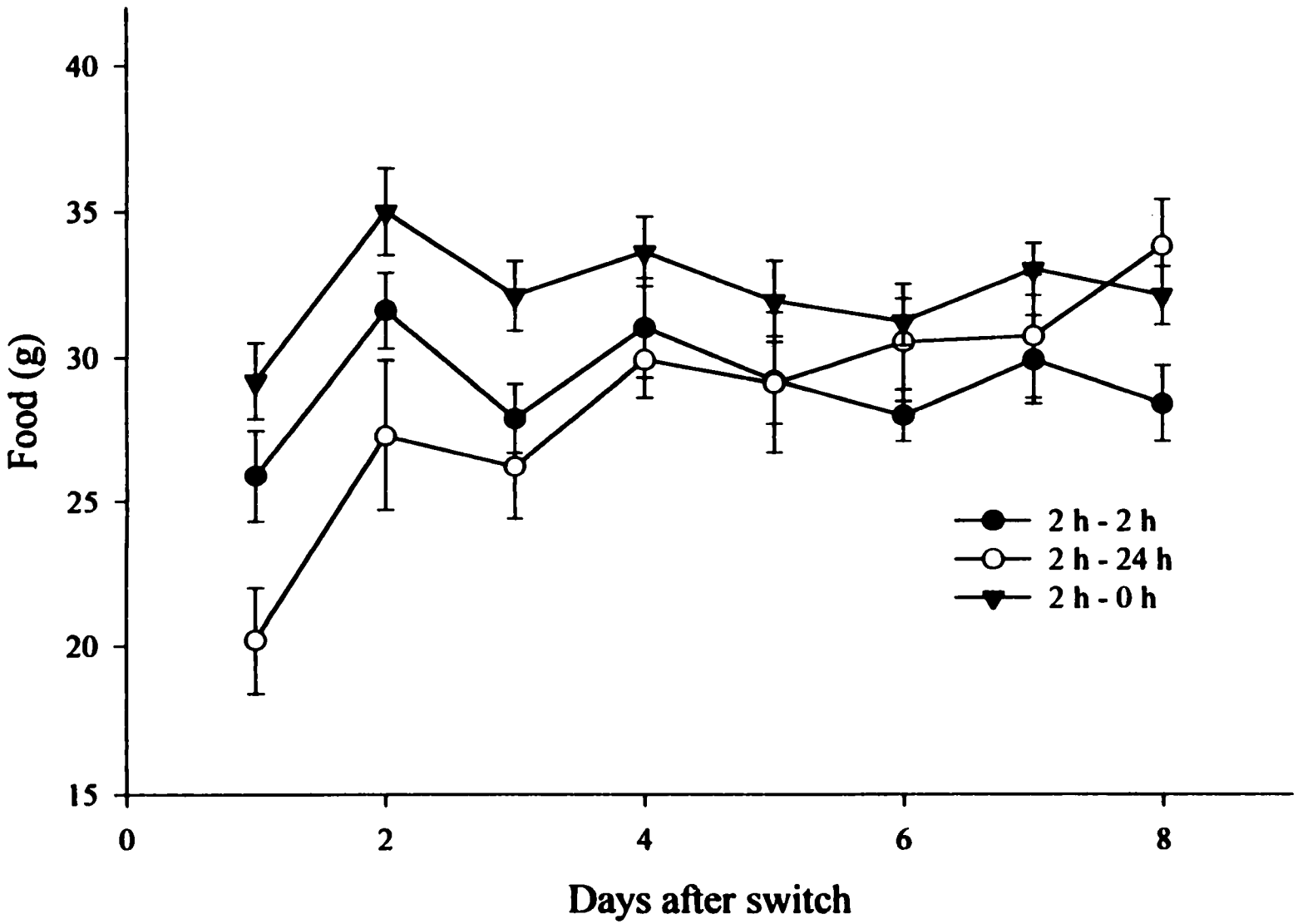

Figure 8 - Feeding in groups moved from 24 h access, Phase II

