Effects of Lighting and Food Restriction Paradigms on Locomotor Activity of the Egyptian Spiny Mouse, *Acomys cahirinus*.

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Introduction

Many mammals exhibit circadian rhythms that are entrainable by a light-dark (LD) cycle. These LD cycles entrain the primary circadian pacemaker, which is called the light-entrainable oscillator (LEO). This oscillator has been well studied in many mammalian species including rats (Brinkhof *et al*, 1998), flying squirrels (DeCoursey, 1960), weasels and the mink (Zielinski, 1986). Results from these studies suggest that light has quantifiable effects on displayed rhythms such that for nocturnal animals, higher intensities of light result in decreased activity and increased *tau*. This is also known as Aschoff's "rule" (Dunlop *et al*, 2007). One species in which this relationship has not been investigated is the Egyptian spiny mouse, *Acomys cahirinus*, and this was one of the goals in this study.

While few studies have been done on *A. cahirinus*, this species exhibits nocturnal behavioral patterns in both the laboratory (Jones *et al*, 2001) and field (Elvert *et al*, 1999). Since these nocturnal rhythms persist in constant darkness (Weaver and Reppert, 1987), they are controlled endogenously by an LEO. However, nothing is known about the effects of LL on this normally nocturnal activity or whether any additional oscillators, independent from the LEO, exist within this species.

Although LEOs have been the most thoroughly studied, recent evidence suggests the presence of a multi-oscillatory system in many mammalian species. One oscillator in particular, the food-entrainable oscillator (FEO), has been studied in several species. For example, honeybees (Frisch *et al*, 1987) display food anticipatory activity (FAA) associated with restricted food availability. When the bees were subjected to one bout of sugar water each day, they quickly learned to visit the food source either during or just before food availability. Similar instances of time-place association have been demonstrated in starlings (Wenger *et al*, 1991), *Sturnus vulgaris*, and garden warblers (Biebach *et al*, 1989), *Sylvia borin*. Furthermore, rats exposed to restricted daily feeding schedules exhibit increased activity and lever-pressing just before the scheduled feeding time(s) in both LD and LL. This activity can even persist for up to five days when food availability is completely eliminated (Boulos *et al*, 1990). Despite clear evidence from other species, the presence of FAA or a FEO has yet to be found in *A. cahirinus*.

This series of experiments focused on the investigation of these issues in *A. cahirinus* and we hypothesized that (1) LL would cause activity changes consistent with Aschoff's "rule" and (2) FAA would be present in both LD and LL, supporting the idea of a separate food-entrainable oscillator.

http://www.hiiret.fi/eng/spiny/care.html

Methods

Animals and Environmental Conditions:

Mice were housed in standard plastic cages (20x20x40cm), each equipped with a running-wheel available to the mice at all times. Groups of six cages were held in light-tight wooden chambers (60x51x186cm) and lighting was provided by two four-foot fluorescent bulbs of 34 Watts and 1400 Lux (Luna-Pro light meter; Gossen, Germany) in each chamber. The chambers were continuously ventilated by a blower system with temperature maintained at 24˚C (±3˚C) and humidity of 54% (± 5%). Each cage was always equipped with a water bottle that was refilled on a regular basis. When they were allowed to feed, the mice were given IAMS Chunks dog food.

Experimental Procedures:

Effects of LD, DD and LL on Activity Rhythms:

Mice (7-13 months) were originally purchased from a commercial supplier (Plymouth Pet & Aquarium; Plymouth, NH) and then bred in the laboratory at Plymouth State University's Natural Science Department (Plymouth, NH). Same-sex mice were housed in pairs under 12:12 LD for at least 15 days prior to any experimentation. Then, at the start of each experiment, mice were additionally entrained to 12:12 LD for at least 10 days while activity was recorded. After the initial LD entrainment period, ten male mice were exposed to 55 days of constant dark (DD). In order to determine if exposure to constant light (LL) can cause "after effects" in *A. cahirinus*, a second experiment exposed mice (n=12; six male, six female) to two periods of DD (11 and 12 days respectively) sandwiched around one period of LL (23 days; 175 lux). In a third experiment, male mice (n=12) were exposed to four sequentially increasing LL intensities (66, 130, 350 and 1400 lux). Each intensity period lasted 21, 21, 32, and 25 days respectively.

