Constant lighting conditions affect sexual behaviour and hormone levels in adult male rats*

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Summary. Adult male Long-Evans rats (90 days of age) were housed in 12 h light : 12 h dark (Group LD), constant white light (Group LL), or constant dark (dim red light) (Group DD) for 60–90 days. The first of two sexual behaviour tests revealed that more (P < 0.05) rats in Group LD achieved intromission than did those in Groups LL and DD. Only 33% of Group LL and 32% of Group DD rats ejaculated. In contrast, all Group LD rats that mounted eventually ejaculated. Males in Group LD achieved more (P < 0.05) ejaculations (mean of both tests combined, 3.4) than did males in Groups LL (1.5) or DD (1.2). Although there were no notable differences amongst the groups in the weights of the accessory sex organs, males in Group DD had higher (P < 0.05) serum androgen levels than did those in Groups LL and LD while Group LL males had higher (P < 0.05) serum prolactin levels than did those in Groups LD and DD. There were no differences in LH or FSH levels. In addition, more rats in Group LL had disrupted activity rhythms than did Group DD rats. These results indicate that housing in constant white or dim red light disrupts sexual behaviour in male rats and may do so through different neuroendocrine mechanisms.

Introduction

Housing prepubescent female rats in constant light accelerates vaginal opening and causes the first period of oestrus to occur earlier than under normal cyclic light–dark conditions (Luce-Clausen & Brown, 1939; Fiske, 1941; Jöchle, 1956). Adult females kept in constant light remain in constant oestrus characterized by prolonged or persistent cornification of the vagina, continual follicular development without ovulation, depressed thyrotrophic and luteinizing hormone concentrations, and continuous secretion of oestrogen from the preovulatory follicles. In addition, these females show almost constant sexual receptivity (Browman, 1937; Hemmingsen & Krarup, 1937; Dempsey & Searles, 1943; Hoffman, 1967; Singh & Greenwald, 1967; Lambert, 1975; Schwartz, 1982). The results of research on the effects of constant light on male rats are less clear-cut and are complicated by inconsistencies. For example, constant light has been reported both to accelerate (Takahashi, Choi & Suzuki, 1971) and to delay (Piacsek, Statham & Goodspeed, 1978) the development of the accessory sex glands in juvenile male rats. Information on whether constant light affects the sexual behaviour of male rats, as it does that of females, has not, to our knowledge, been reported previously.

Materials and Methods

Animals. All male rats used in this study were purchased at 90 days of age from Quebec Breeding Farms and housed individually with food and water freely available. There were 3 groups of 12...
sexually naïve male Long–Evans rats; one group housed in 12 h light:12 h dark (Group LD), one housed in constant light (Group LL), and one housed in constant dark (dim red light) (Group DD) for 60–90 days. To control for subjective day–night (inactive–active) cycles the home cages of rats in Groups LL and DD were equipped with running wheels and individual activity rhythms were recorded on an Esterline–Angus pen recorder.

**Sexual behaviour.** Two sexual behaviour tests were given to each rat, 1 week apart, with one test in the active or dark period and one in the inactive or light period in a counterbalanced order. Males were tested between 3 and 4 h after the onset of the desired activity phase which was determined by examining the pen recordings of individual running-wheel rhythms. One animal from each of Groups LD and DD died before testing began and one LD male was mated with an aggressive female, so his sexual behaviour data were discarded, leaving 10 males in Group LD, 12 in Group LL, and 11 in Group DD.

During the test each male was placed in a 62 × 32 × 32 cm glass observation chamber for 10 min before introducing a soliciting, oestrous female. A 40-W red light illuminated the test apparatus. Sexual behaviour was recorded by the method described by Brown & McFarland (1979) for 45 min or for 3 ejaculations, whichever came first. If the male did not mount within 20 min he was removed from the apparatus and the trial was scored as a failure.

