# SCHEDULED ACTIVITY REORGANIZES CIRCADIAN PHASE IN HAMSTERS UNDER FULL AND SKELETON PHOTOPERIODS

by

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Scheduled Activity Reorganizes Cricadian Phase in Hamsters Under Full and Skeleton Photoperiods

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## Abstract

Circadian rhythms in rodents can be phase shifted by appropriately timed activity bouts or light pulses. To examine the possible interactions of these two stimuli in experiment 1 Syrian hamsters, housed in cages with activity wheels were entrained to a 14:10 full photoperiod (FPP) and were induced to run in the middle of their subjective day. Four of 11 hamsters showed high levels of induced wheel running and prominent and sustained phase delays of nocturnal activity onset (260 $\pm$ 63 min). In Experiment 2 a skeleton photoperiod (SPP, two 30 minute light pulses) was used to better simulate natural dawn and dusk light exposure patterns, and to facilitate observation of transients indicative of possible oscillator decoupling. Adult male Syrian hamsters were entrained to a14:10 LD SPP. Exercise was scheduled at one of 5 phases of the SPP. Some of the animals exercised in the middle of their inactive period (ZT4-7) showed 180° inversion of their activity rhythms. Inversion in one animal appeared to be achieved by partition. Animals that did not invert showed small phase advances (18 $\pm$ 7 min). Hamsters run late in the subjective day to early in the subjective night (ZT9.5-12.5) exhibited a mix of advances (N=7; 63 $\pm$ 24 min) and delays (N=14, -80 $\pm$ 10 min). Exercise near the end of the active period (ZT21-24) caused large phase delays of entrained rhythms (-238 $\pm$ 30 min, t = 8.01). Exercise at other phases late in the activity period (ZT19-21 and ZT20-23) had minimal effects. In addition there

was a significant effect of ZT of wheel confinement on free-running $\tau$ in DD. The ZT21 group showed a significantly longer $\tau$ than the groups run at ZT9.5, 19 and 20. These experiments demonstrate that exercise can have powerful effects on phasing of activity rhythms entrained to both FPPs and SPPs. In addition, these results may be more ecologically valid then past research because SPPs may better simulate patterns of light exposure in nocturnal and diurnal animals, such as humans, that may spend most of the day shielded from natural light.

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Table of Contents

Approval ..... ii
Abstract ..... iii
Acknowledgments ..... v
List of Tables ..... viii
List of Figures ..... ix
General Introduction ..... 1
Relationship between light and activity ..... 4
Experiment 1 ..... 8
Method ..... 8
Data Analysis ..... 9
Results ..... 10
Discussion ..... 11
Experiment 2 ..... 12
Method ..... 13
Data Analysis ..... 14
Results ..... 14
Discussion ..... 17
Experiment 3 ..... 19
Method ..... 19
Results and Discussion ..... 19
General Discussion ..... 20
Phase changes and splitting: 2 oscillator models ..... 20
Activity and light pulses: inhibition and masking responses ..... 27
The nature of the activity zeitgeber ..... 29

Conclusion ..... 30

References ..... 32

List of Tables

Table 1: Group means of wheel revolutions during 3 h of novelty-induced running in a SPP ..... 38

Table 2: Group means for $\tau$ in DD ..... 39

List of Figures

Figure 1: Examples of activity charts for hamsters ran at ZT4 ..... 41

Figure 2: Scatterplot illustrating the relationship between phase shift and wheel running for ZT4 ..... 44

Figure 3: Bar graphs illustrating mean levels of running group phase shifts and group taus. ..... 46

Figure 4: Examples of activity charts for hamster ran at ZT5 ..... 50

Figure 5: Scatterplots illustrating the relationship between phase shift and wheel running for hamsters run in a skeleton photoperiod ..... 55

Figure 6: Examples of activity charts for hamsters ran at ZT9.5, ZT13, ZT19, ZT20 and ZT21 ..... 62

Figure 7: Examples of activity charts for hamsters with morning and evening light pulses removed ..... 70

# Introduction

Traditionally, it has been assumed that circadian pacemakers regulate the timing of rest-activity states and specific behaviors and that the motion of these pacemakers is not subject to modulation by the expression of these states or behaviors. More recently, however, it has become clear that, at least for some rodents, certain behaviors, such as wheel running, can have a dramatic effect on the timing of circadian rhythms. The idea that activity, or associated arousal, might influence circadian rhythms was first hinted at by Aschoff (1960) following his formulation of the "circadian rules"; in nocturnal animals, the brighter the light, the longer the free-running period ($\tau$) of circadian activity rhythms, the shorter the duration of the daily active phase, and the lower the level of activity. Although light was assumed to affect the pacemaker directly, Aschoff did not rule out the possibility that a "reduced level of excitement" in bright light, as opposed to the light itself, might be responsible for slowing the clock. In support of this possibility, Aschoff, Figala & Poppel, (1973) later reported evidence that the free-running $\tau$ of circadian rhythms in hamsters might be altered by access to running wheels. More recent work with rats and mice has shown clearly that $\tau$ is rapidly and chronically shortened by access to a running wheel, indicating that activity or arousal does have a tonic, modulating effect on the circadian pacemaker (Edgar, Martin

& Dement, 1991; Yamada, Shimoda, Ohi, Takahashi & Takahashi, 1988).

In addition to this tonic, or feedback, effect of spontaneous wheel running activity on the $\tau$ of circadian rhythms, a single bout of running activity can acutely phase shift circadian rhythms in constant light (LL) or dark (DD). For example, using the procedure of placing hamsters in a novel wheel to induce wheel running, Reebs and Mrosovsky (1989a) found that 2 h sessions of wheel running caused phase advances when scheduled during the mid-to-late subjective day (the usual inactive period in nocturnal animals) and phase delays when scheduled early in the subjective day. A maximum average phase shift of about .6 h occurred following running bouts induced at circadian time (CT) 6 (i.e., 6 h before the usual onset of the daily active period, designated by convention as CT12). However, in a later study it was found that longer bouts of activity (3 h) resulted in much larger phase shifts (Reebs & Mrosovsky, 1989b), averaging about 3 h when running was induced at CT6. Activity induced shifts are thus easily large enough to stably entrain free-running activity rhythms if produced each day (Mrosovsky, 1988; Reebs & Mrosovsky, 1989a & b).

The importance of locomotor activity or arousal as a circadian zeitgeber or modulator of circadian phase is underscored by evidence that phase shifts induced by a variety of different stimuli are dependent on activity triggered by these stimuli. For

example, dark pulses (Reebs, Lavery & Mrosovsky, 1989; Van Reeth & Turek, 1989), injections of triazolam (Mrosovsky & Salmon, 1990; Turek & Losee-Olsen, 1986), social interactions or litter changes (Mrosovsky, 1988), cold (Mistlberger, Marchant, & Sinclair, 1996) and refeeding after a fast (Mistlberger, Sinclair, Marchant, & Neil, 1996) all can induce arousal and running in hamsters, and all can shift the phase of free-running circadian rhythms. For some of these stimuli, it has been explicitly noted that the larger the running response, the bigger the phase shift (e.g., Mistlberger et al, 1996). Furthermore, the phase-response curves (PRC; the plot that depicts the observed relationship between the circadian time when a zeitgeber is given and the phase shift obtained) for these stimuli are similar to each other, and similar to that for novel wheel-induced activity. If hamsters are prevented from running after triazolam injections (Mrosovsky & Salmon, 1990; Van Reeth & Turek, 1989) or during novel wheel confinement or dark pulses (Reebs, Lavery & Mrosovsky, 1989; Van Reeth & Turek, 1989), phase-shifts are attenuated or absent. Immobilization alone produces negligible or no phase-shifts at most circadian phases (Mrosovsky & Salmon, 1990; Van Reeth, Hinch, Tecco & Turek, 1991). These findings indicate that phase-shifts to these stimuli are at least strongly predicted by the running response. Causal attribution is qualified by observations that some animals may exhibit shifts to arousing non-photic stimuli despite relatively minor running responses (e.g.,

Mistlberger et al, 1996), whereas other animals may exhibit maximal running responses but no shift (Janik & Mrosovsky, 1993; Mrosovsky & Biello, 1994). Whether this variability is due to individual differences in pacemaker sensitivity to activity or to some systematic influence of the context within which activity occurs is unclear.

Relationship between light and activity.

Although both light and activity can produce phase-shifts in hamsters the effects of these two stimuli are very different. The PRC for light shows that nocturnal rodents phase delay when exposed to a light pulse early in its subjective night and phase advance when exposed to light late in its subjective night (Pittendrigh & Daan, 1976a). During the subjective day, light has little effect. In contrast, bouts of locomotor activity cause large phase advances when induced during the mid-to-late subjective day and small phase delays when induced in the subjective day (Mrosovsky, 1988; Reebs & Mrosovsky, 1989a).

Relatively little work has been done to explore how activity and light might interact to affect the phase of circadian rhythms. In one study, hamsters re-entrained to an 8 h advance of the LD cycle more than three times faster if induced to run for 3 h in a novel wheel, beginning 1 h after the onset of the new dark period (or 7 h prior to the hamsters' normal activity start time (Mrosovsky & Salmon, 1987). These hamsters showed average phase-shifts of 7 h by the second day after the LD shift, which is

more than twice that of control hamsters. Analysis of the contribution of light and induced running indicated that changes in light following the LD shift could be expected to account for about 3.1 h of shift, and running about 2.9 h of shift (Reebs & Mrosovsky, 1989a). The combination of the two phase-shifts did not add up to the 7 h shifts observed when light and activity were combined. However, the authors suggested that the total of the effects were close enough to 7 h that they may be considered purely additive, although some synergism is possible (Mrosovsky, 1996).

More recent studies suggest that light and activity in combination may affect circadian rhythms in ways that are not predictable from their respective PRCs. For example, it has been shown that activity may attenuate the effects of light; hamsters that exhibited wheel-running at the time of a light pulse showed smaller phase shifts on average than hamsters that were not active at the time of the pulse (Ralph & Mrosovsky, 1992). Wheel running alone at this circadian phase has little or no phase-shifting effect, thus, the attenuation was not likely due to an offsetting phase shift.

Conversely, light pulses may attenuate or block phase shifts to activity-inducing stimuli, including novel wheels (Mrosovsky, 1991) and triazolam (Joy & Turek, 1992). Light pulses alone at the phases tested had no effect, suggesting that it was not simply producing an independent shift in the opposite direction.

However, transient distortions of the photic PRC immediately following the non-photic stimulus could not be ruled out. In either case, the results indicate that light and activity may interact non-linearly.

Other studies have examined the effects of activity-inducing stimuli on the phase of circadian rhythms in hamsters entrained to standard LD cycles, but these studies have yielded somewhat differing results. One group of studies has yielded results predictable from the non-photic PRC. In these studies, activity inducing stimuli, including exposure of male hamsters to estrous females (Honrado & Mrosovsky, 1989), triazolam injections (Van Reeth & Turek, 1989) and novel wheel confinement (Reebs & St-Coeur, 1994), produced small advances of entrained phase, measured either within animals, or by comparison with other groups stimulated at different phases. Two other studies yielded somewhat different results. In one, hamsters run in novel wheels in the middle of the day did not exhibit significant advances, although hamsters run late in the night did show significant delays of entrained phase (Mistlberger, 1991). The mid-day runners may have failed to shift because they did not run sufficiently (cf. Biello, Janik, & Mrosovsky, 1994; Janik & Mrosovsky, 1993). However, in the other study, hamsters induced to run in the middle of the day, with the lights off to promote activity, exhibited very large delays of entrained phase (Mrosovsky & Janik, 1993). Moreover, when subsequently

recorded in DD, their rhythms were observed to be split, as if scheduled mid-day activity had dissociated the pacemaker into two components. Neither delays nor splitting are predicted from the nonphotic PRC.

Most organisms, including humans, do not live in constant lighting, thus if activity is to be exploited as a means to regulate human circadian rhythms, it will be important to elucidate more clearly the rules by which light cycles and activity combine to set circadian pacemakers. The purpose of the current research, therefore, is to further investigate the effect of scheduled activity on the phase of circadian rhythms in hamsters entrained to LD cycles. In experiment 1 hamsters were entrained to a full 14:10 photoperiod and run in the middle of the light period. In experiment 2, hamsters were entrained to a skeleton photoperiod (SPP), consisting of two 30 minute light pulses 10 h apart. A SPP was used for two reasons: First, SPPs minimize 'masking' effects of light and thus may permit observation of rhythm 'transients' indicative of splitting that might be induced by competing photic and nonphotic inputs to the circadian system. Second, the SPP may be a better representation of natural dawn and dusk light exposure patterns characteristic of nocturnal rodents or even diurnal, urbanized humans (cf. Eastman, 1990; Kripke 1981). Phase-shifts evident under this reduced lighting schedule may thus give more insight into real world effects of scheduled activity on circadian rhythms.

## Experiment 1

In this experiment hamsters were run in the middle of the light period of a full 14:10 LD cycle. Previous studies in which hamsters have been subject to novelty-induced running at this time have, as noted, produced somewhat variable results. One study reported results consistent with small phase advances (Reebs & St-Coeur, 1994), one reported large delays (Mrosovsky & Janik, 1993) and a third reported no significant shift (Mistlberger, 1991b). In addition, one of the studies reported that activity rhythms were split on the first day of DD after 17 days of scheduled activity in LD (Mrosovsky & Janik, 1993), whereas neither of the other two studies observed splitting, despite using a similar protocol of LD followed by DD. In view of these discrepancies, which could be related to differences in light levels utilized, the level of activity induced by wheel confinement, or the availability of a home-cage running wheel, we felt that at least one more examination of the effects of exercise at this time of day was needed.

### Method

Male, Syrian hamsters were obtained from Charles River (Montreal) at approximately 8 weeks of age and were group housed in a colony room with controlled lighting (LD 14:10) and temperature (18°C). After approximately one week the hamsters (N=11) were then transferred to open field cages (92 x 61 x 36 cm) equipped with a nest box (12 x 12 x 12 cm) and running

wheel (36 cm di). The open field cage was constructed of wire mesh and the nest box was constructed of plastic that had been painted to black so no light would be permitted to enter it. The hamsters were allowed to adjust for at least 12 days before the onset of daily sessions of novelty induced activity. Activity was induced for 3 h each day for 17 days by locking the hamsters in a novel running wheel 8 h prior to dark onset, i.e.., at Zeitgeber Time (ZT) 4 (by convention, ZT12 represents dark onset). The animals were maintained in a 14:10 LD cycle during this time. To promote higher levels of activity, light levels were reduced to DD (<1 lux, dim red) during the 3 h novel wheel confinement. After the 17 days of scheduled activity the LD cycle was replaced by DD beginning at the first scotophase after the last session of induced activity, and lasting 10 days.

Data Analysis

Wheel running activity was detected by microswitch closures monitored by microcomputer, using the Activity Counting System interface and software (Simon Fraser University). Activity data were summed and stored in 10 min. bins, and periodically downloaded to a Macintosh for plotting and analyses using *Circadia* (Simon Fraser University).

To assess phase-shifts the mean nocturnal activity onset time for the 10 days prior to scheduled exercise was compared with the activity onset time on the first day of DD following the end of the daily exercise schedule. Activity onsets were detected

by computer and were defined as the first 10 min. bin exceeding 100 wheel revolutions, after 240 min. during which this level was not exceeded. All means are reported ± SE.

To determine if there was a relationship between the size of the phase shift and the amount of activity shown by each animal the mean levels of wheel running expressed during the 3 h of scheduled activity was correlated with the size of the phase shifts. In subsequent experiments, most animals had access to a 17.5 cm wheel, therefore the activity counts for the animals in this study were multiplied by a factor of 2.12 to produce a standardized activity measure appropriate for between group comparisons.