Effects of Food Restriction Paradigms:

Eleven mice (3 female and 8 male; 4-9 weeks) were purchased from the same supplier or from the Laconia Pet Center (Laconia, NH). Again, the mice were initially exposed to a few weeks of standard 12:12 LD, allowing sufficient time for clear locomotor rhythms to be established before the next experiments took place. The remaining experiments studied the effects of food restriction paradigms under LD and LL conditions on wheel-running activity of *A. cahirinus*, during which a two hour period of daytime food restriction was implemented. At 4:30pm, a metal feeder containing 40g of food was hung on the inside of each cage for a total of two hours and promptly removed at 6:30pm. Any excess food that had fallen into the cage was removed as well. The food remaining in the feeder and the cage was weighed and this value subtracted from the original amount to determine the total food consumed for that day per mouse. The next day at 4:30pm, the feeders were again refilled with 40g of food and the process repeated for a total of 33 days. In the next experiment, the feeding schedule was returned to the original 12:12 LD *ad libitum* conditions for 17 days to see if the animals had entrained to the previous RF schedule. Then, the same restricted feeding schedule was implemented under constant light (LL) conditions for 28 days. In the last experiment, *ad libitum* conditions were imposed for 32 days in order to determine whether their rhythms had become entrained and a secondary food-entrainable clock was present.

Data Analysis:

Effects of LD, DD and LL on Activity Rhythms:

The running wheel data were collected by a computer data acquisition system (Drosophila Activity Monitor IV, Trikinetics Waltham, MA) and stored on a Macintosh Computer in five or ten minute intervals. RATMAN (Klemfuss and Clopton, 1993) was used to generate actograms from these files. Since activity data in experiment three were collected in ten minute intervals and RATMAN can only accept data in five minute intervals, ten minute interval data were equally divided into two five minute intervals. Alpha was calculated by using objectively drawn eye-fit lines on the actograms produced by RATMAN. *Tau* was calculated by RATWAVE (Klemfuss and Clopton, 1993) and the resulting *tau* was compared to the activity records. Nine out of 105 values produced by RATWAVE were clearly incorrect values of *tau*. To correct this, *tau* was calculated using the slope of the eye-fit lines in these instances. This procedure had no effect on any statistical analysis.
Total activity for each mouse was calculated by summing the number of wheel rotations per five minute intervals and then dividing by the total number of five minute intervals within each stage of the experiments. *Tau*, alpha, and total activity were calculated for all mice during each of the experiments. Analysis of variance (Super ANOVA, Abacus Concepts, Inc.) was used to determine overall effect ($p<0.05$). Significant differences between means were determined by least square means method ($p<0.05$).

Effects of Food Restriction Paradigms:

All running wheel data were collected by a computer data acquisition system (ClockLab, Actimetrics Evanston, IL) and stored in 5-minute bins. Sequential actograms for each individual subject were visually inspected and Lomb-Scargle periodograms used to calculate tau ($p<0.05$) and determine the presence of significant rhythmicity. Microsoft Office 2003 Excel (Redmond, WA) was used to calculate descriptive statistics and perform Students't-tests to determine overall significance ($p < 0.05$) between daytime and nighttime activity, as well as significant differences between normal and restricted feeding activity levels in LD and LL conditions. Activity in the 1h prior to food restriction was compared in LD and LL to determine if food anticipatory activity had occurred.

