A Behavioral Profile of Bilateral Anophthalmic Mutant Rats

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Abstract: A behavioral profile of hereditary bilateral anophthalmic mutant rat was studied in different light: dark schedules. The control and mutant rats were acclimatized to either a) a 12h light:12h dark cycle or b) continuous darkness or c) continuous illumination. The measurements of spontaneous motor activity with Opto Varimex and behavioral despair in a swim test were conducted. The daily food consumption and plasma glucose levels were also measured. The study indicated that, unlike the control rats, mutants did not exhibit any time dependent change in the spontaneous motor activity in any of the three different lighting conditions. A strong biphasic feeding burst was also not affected by anophthalmia in mutant rats. Our findings on spontaneous motor activity and the feeding pattern are contrary to those in the existing literature.

Key words: anophthalmia, behavioral pattern, circadian rhythms, mutant rats

A mutant with hereditary anophthalmia was recently identified for the first time in a colony of CFY inbred rats. In the mutant rats either one or both eyes and the corresponding optic nerves are absent. The phenotypic changes in these mutants are restricted only to the eye [1]. It is well documented that light, acting through neuroendocrine pathways, plays a role as a stimulator and synchronizer for many physiological and behavioral functions [8]. A prolonged light or dark phase had a marked effect on reproduction, spontaneous motor activity (SMA) and food and water intake [2, 3, 12]. Since the existing literature strongly emphasizes the importance of light: dark cycles in normal rats, a behavioral study was undertaken on bilateral anophthalmic rats, which are totally devoid of primary photoreceptors.

Two-month-old normal sighted control (n=20) and bilateral anophthalmic male rats (n=20) in a weight range of 190–210 grams were acclimatized to each of the following lighting conditions for a period of fifteen days prior to experimentation.

1. 12 hour light (06:00–18:00); 12 hour darkness (18:00–06:00).
2. Continuous darkness.
3. Continuous illumination (300 lux at 1 meter above floor).

Rats in each group were housed in pairs, in polypropylene cages (430 (L) × 270 (W) × 150 mm (H)). All rats were fed on a commercial feed (Lipton India Ltd, Bangalore) and fresh sterile drinking water was provided in glass bottle with a stainless steel nozzle. The animal rooms were maintained at a controlled temperature of 22 ± 2°C and a relative humidity of 55 ± 5%.

In the continuous dark condition, feeding and watering the rats (on alternate days) and cage changing (at weekly intervals) were carried out under red illuminat-
tion, since rats have been shown to exhibit lowest sensitivity in the 605 nm light range [6]. SMA of the individual rats was counted with an Opto Varimex Activity Meter (cage size: 43 × 43 cm Columbus Instruments, Columbus, Ohio 43204, USA) in all three groups. Simultaneously the total number of defecations and urination frequency were manually counted to assess the emotionality of the rats. All the animals were acclimatized for 5 minutes prior to counting of SMA for a further 5 minutes.

As shown in Table 1, in the 12h light : 12h dark schedule, the mutant rats exhibited sustained activity during light and dark phases while the control rats showed a diurnal rhythm in the activity pattern. In these rats the lowest activity was recorded during the light phase and the high activity during the dark phase. Similar diurnal rhythms in the activity pattern of rats were reported earlier [13]. As indicated in Table 1, a significantly high rate of defecation and urination was observed in mutants.

Alteration in the light: Dark condition brought a very significant change in the activity of control rats. Continuous darkness enhanced and continuous illumination drastically reduced the activity. Either continuous darkness or illumination did not alter the activity pattern of mutant rats. Significantly high open field defecation and urination was observed in mutants.

The daily food intake and behavioral despair test were done in rats kept under 12 hour light:12 hour darkness only. The behavioral despair was studied during the light phase between 10:00–11:00h on overnight fasting rats by the method of Porsolt et al. [9]. The rat was forced to swim individually in a glass beaker (height: 30 cm; diameter: 23 cm) containing fresh water 18 cm deep. The temperature of the water was maintained at 25.0 ± 2°C. The animals were subjected to swimming for 5 minutes. The immobility time was recorded for each rat. A rat was judged to be immobile whenever it remained floating passively in the water in a slightly hunched but upright position. Both swimming and immobility time were recorded. On average the mutant rats recorded > 5 minutes of swimming time compared to < 3 minutes for the control rats. During swimming anophthalmic rats did not exhibit any immobility while control rats showed immobility for 122.4 ± 8.4 sec (mean ± SEM). This test indicates that the mutant rat could swim for a longer time because of its higher activity. Normal rats were reported to swim efficiently during the active (dark) phase [5].

In the 12h light: 12h dark cycle, rats have been shown to consume 90% of the total feed in the dark period [11]. In the present study overall food intake by both the groups was negligible until noon, with a gradual increase from afternoon to bulk consumption during the dark phase. It is surprising that non perception of light did not alter the strong periodic feeding burst. We also monitored the blood glucose levels at four different times in a day with an Ames Dextrometer (Miles Laboratories Inc., Japan). The results given in the Table 2 indicate that the food intake pattern and plasma glucose levels were synchronized in both the groups.

Many animals, including man, exhibit a circadian rhythm in behavioral as well as certain physiological functions [7]. Studies on the behavioral pattern in anophthalmic mutants may be useful in understanding the role of extra retinal photoreception in circadian rhythms. Scheuch et al. [10] observed in ZRDCT/An

<table>
<thead>
<tr>
<th></th>
<th>Group</th>
<th>Parameter</th>
<th>Light phase</th>
<th>Dark phase</th>
<th>Continuous darkness</th>
<th>Continuous illumination</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMA</td>
<td>Control</td>
<td>Defecation</td>
<td>1235 ± 161</td>
<td>2281 ± 122</td>
<td>3370 ± 339</td>
<td>969 ± 102</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Urination</td>
<td>3.8 ± 0.4</td>
<td>4.5 ± 0.7</td>
<td>3.0 ± 0.6</td>
<td>2.8 ± 0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SMA</td>
<td>1.8 ± 0.2</td>
<td>2.1 ± 0.3</td>
<td>2.0 ± 0.4</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>Mutant</td>
<td></td>
<td>Defecation</td>
<td>2793 ± 316*</td>
<td>2558 ± 253</td>
<td>2998 ± 236</td>
<td>2010 ± 153*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Urination</td>
<td>6.8 ± 0.6*</td>
<td>7.2 ± 0.9*</td>
<td>6.1 ± 1.0*</td>
<td>5.2 ± 0.5</td>
</tr>
</tbody>
</table>

Values are the mean ± SEM. Duration of monitoring was 5 minutes.

n=18 for SMA studies, n=12 for defecation and urination studies.

* : p<0.05 compared to controls for each parameter by Student’s t-test.
mice that the circadian rhythm was lost only when anophthalmia was associated with hypogenesis of the suprachiasmatic nuclei (SCN). Recently, Iba " reported a clear circadian rhythm in sleep-wakefulness and locomotor activity in Donryu bilateral anophthalmic rats. But the rhythms did not entrain to the light: dark cycle. SCN in these rats did not reveal any gross abnormalities. The preliminary report on SCN histology did not indicate any gross abnormalities (unpublished data), but anophthalmia has no effect on the feeding pattern. Our finding are contrary to those in the existing literature [4, 10], and it is possible that the absence of primary photoreceptors might have resulted in a change in neuroendocrine pathway that regulates the behavioral pattern in mutant rats. Further studies are in progress to elucidate this.

Reference