$\tau$ of free-running rhythms during the first 8 days in DD (excluding days 1-2 if transient cycles were evident) was estimated from the slope of a least squares regression line fit to activity onsets.

Results

During the daily activity schedule, 7 of 11 hamsters showed relatively low levels of induced wheel running during the last week of scheduled activity (1196 ± 321 revs/3 h) and little or no phase shift in the time of nocturnal activity onset as measured on the first day of DD (8 ± 9 min.; e.g., Fig. 1a).

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Insert Figure 1 about here

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However, 2 of these hamsters showed transient delays of activity onset for a week or more during the activity schedule, but these

delays were not sustained when the amount of running induced by daily wheel confinement declined later in the schedule. The other 4 hamsters showed considerably higher levels of induced wheel running (7321 ± 475 revs/3 h) and prominent and sustained phase delays of nocturnal activity onset (260 ± 63 min.; e.g., Fig. 1b). Across all hamsters, phase shifts were strongly related to the amount of induced activity (r=.79, p=.004; Fig. 2).

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Insert Figure 2 about here

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During the first 8 days of DD the hamsters free-ran with a group mean $\tau$ of 23.99 ± .08 min. $\tau$ was not related to phase shifts as measured on the first day of DD (r= .08,), or to the mean daily level of home cage wheel running expressed during DD (r=.16).

Discussion

Consistent with Mrosovsky and Janik (1993), prominent phase delay shifts were observed in the subgroup of hamsters that exhibited high levels of wheel running during the daily cage change sessions. Reebs and St-Couer (1994), by contrast, did not observe a strongly delayed phase of activity onset on the first day of DD. However, in that study, home cage wheel access was not provided until DD onset, which occurred at ZT12 after the last novel wheel session. It appears that most if not all of the hamsters began running soon after the home wheel was activated; this sustained running event may have reset circadian phase to

near this time. Consequently, phase on day 1 of DD in that study may not reflect the true phase of entrainment during the induced activity schedule. Evidence that very large phase shifts can be induced by home cage wheel running on the first day of wheel availability has been reported recently (Gannon & Rea 1995).

Unlike Mrosovsky and Janik (1993), we did not observe evidence of a split state during the first few days of DD in hamsters that showed large phase delays in LD. Conceivably, splitting did occur in LD in our hamsters, but transients may have been masked by lights-on, and recoupling may have somehow been instantaneous on the first day of DD. Alternatively, splitting in LD may have occurred in one study but not the other because of minor methodological differences; e.g., our hamsters were run at ZT4-7, rather than ZT5-8. A third possibility is that splitting in LD occurred in neither study. The split state evident on day 1 of DD in Mrosovsky and Janik (1993) may have been related to their procedure of transferring their animals to a separate room at the beginning of DD. It is not clear how this procedure would induce splitting, but it would explain why in that study there was no evidence of a split state during 2 "probe" tests (i.e., novel wheel session skipped) conducted prior to DD, even though large delays of nocturnal activity were evident by that time.

## Experiment 2

The failure to observe splitting in experiment 1 prompted experiment 2. In this experiment a SPP was employed so as to

minimize 'masking' effects of light that might obscure the observation of transients during a splitting response induced by scheduled activity at ZT5. In addition, activity was scheduled at a range of other ZTs in separate groups to gain further information on how photic and nonphotic zeitgebers might interact at different phase angles.

Method

Six additional groups of male hamsters (8 weeks age) were tested. The first two groups were housed individually either in plastic cages with small running (17 cm) wheels (N=8, 45 x 25 x 20 cm) or in the open field cages with large wheels (36 cm) used in experiment 1 (N=4). The remaining 4 groups of hamsters were all housed in the plastic cages with small wheels.

After stable entrainment to a full 14:10 photoperiod was established, the LD cycle was converted to a SPP, consisting of two 30 min. light pulses (20-40 lux at cage level) separated by 10 h, simulating dawn and dusk, on a background of DD (<1 lux red). The intensity of the light pulses was determined by the minimum amount of light that was required to maintain stable photic entrainment, so as to allow activity to have maximal effects. The animals were left in the SPP for at least 10 days before the daily activity schedule was initiated. Activity was induced for 3 h each day by locking the hamsters in a novel running wheel. Each group of hamsters was subject to wheel confinement at a different ZT. These included ZT5 (N=12); ZT9.5 (N=21); ZT13 (N=23); ZT19 (N=8);

ZT20 (N=14); and ZT21 (N=20). Activity schedules continued for 17 days, after which the SPP was replaced by DD, and the hamsters were left undisturbed, apart from routine maintenance, for 8 - 14 days.

Data Analysis. As in experiment 1 the wheel revolutions for the four animals that ran in 36 cm wheels were multiplied by a factor of 2.12 for analysis. Group differences in level of induced activity, phase of activity onset on the first day of DD, and $\tau$ during DD were assessed by ANOVA and Tukey HSD tests. Pearson product moment correlations were calculated to assess the relationship between the amount of induced running and the size of phase shifts (Bonferroni significance level set at .05/6).

Results

Activity. There was a significant effect of ZT of novel wheel confinement on the amount of running induced in 3 h ($F_{(5,94)}$= 7.325, $p<.001$). All groups ran, on average, about 4000 or more wheel revs/3 h (Table 1, Fig. 3a). The amount of running induced at ZT19-22 was significantly greater than at the other ZTs ($p<.02$).

Insert Table 1 about here

Insert Figure 3 about here

Phase shifts. Phase shifts were significantly related to ZT of novel wheel confinement ($F_{(5,92)}$= 23.2, $p<001$; Fig. 3b). Of 12

hamsters run in the middle of the subjective day (ZT5-8), 5 showed near or complete 180° inversion of their activity rhythms. In 4 cases, clear delaying or advancing transients were not evident; activity during the subjective night ceased, and reappeared about a week later in what was formerly the subjective day (e.g., Fig. 4a). Inversion in the fifth animal, however, appeared to be achieved by splitting, with one component of activity advancing across the "evening" (E) light pulse (the pulse that defined the end of the "day", or inactive period), and a second component of activity delaying across the morning (M) light pulse (the pulse that defined the beginning of the "day"; Fig. 4b). A sixth hamster may represent an intermediate case of splitting with incomplete inversion; in this case one bout of activity appeared to advance and phase jump the E light pulse, whereas a second component delayed several hours but did not jump the M light pulse and assumed a stable, delayed phase position (Fig. 4c). The remaining 6 hamsters did not invert but showed, on average, small phase advances of up to 40 min. (18 ± 7 min.; e.g., Fig. 4d).

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Insert Figure 4 about here

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Phase shifts within the ZT5 group were strongly related to the level of induced activity ($r=.947$, $p<.001$; Fig. 5a). The 5

hamsters that inverted ran, on average, 7991 ± 1112 revs/3 h, whereas those did not invert ran 1165 ± 309 revs/3 h.

Insert Figure 5 about here

Hamsters run late in the subjective day to early in the subjective night (ZT9.5-12.5) exhibited a mix of advances (N=7; 63 ± 24 min.; e.g., Fig. 6a) and delays (N=14, -80 ± 10 min., e.g., Fig. 6b). Phase shifts were significantly related to the level of induced activity (r=.58, p=.005; Fig. 5b). There was no relation between the size and sign of the phase shift and the phase of activity onset prior to the activity schedule.

Hamsters run early in the subjective night (ZT13-16) also exhibited a mix of advances (N=15, 61 ± 18 min. e.g., Fig. 6c) and delays (N=8, -47 ± 19 min.; e.g., Fig. 6d). There was no relation between phase shifts and level of induced activity (Fig. 5c). However, phase shifts were related to the baseline phase angle of entrainment; the more negative the phase angle prior to the activity schedule, the more positive the phase angle on the first day of DD (r=-.62, p=.002).

Insert Figure 6 about here

Hamsters run at two times late in the subjective night (ZT19-22 and ZT20-23) did not exhibit significant group mean

phase shifts, and in all individual cases showed little or no change of entrained phase (e.g., Fig. 5d, e; Fig. 6e, f). However, hamsters run beginning just 1 h later in the subjective night (ZT21-24) showed prominent phase delays of nocturnal activity onset in all 20 cases (-238 ± 30 min., $\tau$ =8.01, p <.001; e.g., Fig. 6g). The size of these phase shifts was not significantly related to the daily amount of induced wheel running (r=.34, p>.05; Fig. 5f).

$\tau$ in DD. There was a significant effect of ZT of wheel confinement on free-running $\tau$ in DD ($F_{(5,93)}$=4.91, p<.001; Table 2, Fig. 3c). The ZT21 group showed a significantly longer $\tau$ than the groups run at ZT9.5, 19 and 20 (p<.05). Within the ZT21 group, there was a significant relation between $\tau$ and phase shift induced by activity; the larger the phase delay, the longer the $\tau$ (r=-.64, p<.001). No other group showed a significant relation between phase shift and $\tau$.

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Insert Table 2 about here

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## Discussion

Consistent with a previous study (Mistlberger, 1991), novelty-induced wheel running in the late subjective night to early subjective day (ZT21-24) was associated with significant delays of entrained phase. However, in the current study the delays were considerably larger (238 ± 28 min. vs. 69 ± 5 min.). Induced running overlapped with the 30 min. M light pulse in this

group. The M light pulse should contribute to photic entrainment by advancing the pacemaker as needed each day. One previous study has provided evidence that phase advances to brief light pulses in hamsters can be attenuated by concurrent, novelty-induced running (Ralph & Mrosovsky, 1992). This raises the possibility that phase delays in the ZT21-24 group were due to nonphotic inhibition of phase advancing effects of light. Among hamsters run from ZT9.5-12.5, most phase delayed, but some phase advanced. Activity in this group overlapped with the E light pulse; advances in these cases could thus be due to inhibition of delays normally induced by this light pulse in the entrained state.

As a first means of evaluating this interpretation, the amount of activity expressed during the E and M light pulses was correlated with the phase of activity onset on day 1 of DD. If activity attenuates phase resetting by light, then ZT9.5 animals that show more running during the E light pulse might show larger phase advances. Conversely, ZT21 hamsters that ran more during the M light pulse might show larger delays. These predictions were not supported. There were no significant correlations between phase shift and amount of running in the light in the ZT9.5, ZT20 or ZT21 groups. The lack of relation between phase and level of activity in the light is underscored by a comparison of delays and advances in the ZT9.5 group; hamsters that phase advanced ran on average 187 ± 32 revs/10 min. (N=7)

during the light pulse, while those that phase delayed ran 171 ± 28 revs/10 min. (N=14), or only about 45 revs more in 30 min. This analysis thus provides no evidence that shifts of entrained phase during the activity schedule can be attributed directly to attenuation of photic resetting by concurrent novelty-induced running. If photic phase resetting was inhibited in any way, this must have been obscured by simultaneous non-photic shifting.

As a second means of evaluating this interpretation, an additional experiment was conducted to determine whether the advances and delays evident in the ZT9.5 and ZT21 groups, respectively, could be simulated by simply removing the E and M pulses, respectively, for 17 days.

## Experiment 3

### Method

Two groups of hamsters used in previous experiments were recorded for 14 days under the SPP and for 17 days during which either the E (N=15 hamsters) or the M (N=14) light pulse was absent.

### Results and Discussion

Following removal of the E light pulse, activity gradually phase advanced in 14/15 hamsters, averaging 130 ± 31 min. by day 17 (e.g., Fig. 7a). By contrast, only 7/21 hamsters in the ZT9.5 group exhibited phase advances, and these advances were, on average, only half as large (64 ± 23 min.; $t = 3.70$, $p = .003$). Combined with the lack of relationship between the amount of

running in the light and the size of phase shifts, this suggests that inhibition of photic delay resetting by running was not a primary contributor to shifts in this group. If it had been, we would have expected to see more animals phase advancing in the ZT9.5 group.

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Insert Figure 7 about here

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Following removal of the M light pulse, activity onset gradually phase delayed in 13/14 cases, but by only about one third as much (-86 ± 25 min.; e.g., Fig. 7b) as in the ZT21 comparison group ($t = 4.58$, $p < .001$). Inhibition of daily photic phase advances by activity thus cannot alone explain the 238 ± 28 min. delays of entrained phase that were characteristic of hamsters induced to run at ZT21-24.

## General Discussion

### Phase changes and splitting: 2-oscillator models.

One objective of this study was to evaluate a specific proposal that scheduled activity can induce splitting in LD (Mrosovsky & Janik, 1993). Of 11 hamsters entrained to a full photoperiod, 6 showed either transient (N=2) or sustained (N=4) phase delays of nocturnal activity onset when induced to run for 3 h in the middle of the light period. During subsequent DD, all of the hamsters free-ran and none exhibited split or clearly bimodal activity rhythms. The phase delays confirm one previous study, in which marked delays were also observed in hamsters confined

to novel cages for 3 h in the middle of the light period (Mrosovsky & Janik, 1993). However, in that study, hamsters with delayed rhythms in LD exhibited split rhythms in DD. These split components fused within a few days. No splitting transients were evident in LD, and there was no evidence of splitting during two probe tests prior to DD. It thus seems possible that splitting in that study was induced in some way by the procedure of transferring animals to DD in a separate room at the end of the activity schedule.

Alternatively, failure to observe split rhythms in DD in the present study may be because of rapid refusion of components, possibly due to our use of dim red light rather than absolute DD. Consequently, additional groups of hamsters were run in a SPP, in hopes of visualizing splitting in process. Of 12 hamsters entrained to a SPP and run in the middle of their subjective day, 5 showed near 180° inversions of their activity rhythms. In 4 cases, no transients were evident, but in one case, activity appeared to split into one advancing and one delaying component which jumped the evening and morning light pulses, respectively, and recoupled in what was formerly the subjective day. One other hamster showed an intermediate response; one component of activity advanced and jumped the evening pulse, whereas the other component delayed but did not jump the morning pulse. These results provide powerful confirmatory evidence that splitting can

be induced by a nonphotic stimulus in animals that are stably entrained to a photic zeitgeber.

The mechanism by which activity can induce splitting in a photically entrained hamster is unclear. The appearance of splitting, during which two bouts of activity transiently express different $\tau$ 's, suggests the existence of two oscillators that can be uncoupled. One possibility is that the circadian system in hamsters contains one oscillator subject to phase control by activity, and a second oscillator subject to phase control by LD. An analogous model, invoking separate light and food-entrainable circadian pacemakers, has been constructed to explain 2 findings: 1. the existence of food-anticipatory rhythms in rats and hamsters following ablation of the light-entrainable circadian pacemaker (the suprachiasmatic nucleus, SCN) (Abe & Rusak, 1992; Mistlberger, 1992; Mistlberger, 1994), and; 2. co-existence of 24 h food anticipatory rhythms and non-24 h free-running rhythms in intact rats and hamsters during daily restricted feeding schedules in LL or DD (Abe & Rusak, 1992; Mistlberger, 1994). Limited evidence that scheduled activity might also synchronize circadian oscillators outside of the SCN is available from studies of SCN ablated hamsters (Mistlberger, 1992), but primarily negative results have been obtained so far from similar studies of rats and mice (Marchant & Mistlberger, 1996; Ruis, Buys, Cambras, & Rietveld, 1989), and this finding thus needs further replication in hamsters to be considered robust. In the present study of intact

hamsters, split rhythms recoupled in phase with the activity schedule; there was no evidence for sustained phase control of one component by scheduled activity and the other component by light. The results thus do not provide evidence for separate oscillators entrained to light and activity.