Results

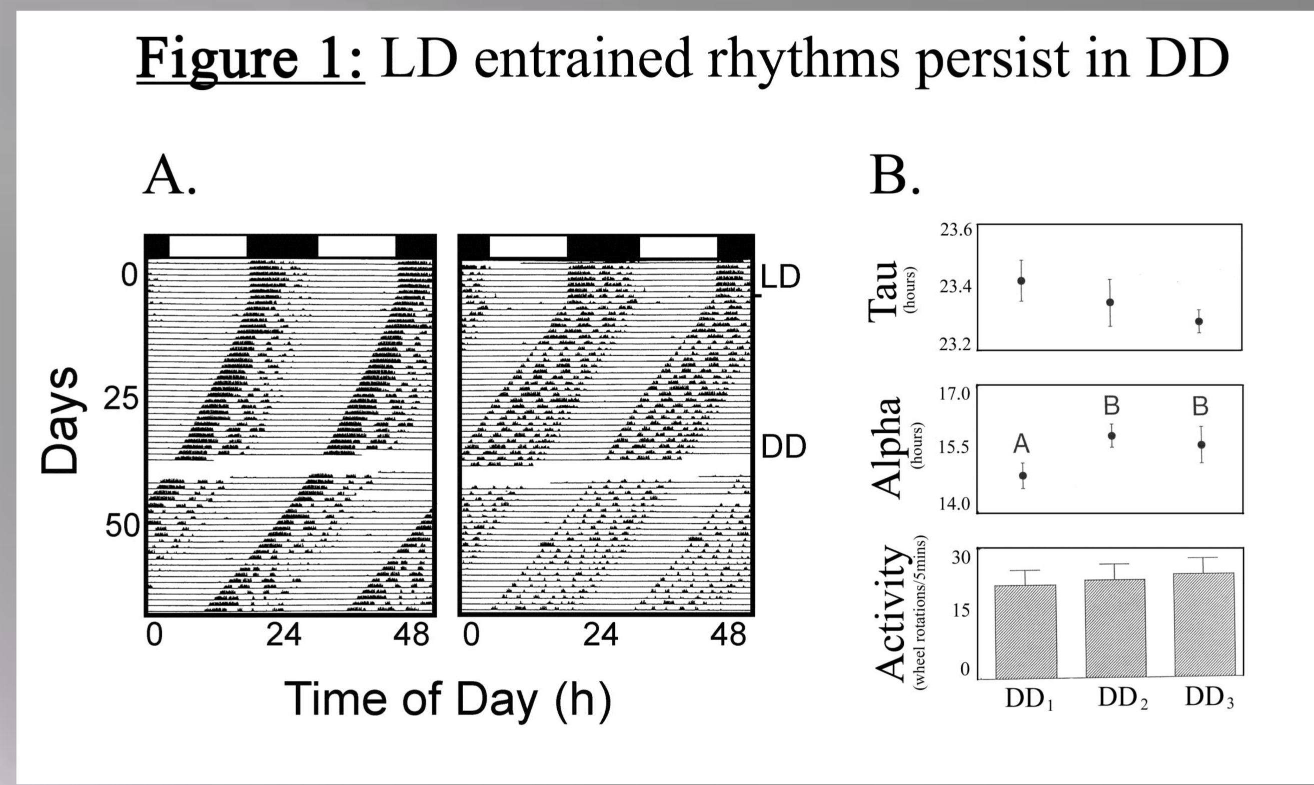

Figure 1: Representative double-plotted actograms (A) and overall results (B) of two *A. cahirinus* exposed to 12:12 Light-Dark (LD) and then constant dark (DD). **A.** Black bars at the top signify the hours of dark; white bars signify hours of light. Each black tick mark indicates a bout of activity recorded of the individual animal. All animals (10/10) entrained to LD and these rhythms persisted in DD with periods less than 24 hours. **B.** Graphical values represent the means ± the standard error of the means. Over time in DD, alpha lengthened significantly ($F(9,18) = 7.19$; $p<0.02$). Neither *tau* ($F(9,18) = 1.53$; $p<0.25$) nor activity ($F(9,18) = 0.38$; $p<0.69$) changed significantly over the 50+ days in DD.

