NEW FINDINGS REGARDING LIGHT INTENSITY
AND ITS EFFECTS AS A ZEITGEBER IN THE
SPRAGUE-DAWLEY RAT

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INTRODUCTION

Circadian rhythmicities are oscillations of
physiological cycles designed to create temporal
organization. Circadian rhythms ensure that
physiological mechanisms are expressed in
proper relationship to each other and the 24
hour day. Light is the main zeitgeber ("time
giver") for biological clocks. The daily
variations in light intensity from dawn to dusk,
and seasonally due to the rotation of the earth,
act upon organisms to give them photoperiodic
information. This entrainment allows them to
vary biologically to prepare for reproduction,
hibernation, migration and the daily adaptations
necessary for survival. In most mammals, the
suprachiasmatic nucleus of the anterior
hypothalamus has been implicated as the
central driving mechanism of circadian
rhythmicity. The photic input from the retina,
via the retino-hypothalamic tract, and
modulation from the pineal gland help regulate
the clock. In this study we investigated the
effects of low light intensity on the circadian
system of the Sprague-Dawley rat. A series of
light intensity experiments were conducted to
determine if a light level of 0.1 Lux will
maintain entrained circadian rhythms of
feeding, drinking, and locomotor activity.

The intensity of light to which an animal is
exposed is one determinant of the length of the
free-running period of a circadian clock in a
constant environment. In 1960, Jurgen
Aschoff showed that more intense light shortens
the free-running period in diurnal organisms,
but lengthens the period in nocturnal animals.
Aschoff further concluded that under more
intense light, the time an animal is active, is
compared with the time it is at rest, increases
in diurnal animals, but decreases in nocturnal
animals. Also, in diurnal species, the total
amount of activity during a free-running
period increases with light intensity, while the
reverse is true in nocturnal species.

METHODS

Groups of six male Sprague-Dawley albino
rats (initial weight 250 g; final weight 350 g)
were obtained from Simonsen Laboratories.
Rats were selected from the lowest shelves on
the racks by the vendor in order to select
animals with exposure to low light intensity,
thus controlling for possible retinal damage.
Typical lighting levels in many animal
vivariums exceed 1000 Lux. At this level it is
now known that retinal damage can occur in
albino rats. It is also important to establish a
light history on the animals, because in
circadian rhythm research, prior exposure to
light can elicit aftereffects lasting up to 100
days.

The rats were housed individually in Nalge
metabolism cages made of lexan and
polycarbonate. These materials pass all
wavelengths of light in the visible spectrum,
and a portion of the ultraviolet spectrum. We
used a full-spectrum fluorescent light source
(Duro-Test Vita-Lite) simulating the spectral
qualities of natural sunlight. The metabolism
cages were housed in individual light tight
cabinets. The fluorescent light sources were
located centrally over each metabolism cage.
Using a calibrated radiometer, light intensities
were adjusted to the appropriate experimental
levels and set no higher than each specific
intensity at the eye level of each animal.

An initial baseline intensity of 10 Lux was
tested because previous studies in laboratory
rodents indicated entrainment at this level,and
above. An experiment was performed utilizing
a light intensity of 5.0 Lux, a level determined
by consensus at a NASA Lighting Requirements
in Microgravity Workshop. Three additional
experiments were performed utilizing 1.0 Lux
and 0.1 Lux (a level less than that of full
moonlight, approximately 0.4 Lux).

The following protocol was used for each
experiment: The rats were acclimated for a
period of 1 week to their new environment at
each light intensity. The light cycle was
adjusted for a 12 hour period of light, starting
at 0700, and 12 hours of darkness beginning at
1900. This 12L:12D cycle at each intensity
was imposed for 3 weeks. Immediately following
the 12L:12D cycle, the rats were exposed to a 2
week period of constant light (LL), At the
conclusion of the LL period, the 12L:12D light
cycle was reinstated for 1 week to re-entrain
the animals. Daily animal care was performed
in the dark at time intervals outside the range.
of circadian entrainment (18 hours before or 33 hours after last maintaining the rats). A final experiment was conducted using 40 Lux for the 12L:12D cycle, followed by an 18-day period of constant darkness (<0.01 Lux) and a return to the LD cycle.

The data format represented below is a raster plot. It is a record of activity over a 24 hour period which is double plotted so that the pattern of the circadian rhythm can easily be seen by the unaided eye. This raster plot visually depicts the difference between the entrained and free-running periods at an intensity of 0.1 lux.

![Raster plot image](image-url)

**Figure 1**

**Table 1. Complex Demodulation Mean Period ± S.E.**

<table>
<thead>
<tr>
<th>Lux</th>
<th>Activity</th>
<th>Drinking</th>
<th>Feeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>24.28±0.01</td>
<td>24.30±0.03</td>
<td>24.29±0.01</td>
</tr>
<tr>
<td>0.1</td>
<td>24.30±0.07</td>
<td>24.45±0.26</td>
<td>24.70±0.05</td>
</tr>
<tr>
<td>1</td>
<td>24.84±0.14</td>
<td>25.29±0.13</td>
<td>25.53±0.06</td>
</tr>
<tr>
<td>5</td>
<td>24.99±0.34</td>
<td>25.50±0.03</td>
<td>25.46±0.13</td>
</tr>
<tr>
<td>10</td>
<td>25.56±0.14</td>
<td>25.85±0.10</td>
<td>25.23±0.63</td>
</tr>
</tbody>
</table>

Period lengths were estimated from linear regression of acrophases (peak of harmonic fit to circadian rhythm) obtained from complex demodulation analyses [Table 1]. Cosinor analysis was utilized to obtain acrophase, mean amplitude, and significance level of the rhythms monitored.

**RESULTS AND DISCUSSION**

Visual inspection of the raster plots clearly indicated that circadian rhythmicity was maintained in all parameters monitored during LD. Results of cosinor analysis showed significant circadian rhythms (alpha=0.05) were detected in all parameters during the control periods.

Furthermore, the results demonstrate that the free-running period length is directly proportional to the logarithm of light intensity [figure 2].

This is consistent with Aschoff's Rule which states that the circadian frequency varies linearly with the logarithm of the intensity. With increasing light intensity, the circadian frequency of dark-active animals decreases (the period lengths).

**CONCLUSION**

These experiments show that extremely low light levels (0.1 lux) can entrain the circadian system of the white laboratory rat. Therefore, the power requirement ² (5.0 lux) specified for Sprague-Dawley rats in microgravity is sufficient to maintain normal circadian rhythmicity.


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