Splitting as observed in the present study might be more parsimoniously explained within the framework of the Pittendrigh and Daan (1976) two-oscillator model. In that scheme, the light entrainable pacemaker is modeled as two coupled oscillators (labeled E and M oscillators) with separate photic PRC's. Under entrained conditions these oscillators and their PRCs are phase displaced and thus differentially stimulated by E and M light. These oscillators may also have nonphotic PRCs, which, presumably, would also be phase displaced. If so, then activity scheduled at some times of the day (e.g., ZT4 or 5), would phase shift one oscillator exclusively or more strongly than the other. The activity pattern illustrated in Fig. 4b (complete splitting with recoupling 180° out of phase) could thus be simulated in the following way; on day 1 of novel wheel confinement, induced running may phase advance the E oscillator instantaneously, causing it to "jump" the E light pulse that follows 5 h later, and which now falls on the advance portion of its photic PRC. The M oscillator, which normally holds a delayed phase position relative to the E oscillator, may be either unstimulated or only weakly stimulated by the scheduled activity. It thus would not

immediately phase advance, and would be prevented from following because it is still subject to phase delay by the E light pulse. The M oscillator might then phase delay on subsequent days due to one or more of several factors, including 1. scheduled activity falling on the delay portion of its nonphotic PRC, 2. coupling forces from the E oscillator in its new phase position near scheduled activity, or 3. its intrinsic $\tau$. These factors would have to be stronger than any advancing effect of the M light pulse, but could be synergistic with delaying effects of this light pulse once the M oscillator photic PRC has been delayed sufficiently.

The activity pattern illustrated in Fig. 4c may be an instructive intermediate case of rhythm dissociation. In this animal, one bout of activity advanced and phase jumped the E light pulse, whereas the second bout of activity delayed a few hours but did not jump the M light pulse. In this case, continued movement of the two components may be halted by daily photic resetting of each. Complete inversion is thus presumably prevented by an opposite action of light. Variability in the degree of splitting may be due to individual differences in: 1. the magnitude of the zeitgebers, i.e., the amount of novelty-induced running and/or the actual light-intensity in each cage; 2. the sensitivity to these Zeitgebers; or 3. the strength of internal oscillator coupling.

If, as suggested, complete inversion can be prevented by an opposing action of light, it seems unlikely that inversion or

splitting would occur in a full photoperiod. This expectation seems consistent with the results of experiment 1. Some of the hamsters showed strong delays during that ZT4 activity schedule, but none showed advancing transients in LD, and none exhibited a split state on the first day of DD. However, if activity rhythms did not split, then it is not clear how to interpret the phase delays. Single or repeated 3 h bouts of novelty-induced running in the mid-subjective day of free-running hamsters invariably cause phase advances, not phase delays (Mrosovsky 1996). Conceivably, partial splitting (e.g., similar to that evident in the intermediate case described above) did occur, but the phase advanced component may have been masked by light, or may have been too weak to drive activity in either LD or subsequent DD. In more typical cases of complete splitting observed in LL, the two bouts of activity are not always equivalent in magnitude (e.g., Boulos & Morin, 1985). A non-behavioral marker of pacemaker phase may be required to resolve this question.

Running induced at ZT9.5 was associated with phase advances of about 60 min. in 7 hamsters, and phase delays of about 80 min. in 14 hamsters. A mix of small phase delays, advances and no responses was also evident in the ZT13 group. Variability of responses suggests that these ZTs straddled the transition from phase advance to phase delay or dead zones of the non-photic PRC. Variability may also reflect small differences in

the initial phase angle of photic entrainment; this appeared to be the case in the ZT13 group.

Running induced at ZT19 and ZT20 produced virtually no phase changes, despite very high levels of running. This is consistent with evidence that this is a relatively dead zone of the nonphotic PRC (Mrosovsky, 1996). It also suggests that the mix of small advances and delays observed in the ZT9.5 and ZT13 groups was not a reflection of changes in pacemaker precision and stability caused by some non-specific (i.e., phase independent) effect of behavioral activation or increased wheel running.

Running induced at ZT21 produced large phase delays in all of the hamsters tested. These are all the more striking given the lack of response to activity induction beginning just 1 or 2 h earlier. The abruptness of the change from no response to large delay may define a transition point in the nonphotic PRC at about ZT23-24 (the first hour of the subjective day). This confirms a result obtained in an earlier study using a full photoperiod (Mistlberger, 1991). The effect was larger here presumably because the photic zeitgeber was weaker. The long free-running $\tau$ in this group, relative to the other groups, also confirms the previous study, and is consistent with, and may be causal to, the delayed phase angle of entrainment evident in the SPP.

Within the framework of the 2-oscillator model, the absence of any kind of rhythm dissociation in the ZT9.5, 13, 19, 20 and 21 groups would suggest that nonphotic stimulation at these phases

did not produce strong differential stimulation of E and M oscillators.

Activity and light pulses: inhibition and masking responses.

Activity is associated with increased serotonin (5HT) release in the SCN (Shioiri, Takahashi, Yamada, & Takahashi, 1991), and 5HT and its pharmacological agonists attenuate SCN cellular and pacemaker responses to photic stimulation (Rea, Glass & Colwell, 1994; Selim, Glass, Hauser, & Rea, 1993). Consequently, activity per se may be expected to attenuate pacemaker responses to light. Some evidence for this is available (Ralph & Mrosovsky, 1992). However, there was no clear evidence that such attenuation could explain in any simple way the phase changes to activity observed in ZT9.5 and ZT21 hamsters in the present study, although activity did overlap with one light pulse in these groups. Activity also overlapped with light in the ZT20 group, but no phase changes were evident in these animals. Conceivably, activity may have inhibited photic phase resetting in the ZT20 group, but may have also directly induced a phase advance that effectively substituted for the attenuated light-induced advance. This possibility is considered remote. Activity did not overlap with light in the ZT19 condition. The complete absence of phase shifts in that group thus defines ZT19-22 as a dead zone. ZT21-24 clearly contains a delay zone, given the large phase delays evident in all of those hamsters. Consequently, it is highly unlikely that ZT20-23 contains an advance zone for nonphotic shifting. It is instead

more likely that ZT20-23 is a dead zone, and that activity had little or no effect on daily phase advance resetting provided by the M light pulse. If activity can inhibit phase shift responses to light, this effect must be relatively weak in the entrained state.

Recent studies suggest that not only might activity inhibit shifts to light, but light may inhibit shifts to activity (Joy & Turek, 1992; Mrosovsky, 1991). The present results may thus be interpreted from a different perspective. The failure of activity induced in the ZT19 and ZT20 conditions to alter entrained phase may not define a dead zone, but may instead reflect inhibitory effects of the light pulse occurring at or near the end of the daily induced activity bout. The ZT21 condition may escape this effect because fully 2 h of activity occurred after the light pulse. Additional studies will be necessary to explore this interpretation. Mutually inhibitory effects of light and activity on pacemaker resetting in the entrained state may, if substantiated, add considerable complexity to modeling photic-nonphotic interactions under different dual zeitgeber schedules.

In addition to its pacemaker mediated effects, light also has direct effects on behavior, and in nocturnal rodents is generally observed to reduce activity. Consistent with this expectation, we observed reductions of novel wheel running during the 30 min. daily light pulses in all but one of the ZT9.5 and ZT20 hamsters (group mean reductions of 51% and 26%, respectively, by comparison with the 30 min. immediately preceding the light

pulse). Surprisingly, this masking effect of light was much weaker in the ZT21 group; 9/24 hamsters actually showed increased activity when the light came on, and overall, the change with respect to the previous 30 min. was only -1.1 ± 4.7%. A teleological explanation for this result might be as follows; light observed in the E presumably signals to a nocturnal animal that it is too soon to leave the den. This light induces a phase delay, but should also acutely inhibit activity to prevent premature foraging at that time (cf. DeCoursey, 1986). Light observed in the M presumably has a very different significance; it signals to the nocturnal animal that it has overstayed its open field activity. Inhibition of activity by light at this time would not be adaptive; it should instead stimulate activity to facilitate return to the den. Presumably, some transition point would exist where light changes from inhibitory to excitatory in its acute behavioral effect. This could explain the difference between the ZT20 and ZT21 groups, if the transition occurs near the ZT20-21 zone. This observation is, to our knowledge, novel, and will require replication before any attempt at a mechanistic analysis.

The nature of the activity zeitgeber.

In 3 of 7 groups there was a significant relation between phase shifts and the amount of running induced by novel wheel confinement. In 2 other groups the correlations were better than .50 but only marginally significant. Previous studies have also found positive correlations between wheel revolutions and phase-

shifting (Mrosovsky, 1996), which led to the suggestion that activity itself was responsible for the phase-shifts. However, other studies have shown that in some animals, even high levels of running activity do not result in phase-shifts (Janik & Mrosovsky, 1993; Mrosovsky & Biello, 1994). This has prompted suggestions that it is not the activity per se that is responsible for phase-shifting, but the motivational context within which activity is exhibited (Janik & Mrosovsky, 1993; Mrosovsky & Biello, 1994). The present study does not shed any light on this issue, but does reinforce the notion that wheel running, if not directly causal to phase shifting, is highly predictive, because we did not see substantive shifting in any animal that failed to run in the novel wheels.

Conclusion

Light may be the primary zeitgeber for phase control of circadian rhythms in mammals, but as this study and others clearly demonstrate, non-photic events can have powerful modulating effects. The present results indicate that under full photoperiods, running induced in the middle of the usual inactive phase can strongly delay nocturnal activity, whereas in a SPP, complete inversions may occur. These results raise the possibility that similar reorganization of circadian phase might be effected in humans by strong non-photic stimuli associated with physical activity at certain circadian phases. Such effects may be particularly prevalent when photic zeitgebers are weaker, as

might occur during short winter days, or, transiently, on certain shift rotations or following jet travel.

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Table 1

Group means of no. of wheel revolutions during 3 h of novelty-induced running in a SPP.

| Group | Mean (± SE) | Range |
|---|---|---|
| ZT5-8 | 3976 ± 1114 | 68 to 9692 |
| ZT9.5-12.5 | 4313 ± 315 | 2025 to 6375 |
| ZT13-17 | 5097 ± 279 | 2986 to 7864 |
| ZT19-21 | 8385 ± 683 | 2591 to 8723 |
| ZT20-23 | 4415 ± 276 | 2986 to 6106 |
| ZT21-24 | 5865 ± 359 | 2444 to 8894 |

Table 2

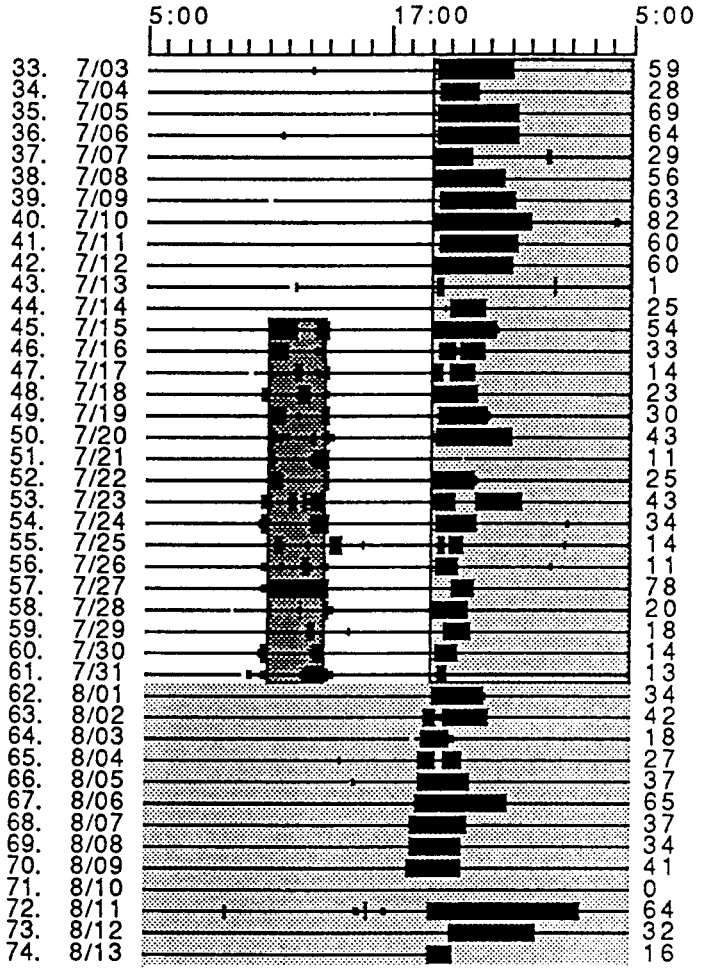
Group means for $\tau$ in DD.

| Group | $\tau$ in DD (± SE) |
|---|---|
| ZT5-8 | 24.08 ± .08 |
| ZT9.5-12.5 | 24.05 ± .03* |
| ZT13-17 | 24.09 ± .03 |
| ZT19-21 | 23.90 ± .04* |
| ZT20-23 | 24.01 ± .03* |
| ZT21-24 | 24.22 ± .04 |

*significantly different from ZT21, $p<.05$.

Figure 1. Single plotted wheel-running activity charts of two hamsters subjected to novelty induced running at ZT4. Each line represents 24 h, with time plotted from left to right in consecutive 10-min. bins. Vertical deflections indicate bins during which wheel revolutions were registered. Animals were recorded in LD (all shaded areas are dim red light and heavily shaded area is 3 h of exercise): a) no phase shift; b) large phase delay

Figure 1a


5:00
17:00
5:00
33. 7/03 59
34. 7/04 28
35. 7/05 69
36. 7/06 64
37. 7/07 29
38. 7/08 56
39. 7/09 63
40. 7/10 82
41. 7/11 60
42. 7/12 60
43. 7/13 1
44. 7/14 25
45. 7/15 54
46. 7/16 33
47. 7/17 14
48. 7/18 23
49. 7/19 30
50. 7/20 43
51. 7/21 11
52. 7/22 25
53. 7/23 43
54. 7/24 34
55. 7/25 14
56. 7/26 11
57. 7/27 78
58. 7/28 20
59. 7/29 18
60. 7/30 14
61. 7/31 13
62. 8/01 34
63. 8/02 42
64. 8/03 18
65. 8/04 27
66. 8/05 37
67. 8/06 65
68. 8/07 37
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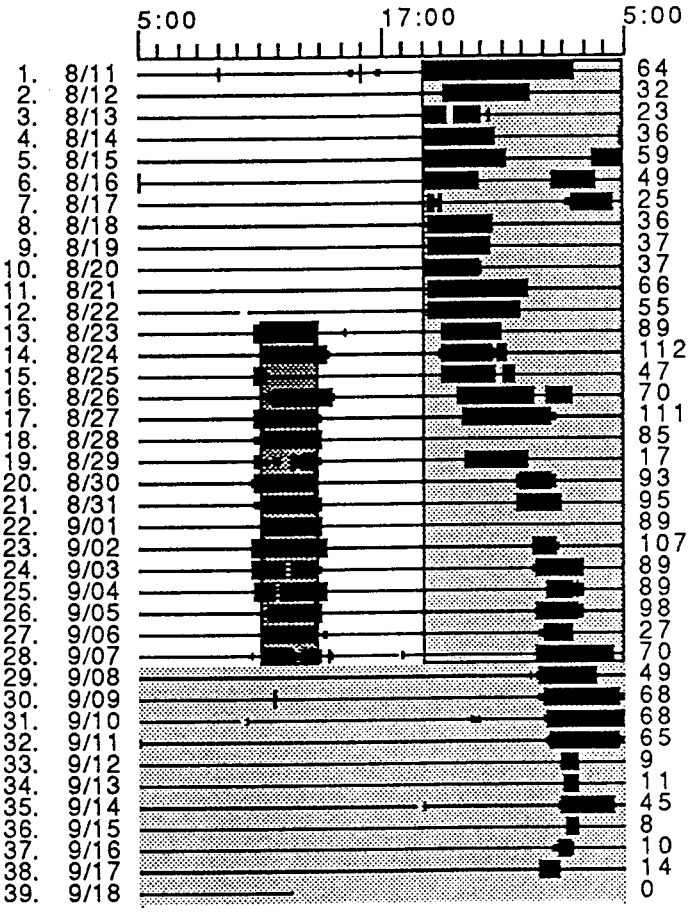
70. 8/09 41
71. 8/10 0
72. 8/11 64
73. 8/12 32
74. 8/13 16