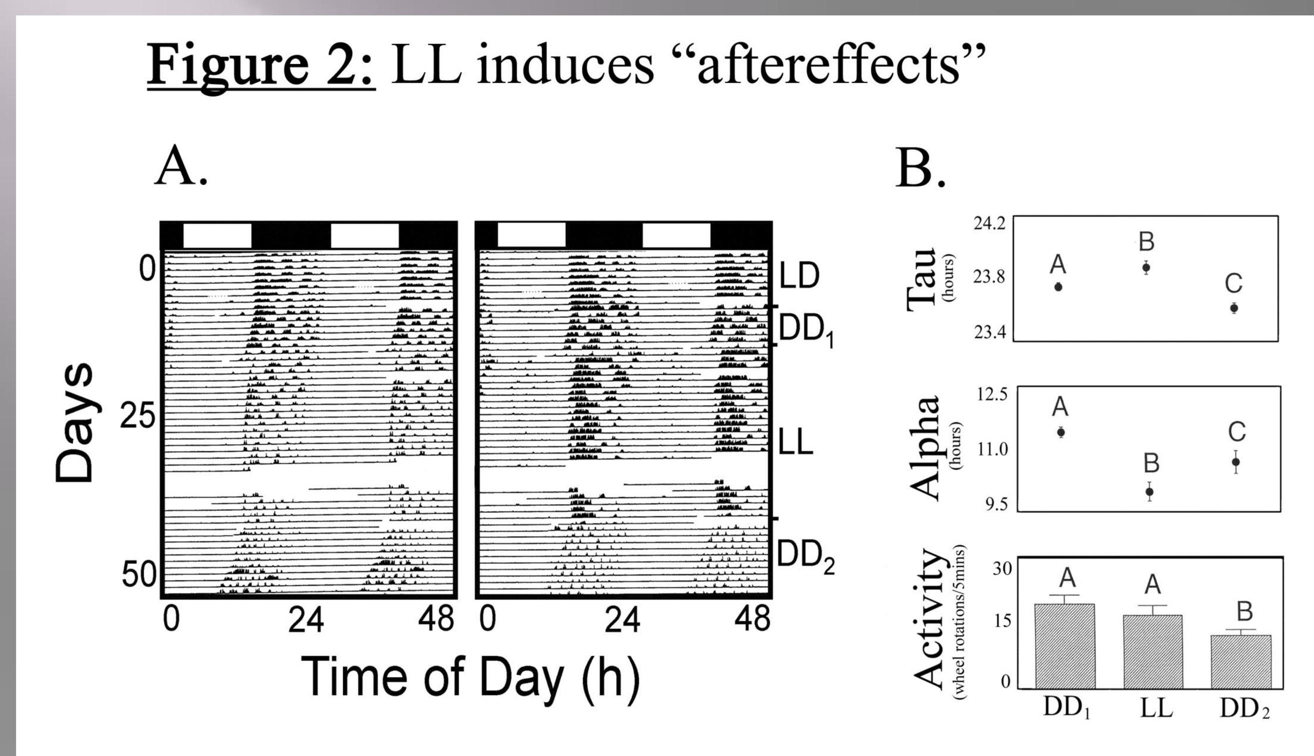

Figure 2: Representative actograms (A) and overall results (B) of two different mice entrained to LD and then exposed to two periods of DD sandwiched around a period of constant light (LL; 175 lux). **B.** From DD_1 to LL, a significant decrease in alpha occurred ($F(11,22) = 11.18$; $p<0.003$). When LL was subsequently changed to DD_2 alpha increased significantly, but not to its original DD_1 value. *Tau* was less than 24 hours in DD_1 but became significantly longer ($F(11,22) = 21.46$; $p<0.0001$) upon LL exposure. When the photic conditions reverted back to DD (DD_2), there was a significant shortening in *tau* compared to both LL and DD_1 *taus*. In addition, activity decreased significantly from LL to DD_2 ($F(11,22) = 8.43$; $p<0.003$).