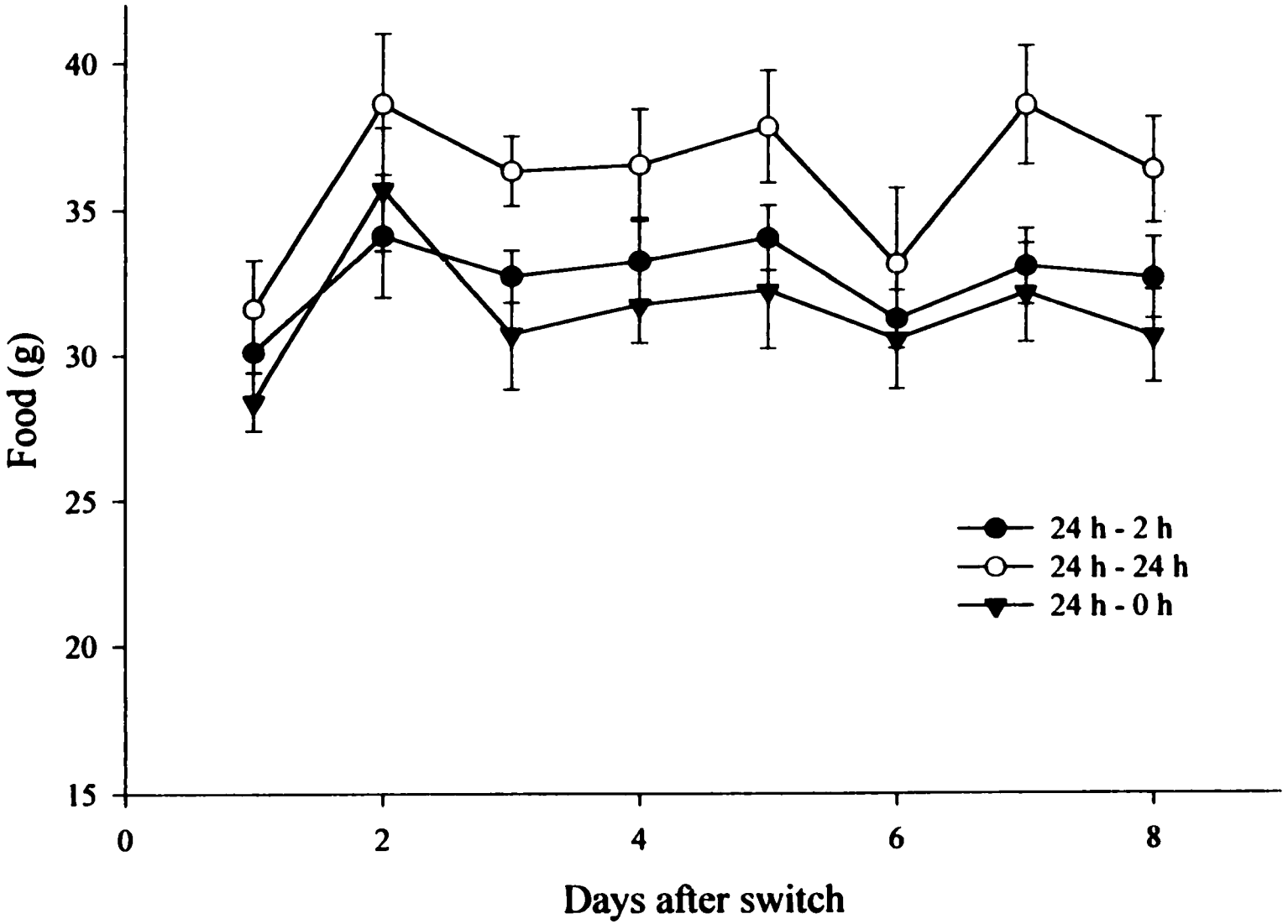

Figure 9 - Experiment 2, 2 hour running totals, day vs. night