Figure 1b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17:00
5:00
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2. 8/12 32
3. 8/13 23
4. 8/14 36
5. 8/15 59
6. 8/16 49
7. 8/17 25
8. 8/18 36
9. 8/19 37
10. 8/20 37
11. 8/21 66
12. 8/22 55
13. 8/23 89
14. 8/24 112
15. 8/25 47
16. 8/26 70
17. 8/27 111
18. 8/28 85
19. 8/29 17
20. 8/30 93
21. 8/31 95
22. 9/01 89
23. 9/02 107
24. 9/03 89
25. 9/04 89
26. 9/05 98
27. 9/06 27
28. 9/07 70
29. 9/08 49
30. 9/09 68
31. 9/10 68
32. 9/11 65
33. 9/12 9
34. 9/13 11
35. 9/14 45
36. 9/15 8
37. 9/16 10
38. 9/17 14
39. 9/18 0

Figure 2. Scatterplot illustrating the relationship between phase shift on Day 1 of DD and wheel running during 3h of novel wheel confinement

Figure 2

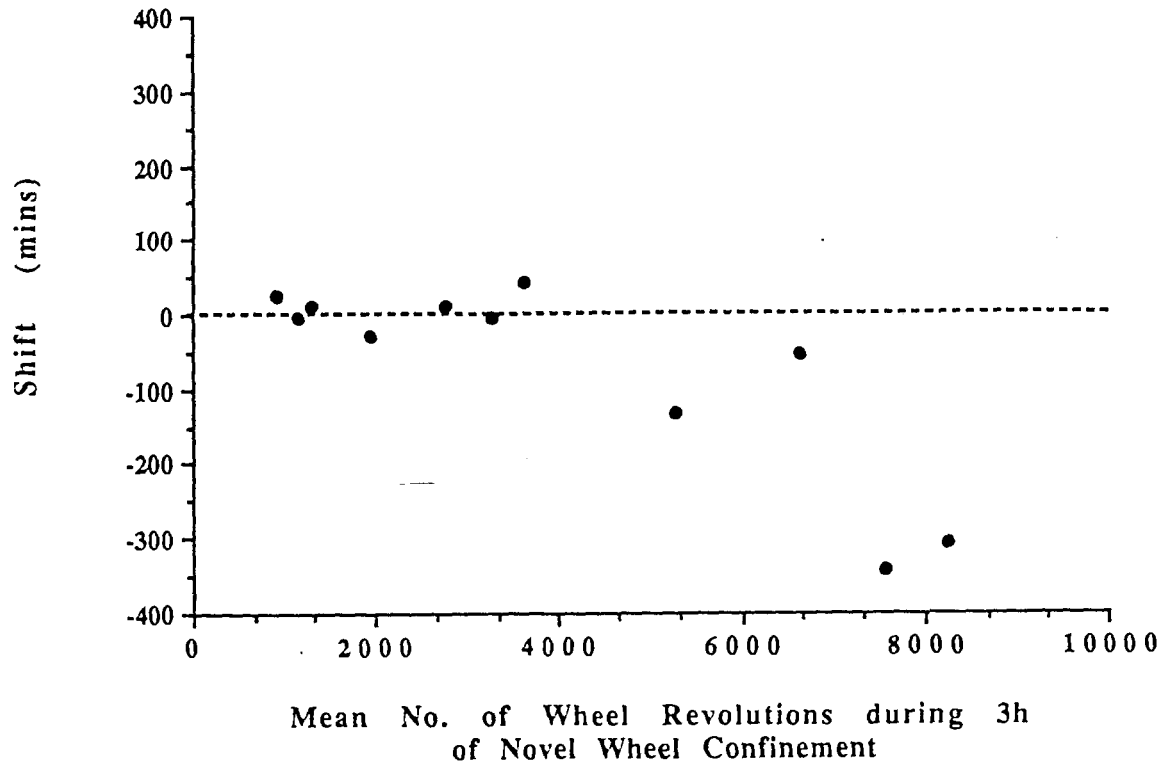

400
300
200
100
0
-100
-200
-300
-400
Shift (mins)
0
2000
4000
6000
8000
10000
Mean No. of Wheel Revolutions during 3h
of Novel Wheel Confinement

Figure 3. Bar graphs illustrating mean (± SE) a) levels of running; b) group phase shifts; c) group taus.

Figure 3a

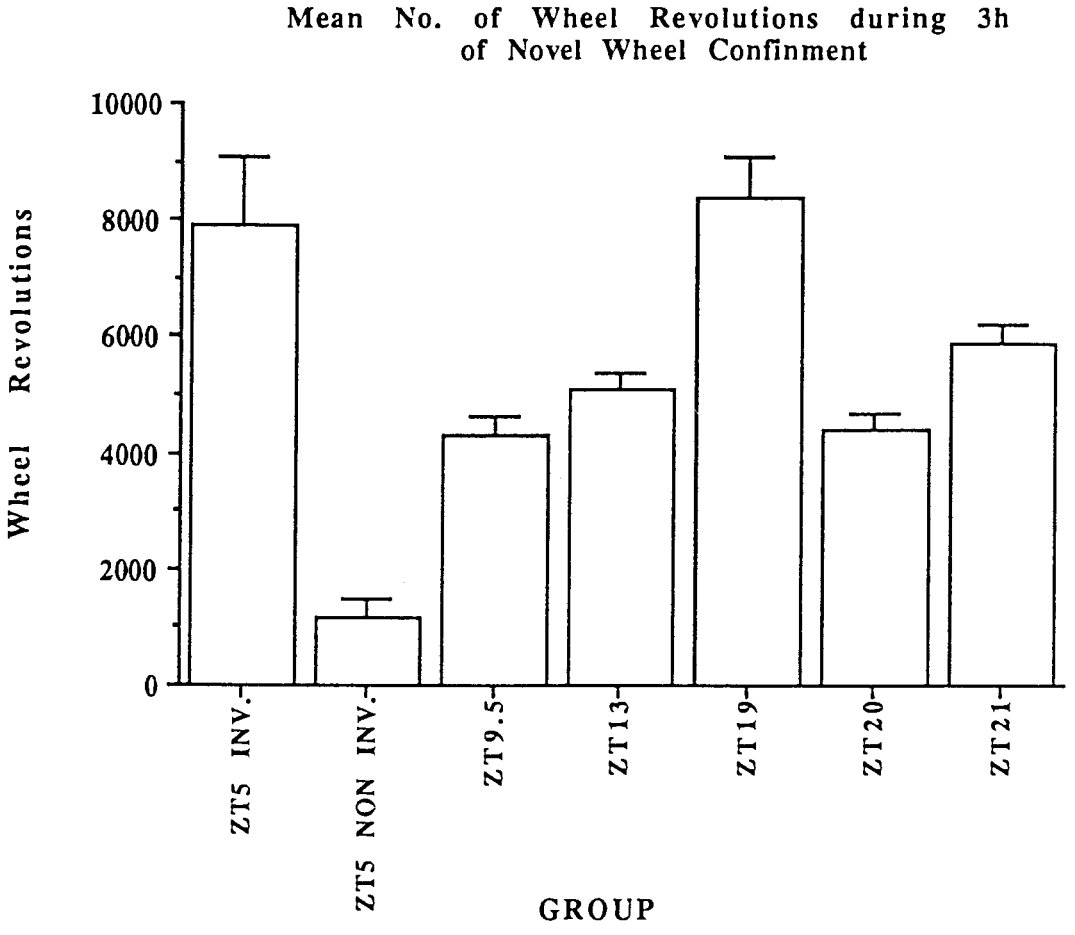

Mean No. of Wheel Revolutions during 3h of Novel Wheel Confinment
Wheel Revolutions
10000
8000
6000
4000
2000
0
ZT5 INV.
ZT5 NON INV.
ZT9.5
ZT13
ZT19
ZT20
ZT21
GROUP

Figure 3b

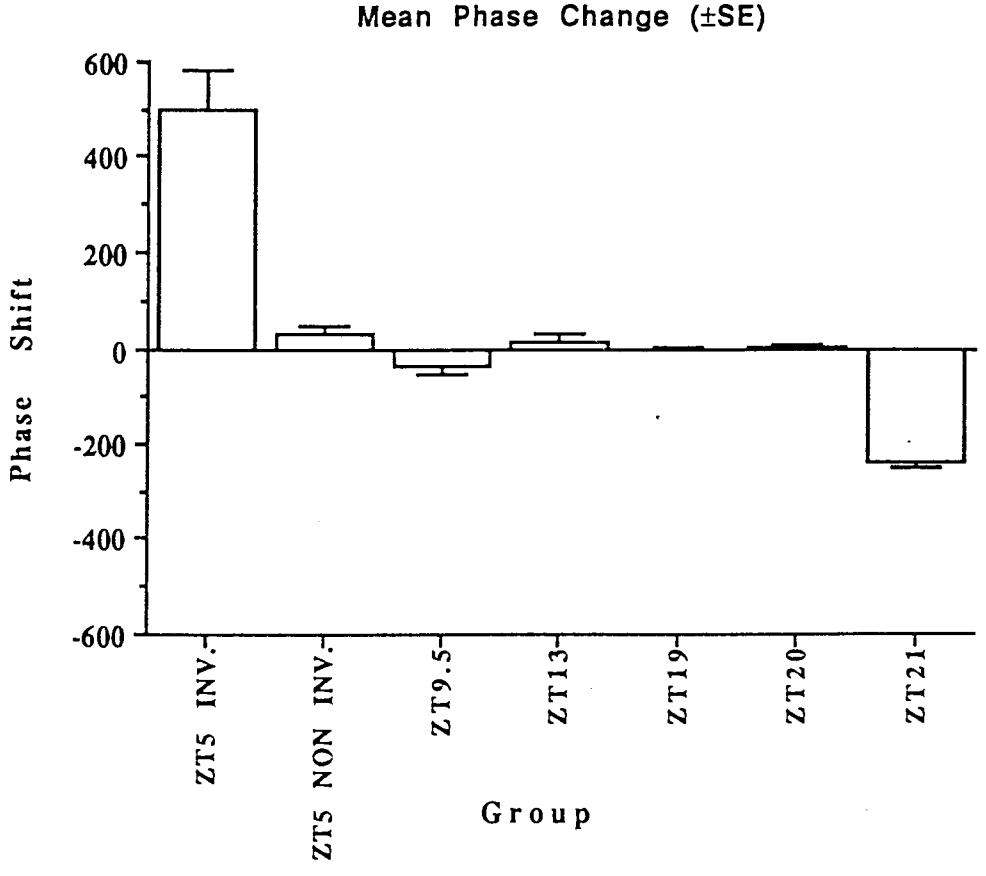

Mean Phase Change (±SE)
600
400
200
0
-200
-400
-600
Phase Shift
ZT5 INV.
ZT5 NON INV.
ZT9.5
ZT13
ZT19
ZT20
ZT21
Group

Figure 3c

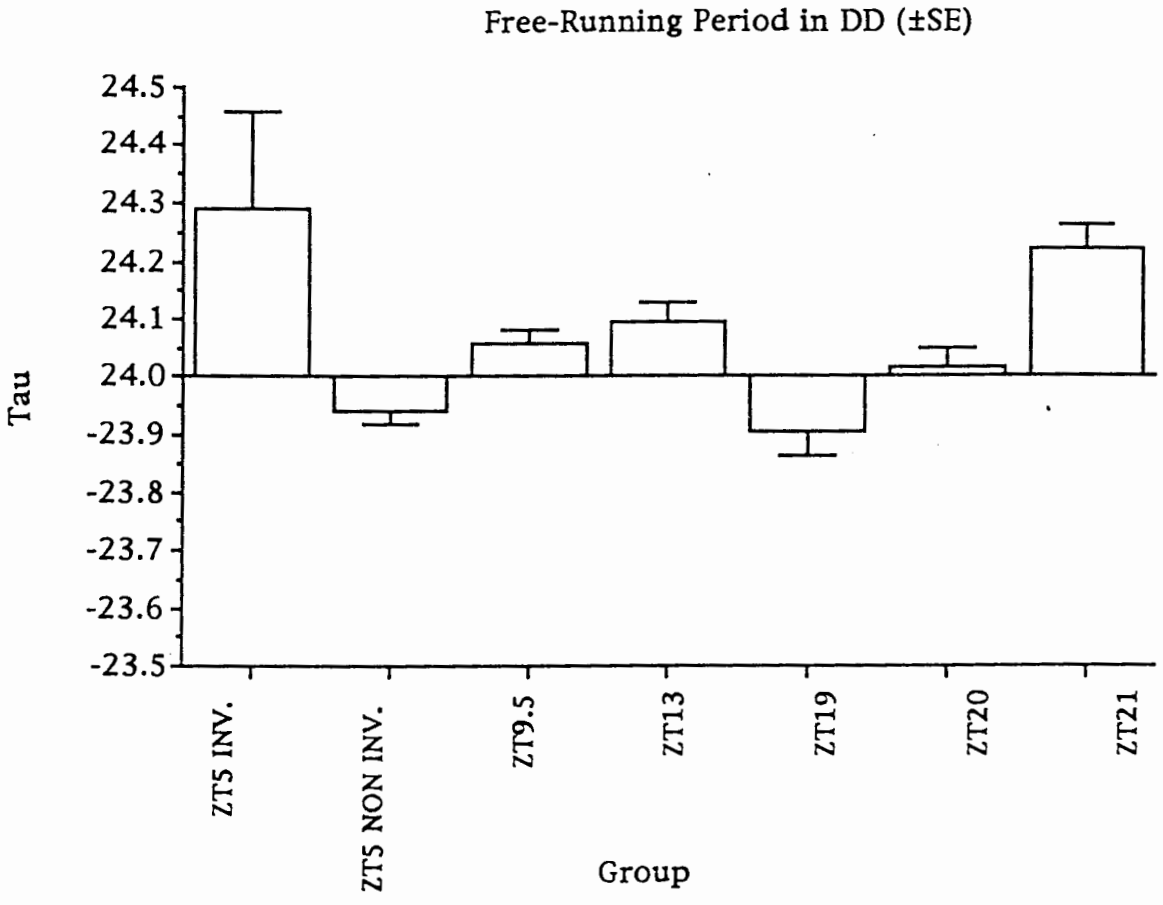

Free-Running Period in DD (±SE)
Tau
24.5
24.4
24.3
24.2
24.1
24.0
-23.9
-23.8
-23.7
-23.6
-23.5
ZT5 INV.
ZT5 NON INV.
ZT9.5
ZT13
ZT19
ZT20
ZT21
Group

Figure 4. Double plotted wheel-running activity charts of four hamsters subjected to novelty induced running at ZT5. Each line represents 48 h, with time plotted from left to right in consecutive 10-min. bins. Vertical deflections indicate bins during which wheel revolutions were registered (all shaded areas are dim red light and heavily shaded area is 3 h of exercise): a) inversion without transients; b) inversion with splitting; c) partial dissociation, with advance; d) no shift.