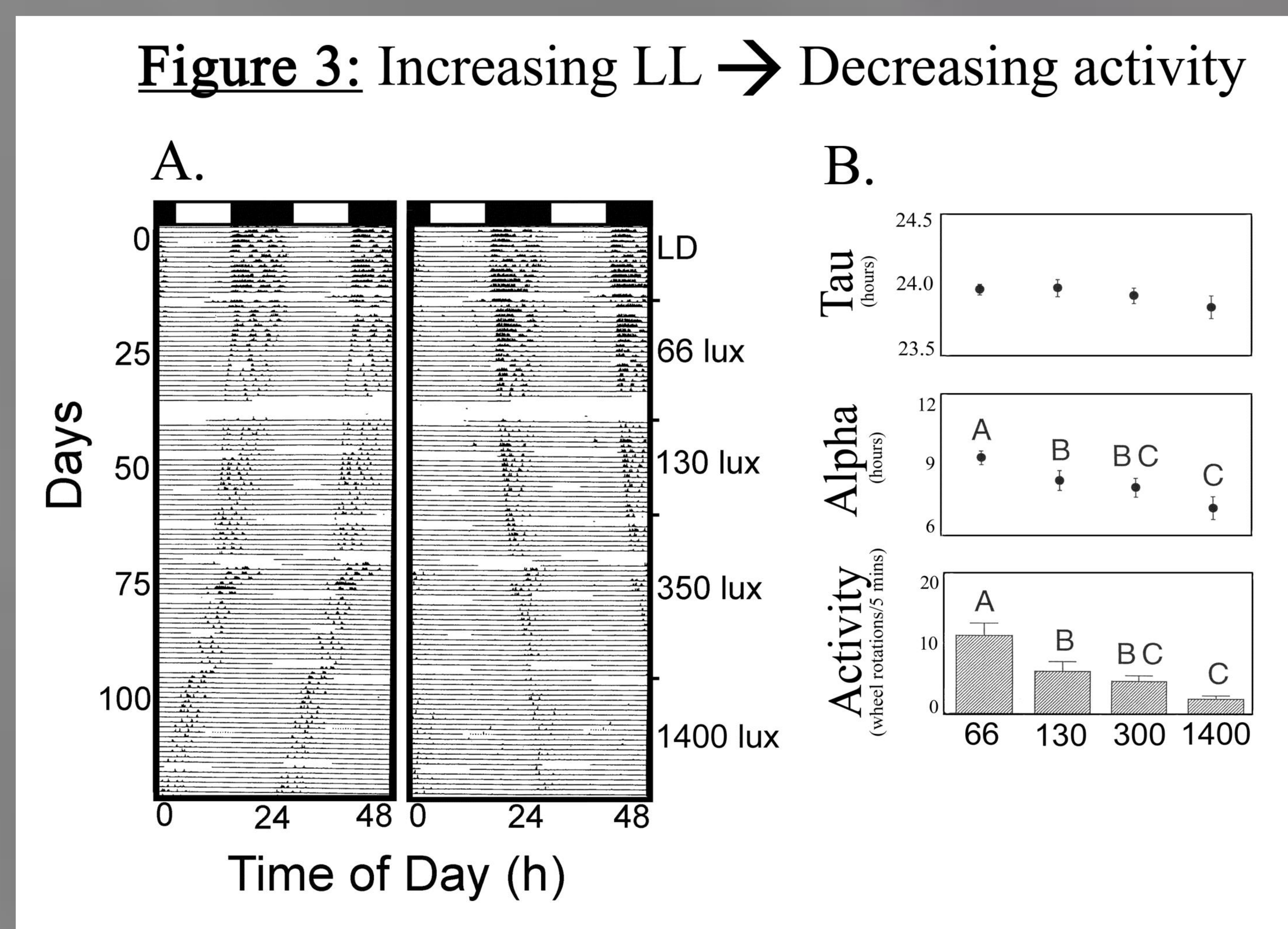

Figure 3: Representative actograms (A) and overall results (B) of two mice entrained to LD and then exposed to increasing LL intensities (66 to 1400 lux). **B.** As LL intensity increased, a significant decrease in both alpha ($F(11,33) = 8.20$; $p<0.0009$) and running-wheel activity ($F(11,33) = 18.86$; $p<0.0001$) occurred However, *tau* was not significantly affected.