**Hormone analysis.** At 1 week after the second behavioural test, blood samples were taken by cardiac puncture under halothane anaesthesia and a second sample was taken 12 h later. Half of the initial samples were procured during the rats’ active phase and half during their inactive phase. Plasma was analysed for androgen, prolactin, luteinizng hormone (LH) and follicle-stimulating hormone (FSH) concentrations by radioimmunoassay with a double-blind procedure.

Androgens were measured by radioimmunoassay as described by Moger & Armstrong (1974) using a testosterone antibody (B-8) which cross-reacts with 17β-hydroxy-5α-androstane-3-one (98%), 5α-androstane-3α,17β-diol (58%), and 5α-androstane-3β,17β-diol (24%). Results of this assay are therefore expressed as androgen concentration. Sensitivity of the assay (≥ 2 s.d. less than B₀ binding) was 10 pg/tube and the intra-assay coefficient of variation at 50% displacement was approximately 8%. The efficiency of testosterone extraction from serum exceeded 85%. LH, FSH and prolactin were assayed with reagents provided by Dr A. F. Parlow and the NIADDK. LH and FSH were assayed as described by Moger (1975) and concentrations are expressed in terms of the RP-1 preparations. Sensitivities of the LH and FSH assays were 4 and 8 ng/tube, respectively, and the intra-assay coefficient of variation for both assays was 5-8%. Prolactin was assayed with the same protocol as LH and FSH and the results expressed in terms of the RP-2 preparation. Sensitivity was 0.08 ng prolactin/tube and the intra-assay coefficient of variation was 6%. All samples from this experiment were analysed in a single assay. Because some animals died after the first blood sample was taken and not enough blood was available in some samples for all assays, the sample sizes for the hormone assays vary (see Table 4).

**Accessory sex organ weights.** After the second blood sample, the rats were killed and the testes, epididymides, ventral prostate gland and seminal vesicles of 9 males in each group were removed and weighed.

**Results**

**Sexual behaviour**

On both sexual behaviour tests more males in Group LD ejaculated than did those in Groups LL or DD. More Group LD males achieved intromission than did males in Groups LL or DD males in Test 1 but there were no differences amongst the groups in the number of males mounting during each test (see Table 1).
Table 1. Numbers of males in Groups LD, LL and DD which mounted, achieved intromission, and ejaculated during Tests 1 and 2 (tests lasted 45 min or for 3 ejaculations, whichever came first)

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Mounts</th>
<th>Intromissions</th>
<th>Ejaculations</th>
<th>Mounts</th>
<th>Intromissions</th>
<th>Ejaculations</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD</td>
<td>10</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>LL</td>
<td>12</td>
<td>6</td>
<td>3</td>
<td>3</td>
<td>8</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>DD</td>
<td>11</td>
<td>9</td>
<td>5</td>
<td>4</td>
<td>8</td>
<td>7</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 2. Mean (± s.e.m.) ejaculation frequency for males in Groups LD, LL and DD in the active or dark phase and inactive or light phase of their light:dark cycle averaged over Tests 1 and 2 (tests lasted 45 min or for 3 ejaculations, whichever came first)

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>Active phase</th>
<th>Inactive phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD</td>
<td>10</td>
<td>2·30 ± 0·42</td>
<td>1·10 ± 0·28</td>
</tr>
<tr>
<td>LL</td>
<td>12</td>
<td>0·92 ± 0·35</td>
<td>0·58 ± 0·28</td>
</tr>
<tr>
<td>DD</td>
<td>11</td>
<td>0·82 ± 0·36</td>
<td>0·36 ± 0·31</td>
</tr>
</tbody>
</table>

A 2 × 3 split-plot analysis of variance (Kirk, 1968) indicated a significant difference in ejaculation frequency between groups (F = 3·97; d.f. = 2,30; P < 0·05) and a significant effect of testing in the active or inactive phase (F = 10·84; d.f. = 1,30; P < 0·01) with a non-significant interaction (F = 1·88, d.f. = 2,30). Tests of simple main effects showed a significant difference in ejaculation frequency amongst groups when tested in the active phase (F = 11·45; d.f. = 1,60; P < 0·01) but no difference amongst groups when tested in the inactive phase (F = 2·38; d.f. = 1,60). Post-hoc analysis indicated that males in Group LD achieved more ejaculations than did males in Groups LL or DD (Scheffé: F = 7·77; d.f. = 2,30; P < 0·05; see Table 2).