Figure 4a

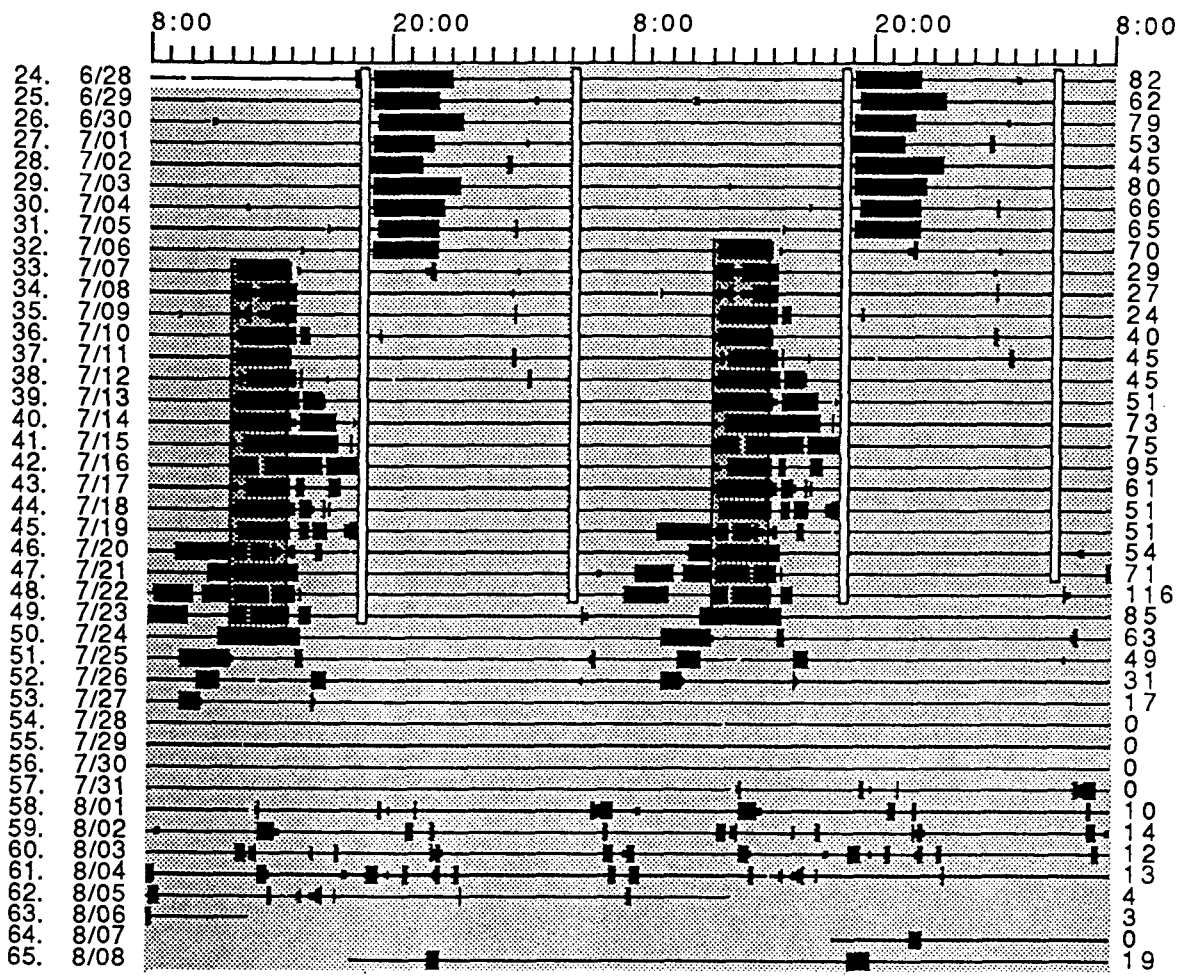

8:00
20:00
8:00
20:00
8:00
24. 6/28 82
25. 6/29 62
26. 6/30 79
27. 7/01 53
28. 7/02 45
29. 7/03 80
30. 7/04 66
31. 7/05 65
32. 7/06 70
33. 7/07 29
34. 7/08 27
35. 7/09 24
36. 7/10 40
37. 7/11 45
38. 7/12 45
39. 7/13 51
40. 7/14 73
41. 7/15 75
42. 7/16 95
43. 7/17 61
44. 7/18 51
45. 7/19 51
46. 7/20 54
47. 7/21 71
48. 7/22 116
49. 7/23 85
50. 7/24 63
51. 7/25 49
52. 7/26 31
53. 7/27 17
54. 7/28 0
55. 7/29 0
56. 7/30 0
57. 7/31 0
58. 8/01 10
59. 8/02 14
60. 8/03 12
61. 8/04 13
62. 8/05 4
63. 8/06 3
64. 8/07 0
65. 8/08 19

Figure 4b

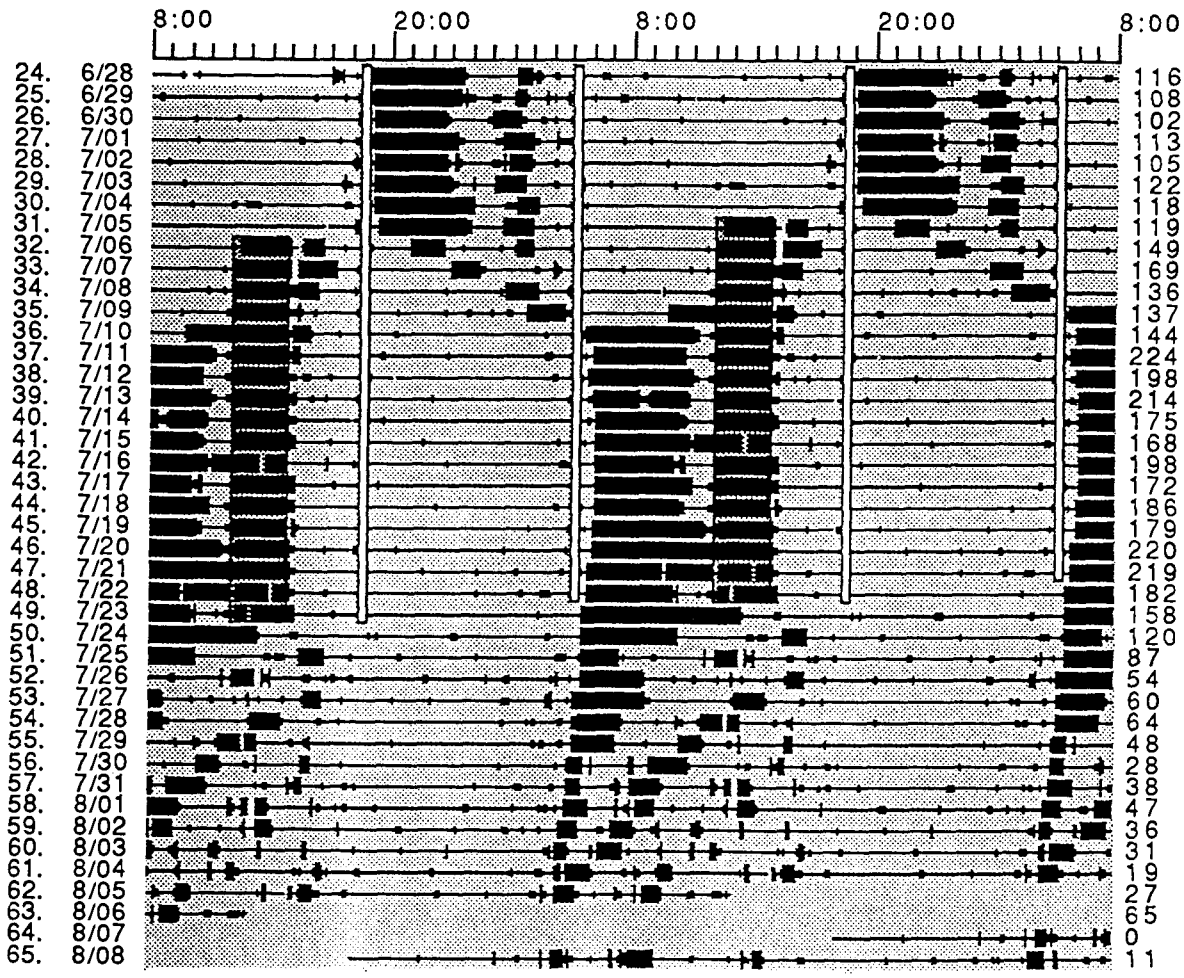

8:00
20:00
8:00
20:00
8:00
24. 6/28 116
25. 6/29 108
26. 6/30 102
27. 7/01 113
28. 7/02 105
29. 7/03 122
30. 7/04 118
31. 7/05 119
32. 7/06 149
33. 7/07 169
34. 7/08 136
35. 7/09 137
36. 7/10 144
37. 7/11 224
38. 7/12 198
39. 7/13 214
40. 7/14 175
41. 7/15 168
42. 7/16 198
43. 7/17 172
44. 7/18 186
45. 7/19 179
46. 7/20 220
47. 7/21 219
48. 7/22 182
49. 7/23 158
50. 7/24 120
51. 7/25 87
52. 7/26 54
53. 7/27 60
54. 7/28 64
55. 7/29 48
56. 7/30 28
57. 7/31 38
58. 8/01 47
59. 8/02 36
60. 8/03 31
61. 8/04 19
62. 8/05 27
63. 8/06 65
64. 8/07 0
65. 8/08 11

Figure 4c

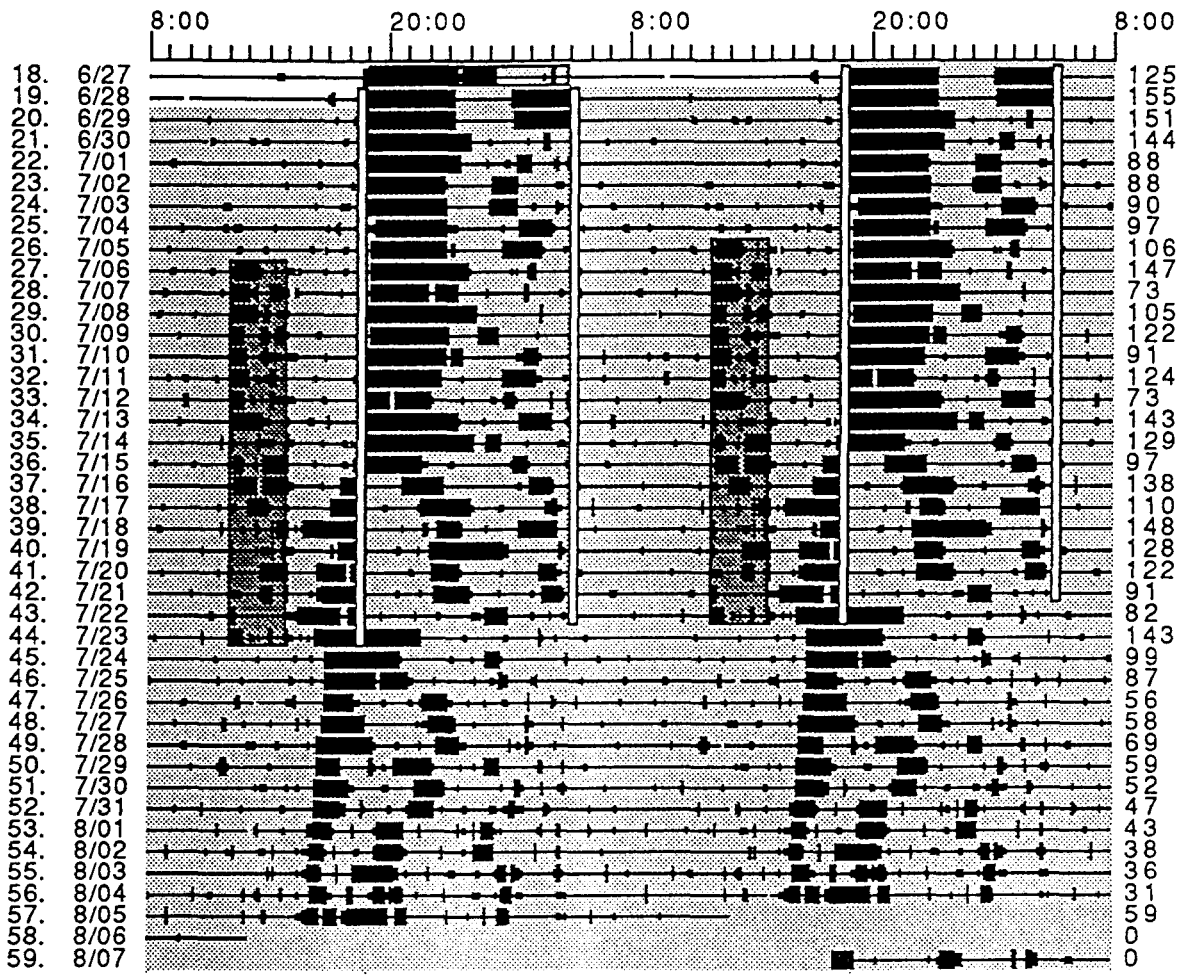

8:00
20:00
8:00
20:00
8:00
18. 6/27 125
19. 6/28 155
20. 6/29 151
21. 6/30 144
22. 7/01 88
23. 7/02 88
24. 7/03 90
25. 7/04 97
26. 7/05 106
27. 7/06 147
28. 7/07 73
29. 7/08 105
30. 7/09 122
31. 7/10 91
32. 7/11 124
33. 7/12 73
34. 7/13 143
35. 7/14 129
36. 7/15 97
37. 7/16 138
38. 7/17 110
39. 7/18 148
40. 7/19 128
41. 7/20 122
42. 7/21 91
43. 7/22 82
44. 7/23 143
45. 7/24 99
46. 7/25 87
47. 7/26 56
48. 7/27 58
49. 7/28 69
50. 7/29 59
51. 7/30 52
52. 7/31 47
53. 8/01 43
54. 8/02 38
55. 8/03 36
56. 8/04 31
57. 8/05 59
58. 8/06 0
59. 8/07 0

Figure 4d

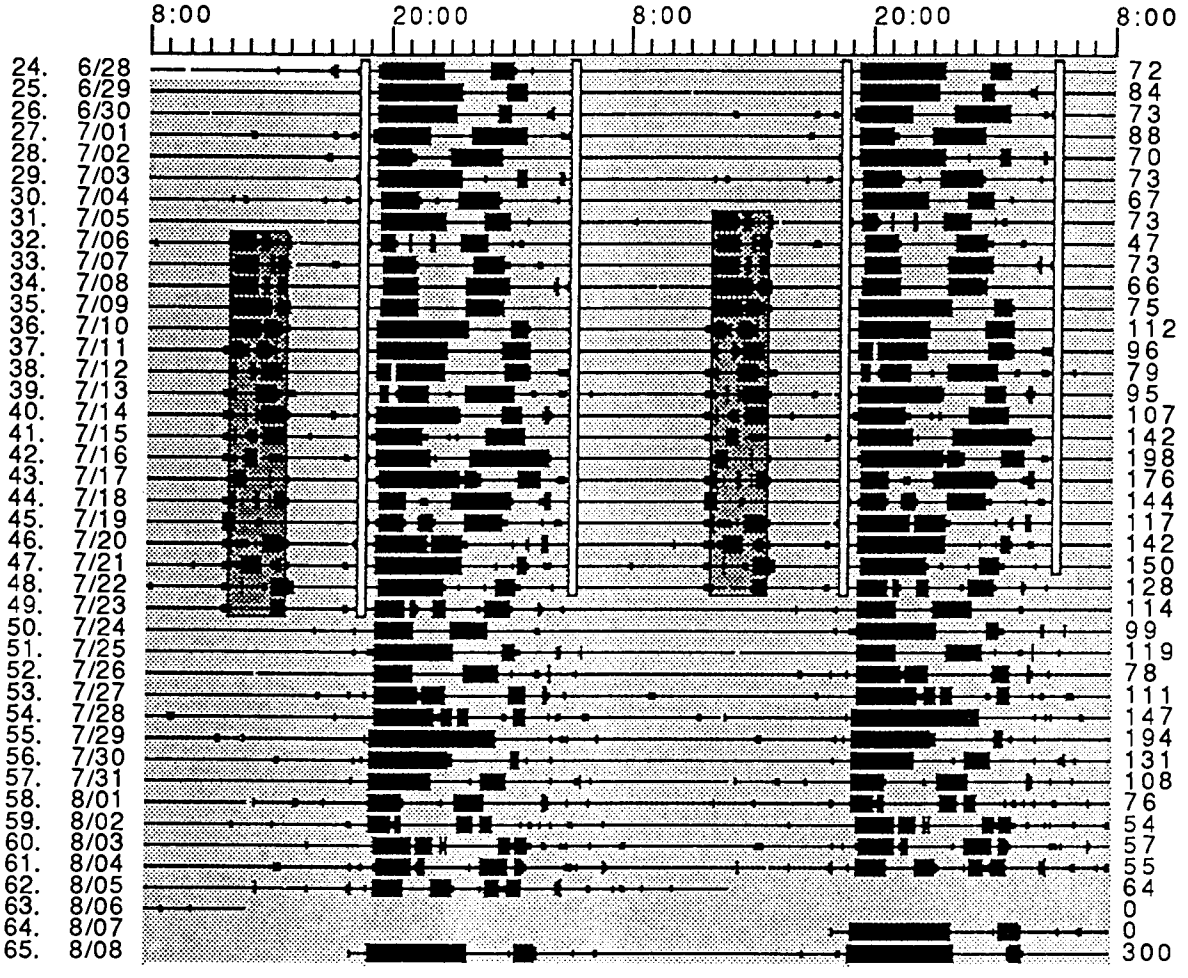

8:00
20:00
8:00
20:00
8:00
24. 6/28 72
25. 6/29 84
26. 6/30 73
27. 7/01 88
28. 7/02 70
29. 7/03 73
30. 7/04 67
31. 7/05 73
32. 7/06 47
33. 7/07 73
34. 7/08 66
35. 7/09 75
36. 7/10 112
37. 7/11 96
38. 7/12 79
39. 7/13 95
40. 7/14 107
41. 7/15 142
42. 7/16 198
43. 7/17 176
44. 7/18 144
45. 7/19 117
46. 7/20 142
47. 7/21 150
48. 7/22 128
49. 7/23 114
50. 7/24 99
51. 7/25 119
52. 7/26 78
53. 7/27 111
54. 7/28 147
55. 7/29 194
56. 7/30 131
57. 7/31 108
58. 8/01 76
59. 8/02 54
60. 8/03 57
61. 8/04 55
62. 8/05 64
63. 8/06 0
64. 8/07 0
65. 8/08 300