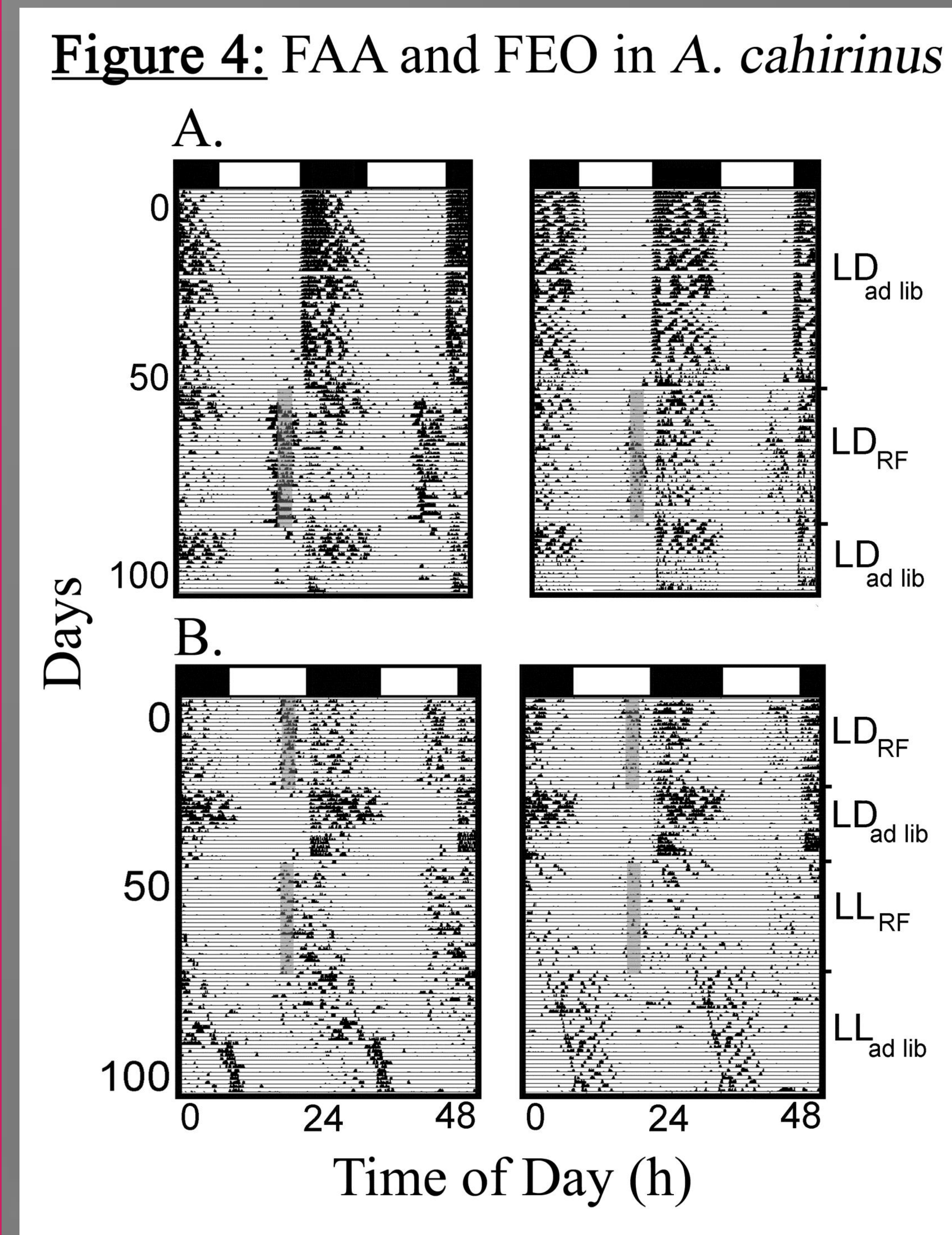

Figure 4: Representative actograms showing the effects of food restriction (RF) paradigms in LD (A) and LL (B) on running-wheel activity of two mice. The shaded grey region indicates a 2h period of restricted feeding (16:30 – 18:30 h). **A.** When the mice were exposed to 12:12 LD *ad libitum* conditions, all mice (11/11) individuals showed significant nocturnal activity. When the LD entrained mice were then exposed to a two-hour period of RF during the daytime, 10/11 (91%) individuals still exhibited significantly more activity during the nighttime – i.e. – they were still generally nocturnally active. However, all (11/11) mice also showed a significant increase in daytime activity during the 1 hour prior to food availability. This increase in diurnal activity persisted in 4/11 animals when food restriction was discontinued and *ad libitum* conditions reinstated. **B.** Under LL, the main activity bouts of most animals (9/11) exhibited no coordination with the restricted feeding while 2/11 animals appeared to entrain to the restricted feeding. Activity, *taus*, ($t=0.12$; $p < 0.05$) in LL food restriction and LL *ad libitum* were not significantly different.

Conclusions

- Consistent with Aschoff's "rule", increased LL intensities resulted in decreased activity.
- LL induces "after effects" on activity rhythms.
- Results are consistent with the presence of a separate FEO in this species:
  - FAA was present during periods of restricted feeding.
  - Activity associated with FAA persisted in *ad libitum* conditions.

Acknowledgements

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