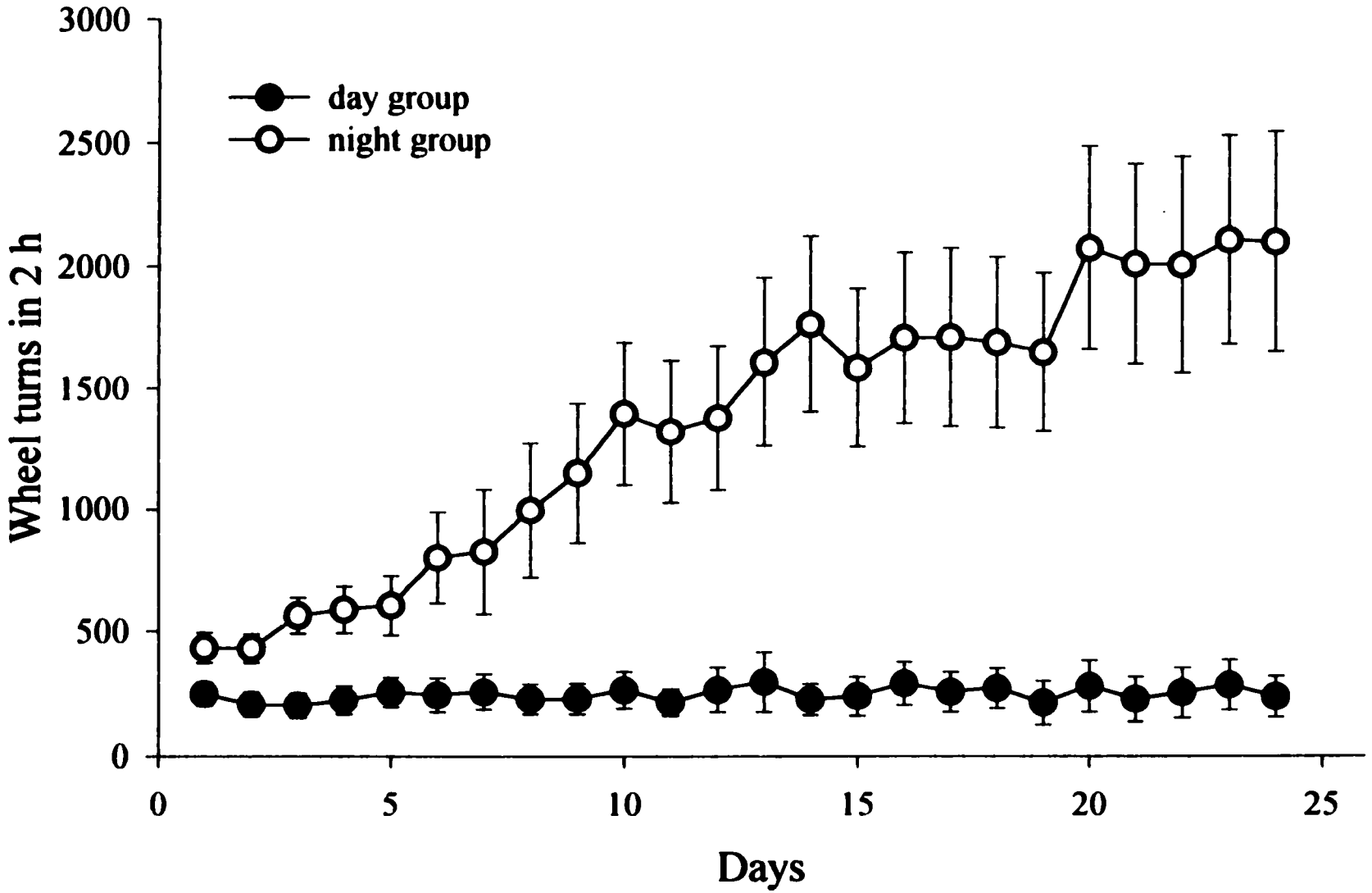

Figure 10 - Experiment 2, Feeding in day, night and no wheel groups