Figure 5. Scatterplots illustrating the relationship between phase shift on Day 1 of DD and wheel running during 3h of novel wheel confinement; a) ZT5; b) ZT9.5; c) ZT13; d) ZT19; e) ZT20; f) ZT21.

Figure 5a

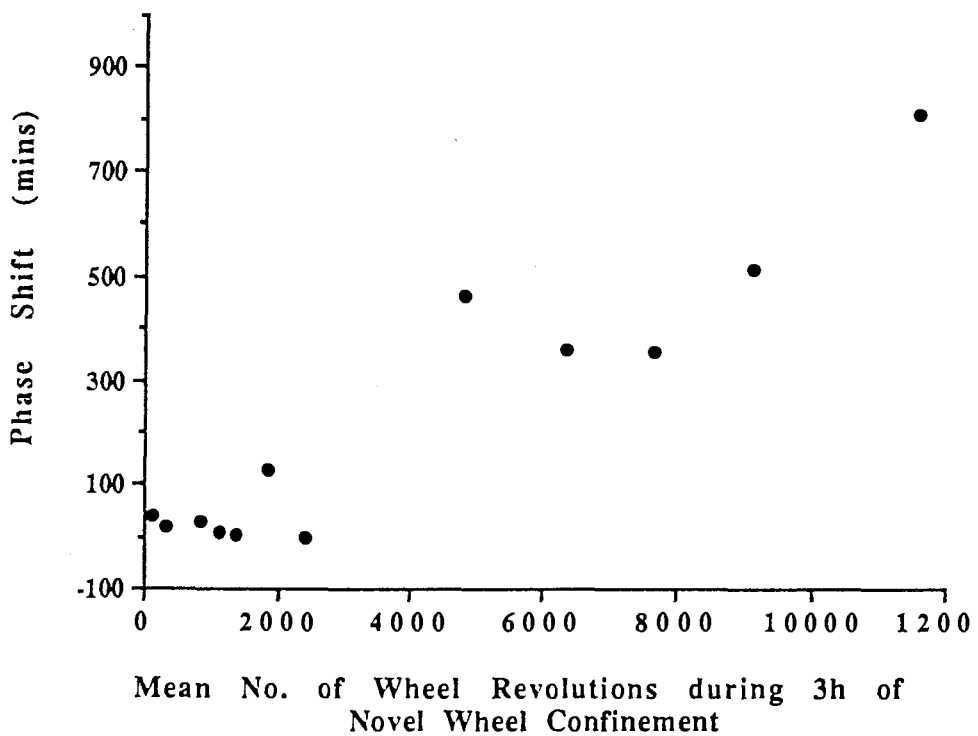

Phase Shift (mins)
900
700
500
300
100
-100
0
2000
4000
6000
8000
10000
12000
Mean No. of Wheel Revolutions during 3h of Novel Wheel Confinement

Figure 5b

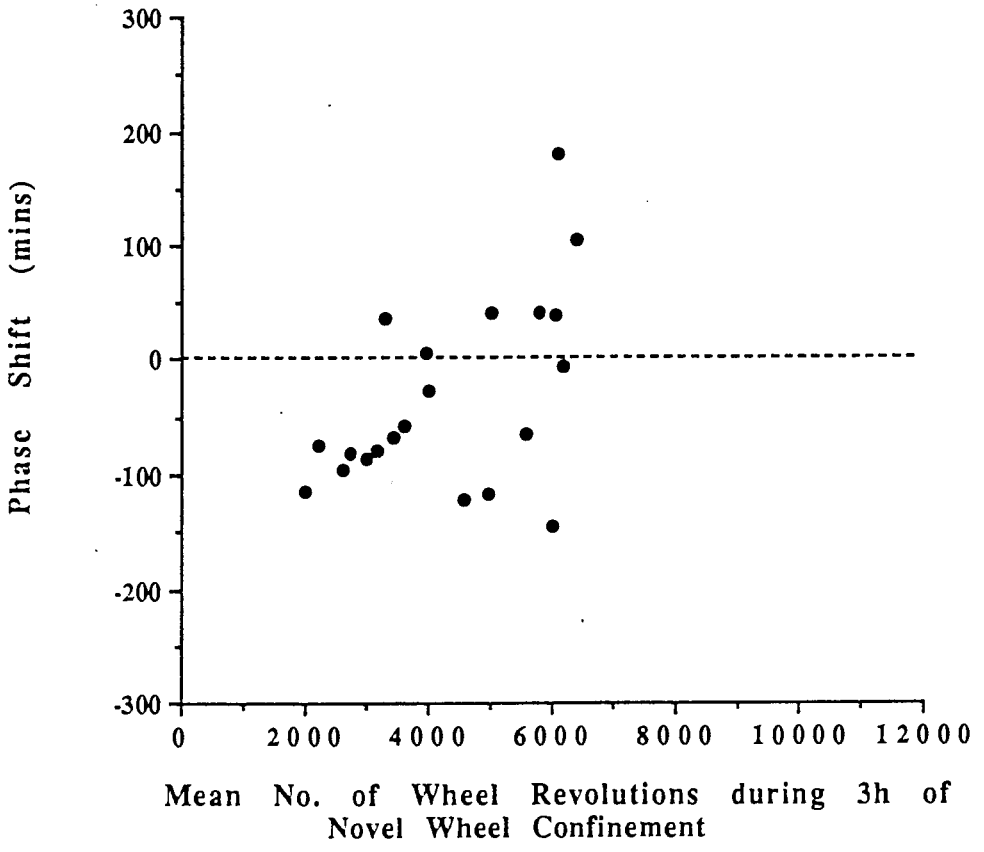

300
200
100
0
-100
-200
-300
Phase Shift (mins)
0
2000
4000
6000
8000
10000
12000
Mean No. of Wheel Revolutions during 3h of Novel Wheel Confinement

Figure 5c

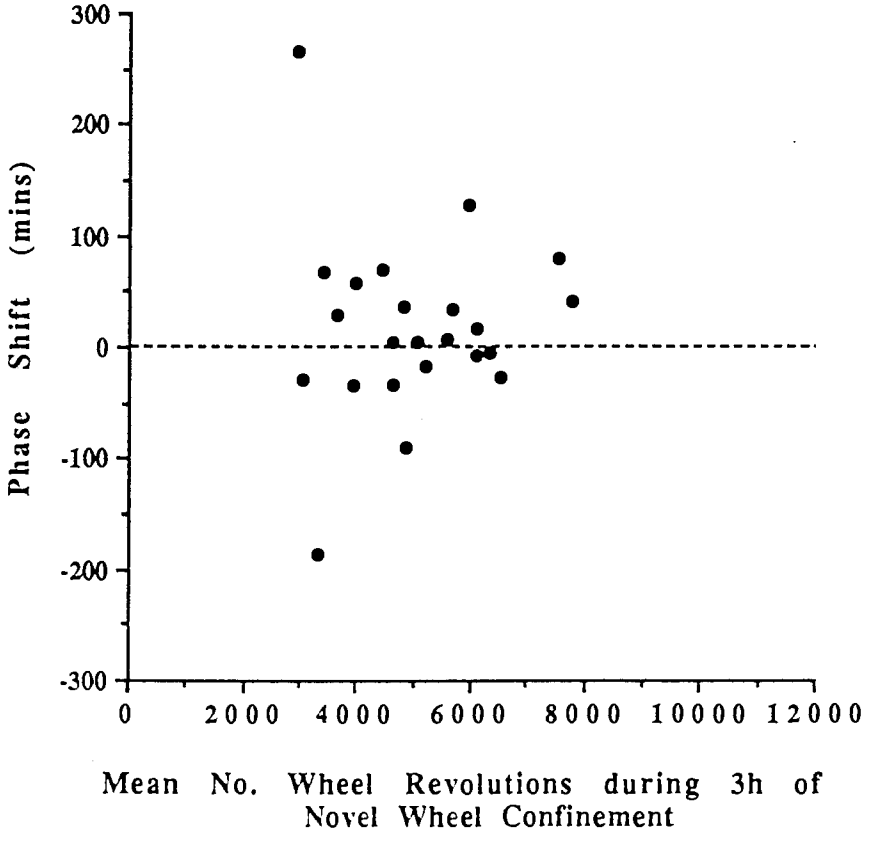

300
200
100
0
-100
-200
-300
Phase Shift (mins)
0
2000
4000
6000
8000
10000
12000
Mean No. Wheel Revolutions during 3h of Novel Wheel Confinement

Figure 5d

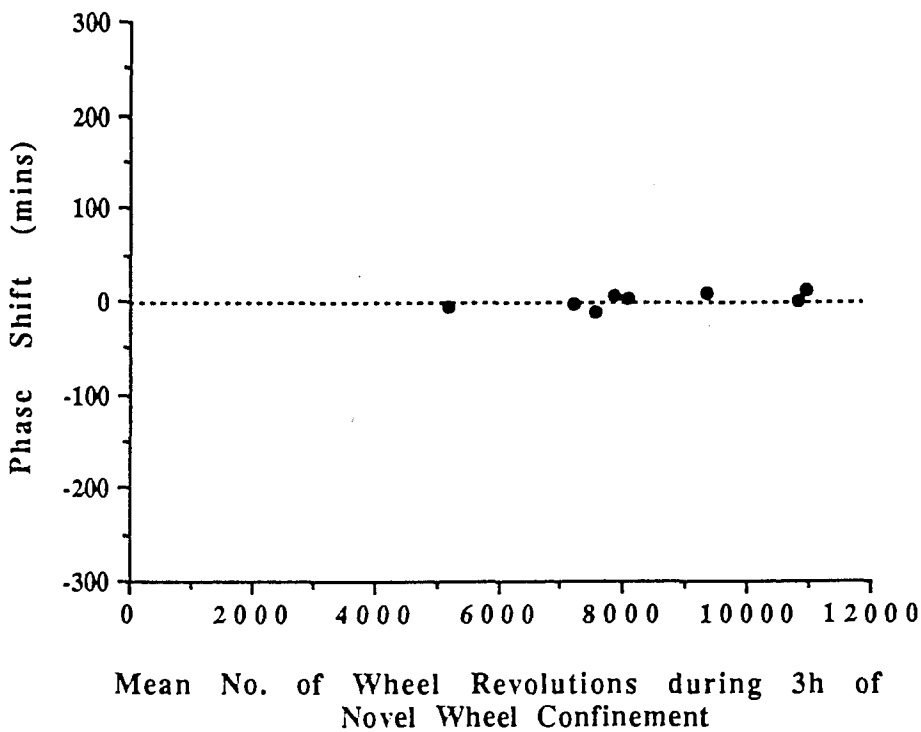

300
200
100
0
-100
-200
-300
Phase Shift (mins)
0
2000
4000
6000
8000
10000
12000
Mean No. of Wheel Revolutions during 3h of Novel Wheel Confinement

Figure 5e

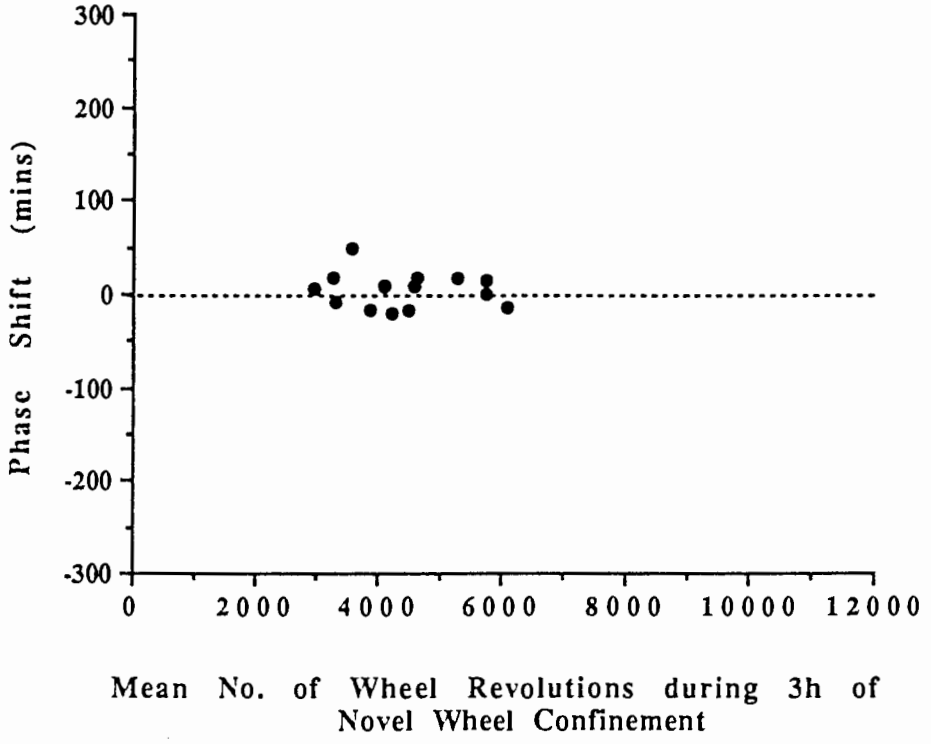

300
200
100
0
-100
-200
-300
Phase Shift (mins)
0
2000
4000
6000
8000
10000
12000
Mean No. of Wheel Revolutions during 3h of Novel Wheel Confinement

Figure 5f

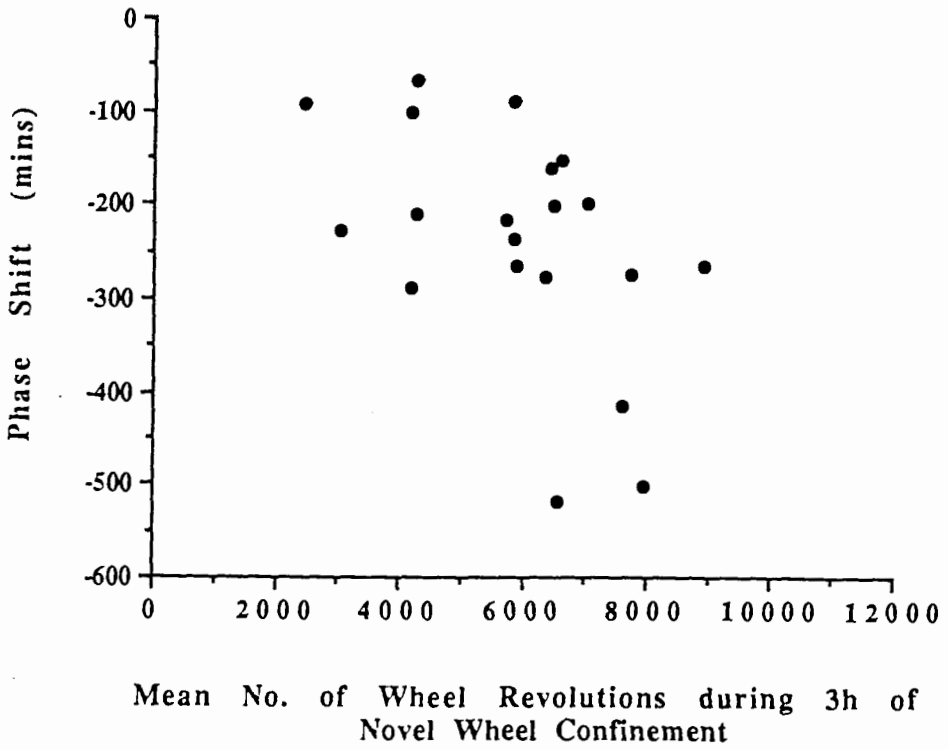

Phase Shift (mins)
0
-100
-200
-300
-400
-500
-600
0
2000
4000
6000
8000
10000
12000
Mean No. of Wheel Revolutions during 3h of Novel Wheel Confinement

Figure 6. Single plotted wheel-running activity charts of hamsters subjected to novelty induced running. Each line represents 24 h, with time plotted from left to right in consecutive 10-min. bins. Vertical deflections indicate bins during which wheel revolutions were registered (all shaded areas are dim red light and heavily shaded area is 3 h of exercise): a) ZT9.5 (advance); b) ZT9.5 (delay); c) ZT13 (advance); d) ZT13 (delay); e) ZT19; f) ZT20; g) ZT21. Each line represents 24h, with time plotted from left to right in consecutive 10-min. bins. Vertical deflections indicate bins during which wheel revolutions were registered.

Figure 6a

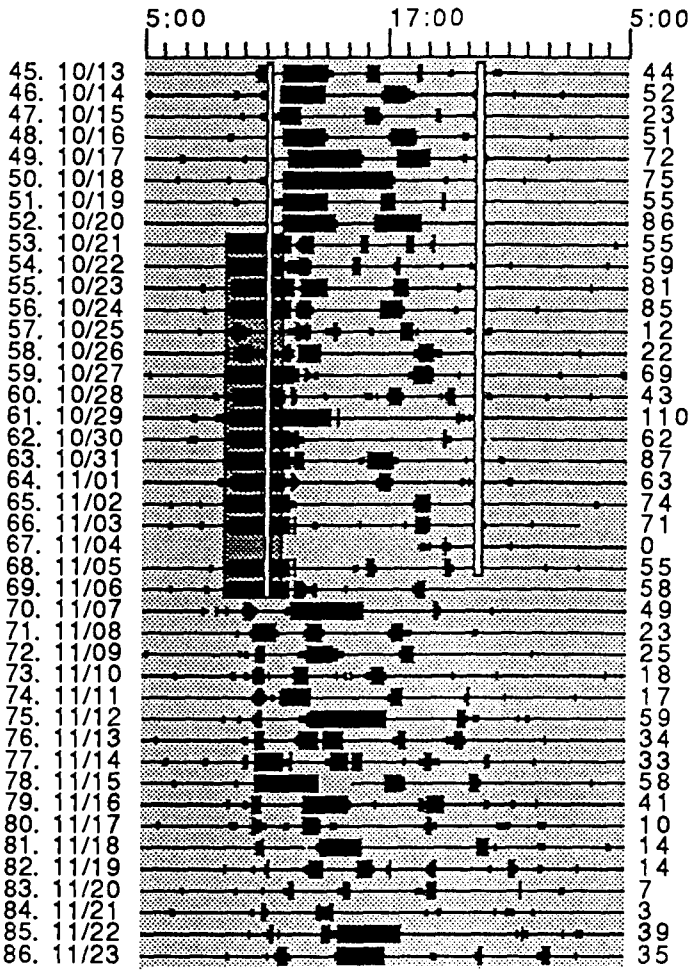

5:00
17:00
5:00
45. 10/13 44
46. 10/14 52
47. 10/15 23
48. 10/16 51
49. 10/17 72
50. 10/18 75
51. 10/19 55
52. 10/20 86
53. 10/21 55
54. 10/22 59
55. 10/23 81
56. 10/24 85
57. 10/25 12
58. 10/26 22
59. 10/27 69
60. 10/28 43
61. 10/29 110
62. 10/30 62
63. 10/31 87
64. 11/01 63
65. 11/02 74
66. 11/03 71
67. 11/04 0
68. 11/05 55
69. 11/06 58
70. 11/07 49
71. 11/08 23
72. 11/09 25
73. 11/10 18
74. 11/11 17
75. 11/12 59
76. 11/13 34
77. 11/14 33
78. 11/15 58
79. 11/16 41
80. 11/17 10
81. 11/18 14
82. 11/19 14
83. 11/20 7
84. 11/21 3
85. 11/22 39
86. 11/23 35

Figure 6b

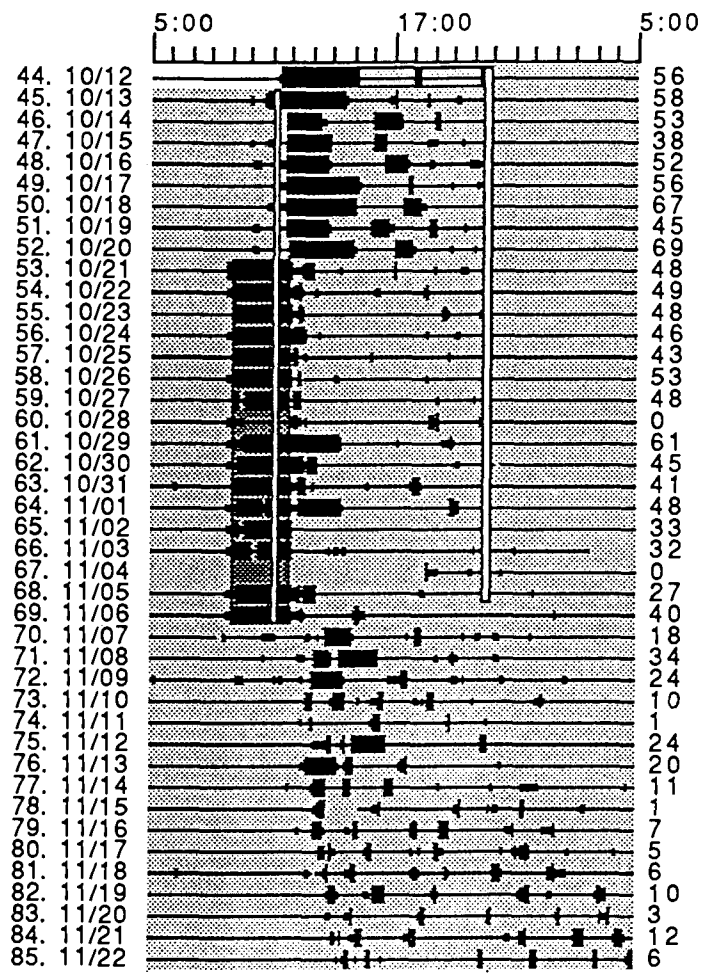

5:00
17:00
5:00
44. 10/12 56
45. 10/13 58
46. 10/14 53
47. 10/15 38
48. 10/16 52
49. 10/17 56
50. 10/18 67
51. 10/19 45
52. 10/20 69
53. 10/21 48
54. 10/22 49
55. 10/23 48
56. 10/24 46
57. 10/25 43
58. 10/26 53
59. 10/27 48
60. 10/28 0
61. 10/29 61
62. 10/30 45
63. 10/31 41
64. 11/01 48
65. 11/02 33
66. 11/03 32
67. 11/04 0
68. 11/05 27
69. 11/06 40
70. 11/07 18
71. 11/08 34
72. 11/09 24
73. 11/10 10
74. 11/11 1
75. 11/12 24
76. 11/13 20
77. 11/14 11
78. 11/15 1
79. 11/16 7
80. 11/17 5
81. 11/18 6
82. 11/19 10
83. 11/20 3
84. 11/21 12
85. 11/22 6

Figure 6c

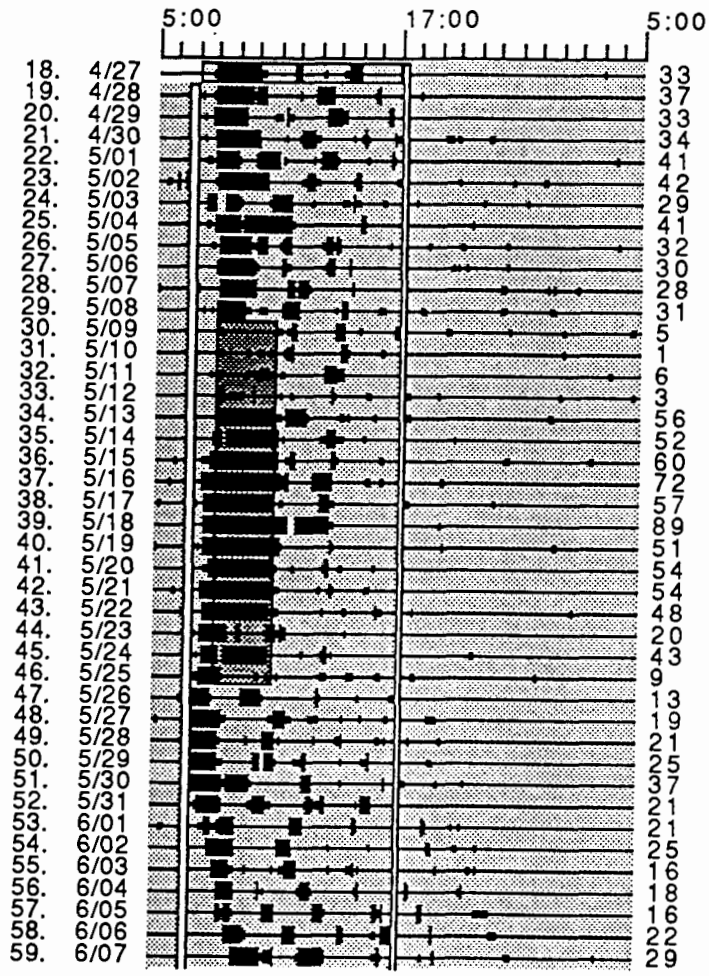

5:00
17:00
5:00
18. 4/27 33
19. 4/28 37
20. 4/29 33
21. 4/30 34
22. 5/01 41
23. 5/02 42
24. 5/03 29
25. 5/04 41
26. 5/05 32
27. 5/06 30
28. 5/07 28
29. 5/08 31
30. 5/09 5
31. 5/10 1
32. 5/11 6
33. 5/12 3
34. 5/13 56
35. 5/14 52
36. 5/15 60
37. 5/16 72
38. 5/17 57
39. 5/18 89
40. 5/19 51
41. 5/20 54
42. 5/21 54
43. 5/22 48
44. 5/23 20
45. 5/24 43
46. 5/25 9
47. 5/26 13
48. 5/27 19
49. 5/28 21
50. 5/29 25
51. 5/30 37
52. 5/31 21
53. 6/01 21
54. 6/02 25
55. 6/03 16
56. 6/04 18
57. 6/05 16
58. 6/06 22
59. 6/07 29

Figure 6d

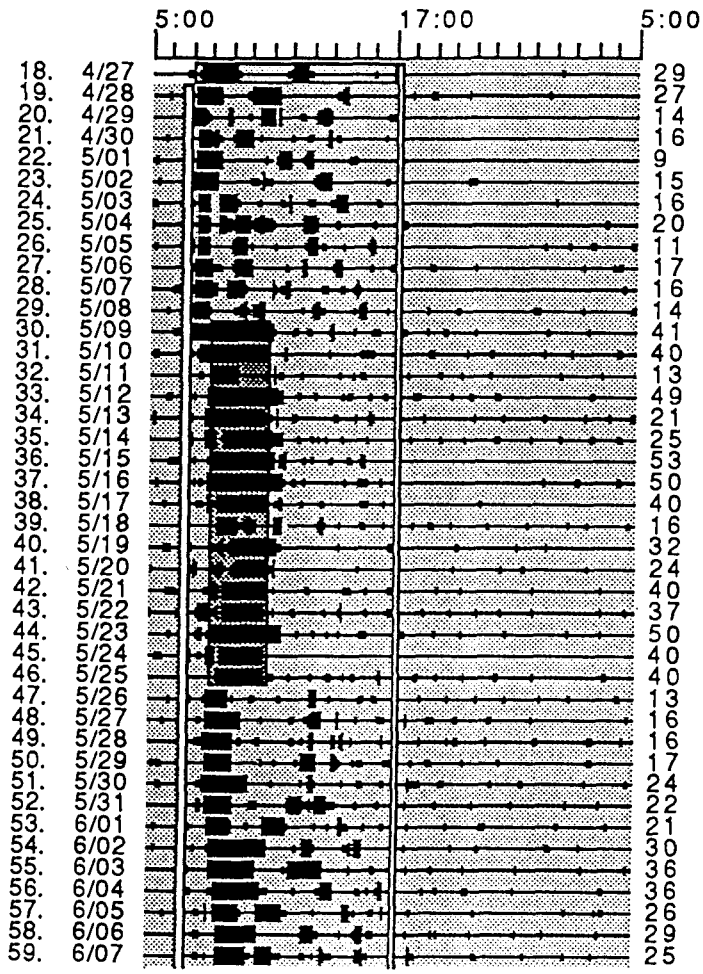

5:00
17:00
5:00
18. 4/27 29
19. 4/28 27
20. 4/29 14
21. 4/30 16
22. 5/01 9
23. 5/02 15
24. 5/03 16
25. 5/04 20
26. 5/05 11
27. 5/06 17
28. 5/07 16
29. 5/08 14
30. 5/09 41
31. 5/10 40
32. 5/11 13
33. 5/12 49
34. 5/13 21
35. 5/14 25
36. 5/15 53
37. 5/16 50
38. 5/17 40
39. 5/18 16
40. 5/19 32
41. 5/20 24
42. 5/21 40
43. 5/22 37
44. 5/23 50
45. 5/24 40
46. 5/25 40
47. 5/26 13
48. 5/27 16
49. 5/28 16
50. 5/29 17
51. 5/30 24
52. 5/31 22
53. 6/01 21
54. 6/02 30
55. 6/03 36
56. 6/04 36
57. 6/05 26
58. 6/06 29
59. 6/07 25

Figure 6e

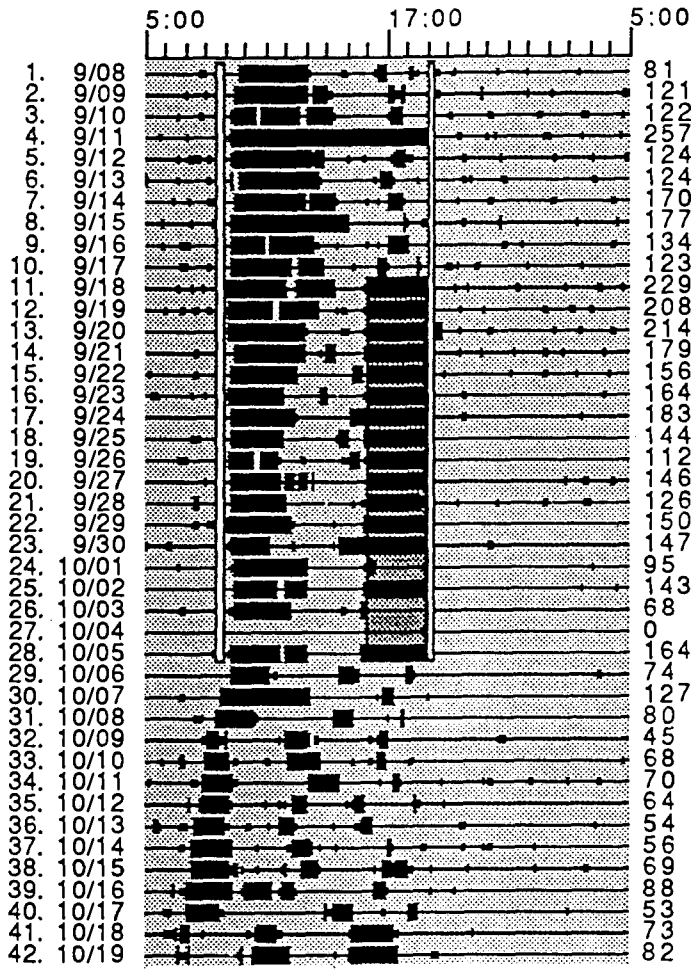

5:00
17:00
5:00
1. 9/08 81
2. 9/09 121
3. 9/10 122
4. 9/11 257
5. 9/12 124
6. 9/13 124
7. 9/14 170
8. 9/15 177
9. 9/16 134
10. 9/17 123
11. 9/18 229
12. 9/19 208
13. 9/20 214
14. 9/21 179
15. 9/22 156
16. 9/23 164
17. 9/24 183
18. 9/25 144
19. 9/26 112
20. 9/27 146
21. 9/28 126
22. 9/29 150
23. 9/30 147
24. 10/01 95
25. 10/02 143
26. 10/03 68
27. 10/04 0
28. 10/05 164
29. 10/06 74
30. 10/07 127
31. 10/08 80
32. 10/09 45
33. 10/10 68
34. 10/11 70
35. 10/12 64
36. 10/13 54
37. 10/14 56
38. 10/15 69
39. 10/16 88
40. 10/17 53
41. 10/18 73
42. 10/19 82

Figure 6f

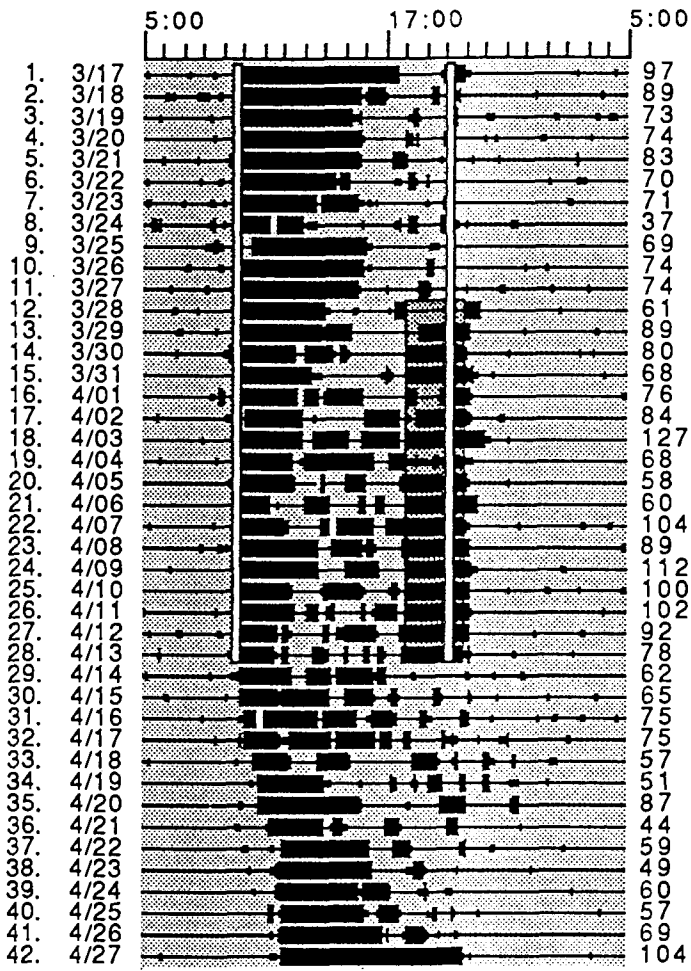

5:00
17:00
5:00
1. 3/17 97
2. 3/18 89
3. 3/19 73
4. 3/20 74
5. 3/21 83
6. 3/22 70
7. 3/23 71
8. 3/24 37
9. 3/25 69
10. 3/26 74
11. 3/27 74
12. 3/28 61
13. 3/29 89
14. 3/30 80
15. 3/31 68
16. 4/01 76
17. 4/02 84
18. 4/03 127
19. 4/04 68
20. 4/05 58
21. 4/06 60
22. 4/07 104
23. 4/08 89
24. 4/09 112
25. 4/10 100
26. 4/11 102
27. 4/12 92
28. 4/13 78
29. 4/14 62
30. 4/15 65
31. 4/16 75
32. 4/17 75
33. 4/18 57
34. 4/19 51
35. 4/20 87
36. 4/21 44
37. 4/22 59
38. 4/23 49
39. 4/24 60
40. 4/25 57
41. 4/26 69
42. 4/27 104

Figure 6g

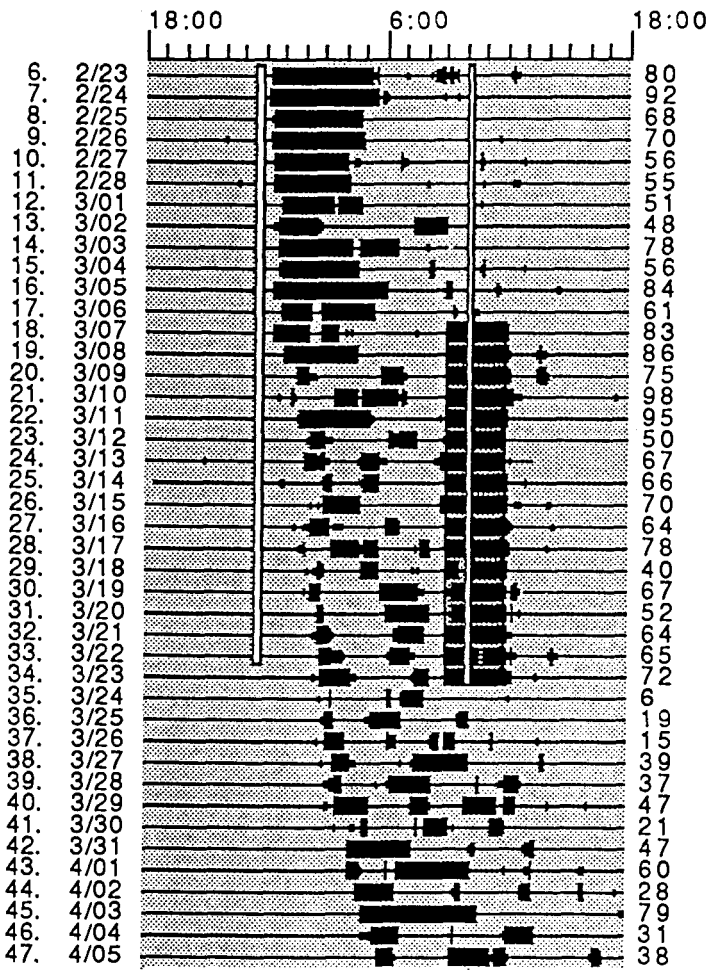

18:00
6:00
18:00
6. 2/23 80
7. 2/24 92
8. 2/25 68
9. 2/26 70
10. 2/27 56
11. 2/28 55
12. 3/01 51
13. 3/02 48
14. 3/03 78
15. 3/04 56
16. 3/05 84
17. 3/06 61
18. 3/07 83
19. 3/08 86
20. 3/09 75
21. 3/10 98
22. 3/11 95
23. 3/12 50
24. 3/13 67
25. 3/14 66
26. 3/15 70
27. 3/16 64
28. 3/17 78
29. 3/18 40
30. 3/19 67
31. 3/20 52
32. 3/21 64
33. 3/22 65
34. 3/23 72
35. 3/24 6
36. 3/25 19
37. 3/26 15
38. 3/27 39
39. 3/28 37
40. 3/29 47
41. 3/30 21
42. 3/31 47
43. 4/01 60
44. 4/02 28
45. 4/03 79
46. 4/04 31
47. 4/05 38

Figure 7. Single plotted wheel-running activity charts of hamsters with one light pulse of SPP removed. Each line represents 24 h, with time plotted from left to right in consecutive 10-min. bins. Vertical deflections indicate bins during which wheel revolutions were registered (all shaded areas are dim red light): A. E light pulse removed; B. M light pulse removed.

Figure 7a

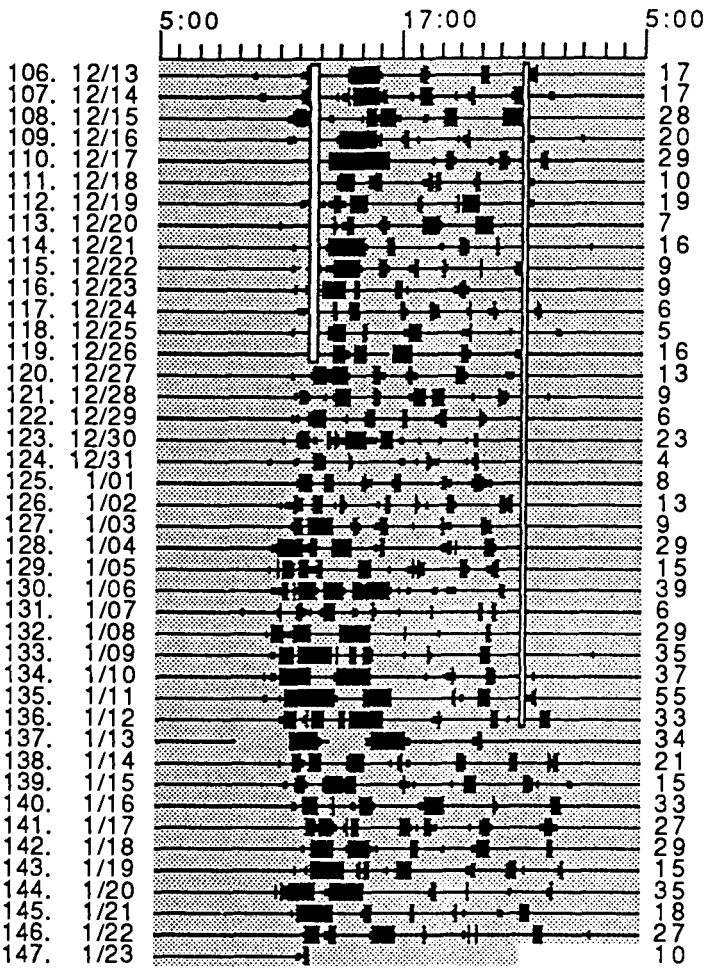
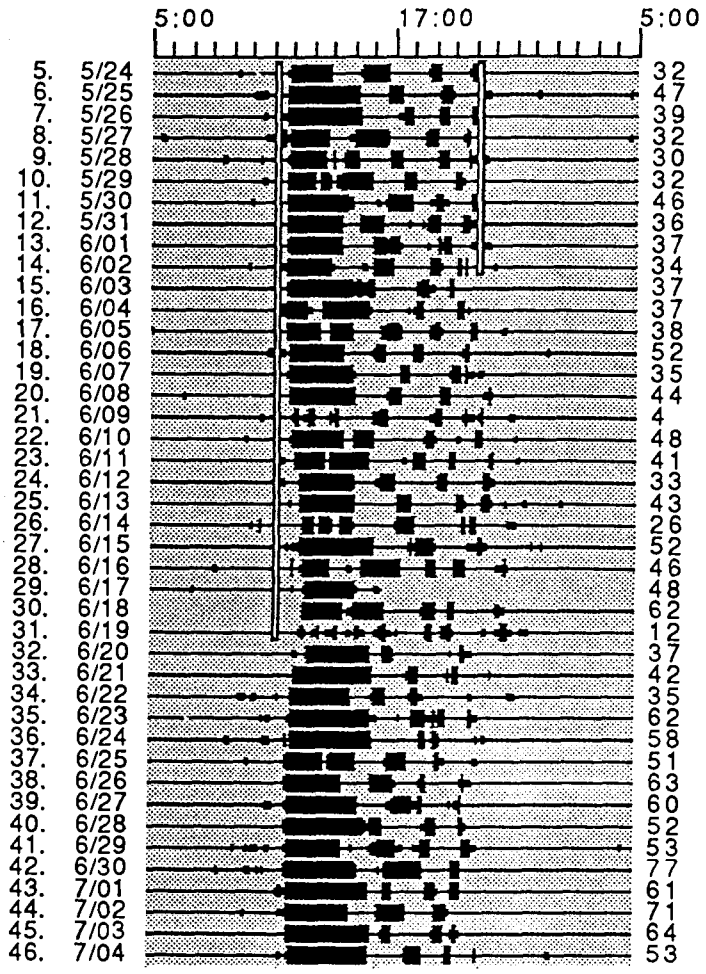

5:00
17:00
5:00
106. 12/13 17
107. 12/14 17
108. 12/15 28
109. 12/16 20
110. 12/17 29
111. 12/18 10
112. 12/19 19
113. 12/20 7
114. 12/21 16
115. 12/22 9
116. 12/23 9
117. 12/24 6
118. 12/25 5
119. 12/26 16
120. 12/27 13
121. 12/28 9
122. 12/29 6
123. 12/30 23
124. 12/31 4
125. 1/01 8
126. 1/02 13
127. 1/03 9
128. 1/04 29
129. 1/05 15
130. 1/06 39
131. 1/07 6
132. 1/08 29
133. 1/09 35
134. 1/10 37
135. 1/11 55
136. 1/12 33
137. 1/13 34
138. 1/14 21
139. 1/15 15
140. 1/16 33
141. 1/17 27
142. 1/18 29
143. 1/19 15
144. 1/20 35
145. 1/21 18
146. 1/22 27
147. 1/23 10

Figure 7b


5:00
17:00
5:00
5. 5/24 32
6. 5/25 47
7. 5/26 39
8. 5/27 32
9. 5/28 30
10. 5/29 32
11. 5/30 46
12. 5/31 36
13. 6/01 37
14. 6/02 34
15. 6/03 37
16. 6/04 37
17. 6/05 38
18. 6/06 52
19. 6/07 35
20. 6/08 44
21. 6/09 4
22. 6/10 48
23. 6/11 41
24. 6/12 33
25. 6/13 43
26. 6/14 26
27. 6/15 52
28. 6/16 46
29. 6/17 48
30. 6/18 62
31. 6/19 12
32. 6/20 37
33. 6/21 42
34. 6/22 35
35. 6/23 62
36. 6/24 58
37. 6/25 51
38. 6/26 63
39. 6/27 60
40. 6/28 52
41. 6/29 53
42. 6/30 77
43. 7/01 61
44. 7/02 71
45. 7/03 64
46. 7/04 53