Because many males which failed to ejaculate did not mount the female (see Table 1), sample sizes for other measures of copulatory behaviour are variable and statistical analyses were done using the Kruskal–Wallis analysis of variance (Siegel, 1956).

When all males that mounted or intromitted were considered, there were no differences in mount latencies or intromission latencies in Test 1 or Test 2, nor were there significant differences in mount frequency over the first 30 min of the tests (Table 3). There was, however, a significant difference in intromission frequency in Test 1, with males in Group LD intromitting more than males in Groups LL and DD. The inter-intromission intervals differed significantly in Test 2, with males in Group DD having longer intervals between intromissions than males in Groups LD or LL (Table 3).

Other measures of copulatory behaviour are calculated only for males which ejaculated and, as might be expected, there were no differences amongst groups on any of these measures (ejaculation latency, post-ejaculatory interval, mount frequency and intromission frequency) on the first ejaculatory series of the first or second test (Table 3).

Hormone analyses

Table 4 shows that males in Group DD had higher serum levels of androgen than did males in Groups LD and LL (F = 25·49; d.f. = 2,31; P < 0·05). Males in Group LL had higher serum levels of prolactin than did males in Groups LD and DD (F = 3·85; d.f. = 2,29; P < 0·05). There were no differences in LH (F = 0·15; d.f. = 2,20) or FSH (F = 3·34; d.f. = 2,25) concentrations in the 3 groups.
Table 3. Median scores (with the no. of rats in parentheses) for measures of sexual behaviour for those males in each group which achieved mounts, intromissions or ejaculations in Tests 1 and 2.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Test</th>
<th>Group LD</th>
<th>Group LL</th>
<th>Group DD</th>
<th>( \chi^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mount latency (sec)</td>
<td>1</td>
<td>46-0 (8)</td>
<td>87-5 (6)</td>
<td>125-0 (9)</td>
<td>0-50</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>8-0 (7)</td>
<td>21-0 (8)</td>
<td>10-0 (8)</td>
<td>2-32</td>
</tr>
<tr>
<td>Intromission latency (sec)</td>
<td>1</td>
<td>66-0 (8)</td>
<td>49-0 (3)</td>
<td>58-0 (5)</td>
<td>0-77</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>32-0 (7)</td>
<td>32-0 (6)</td>
<td>16-0 (7)</td>
<td>0-85</td>
</tr>
<tr>
<td>Mount frequency (30 min)</td>
<td>1</td>
<td>29-5 (10)</td>
<td>0-50 (12)</td>
<td>6-0 (11)</td>
<td>4-75</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>36-0 (10)</td>
<td>20-0 (12)</td>
<td>7-0 (11)</td>
<td>0-86</td>
</tr>
<tr>
<td>Intromission frequency (30 min)</td>
<td>1</td>
<td>19-5 (10)</td>
<td>0-0 (12)</td>
<td>0-0 (11)</td>
<td>6-61*</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>22-5 (10)</td>
<td>5-5 (12)</td>
<td>3-0 (11)</td>
<td>3-04</td>
</tr>
<tr>
<td>Intromission interval (sec)</td>
<td>1</td>
<td>33-8 (8)</td>
<td>43-6 (3)</td>
<td>66-0 (5)</td>
<td>3-61</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>34-1 (7)</td>
<td>46-6 (6)</td>
<td>133-6 (6)</td>
<td>6-34*</td>
</tr>
<tr>
<td>Ejaculation latency (sec)</td>
<td>1</td>
<td>390-5 (8)</td>
<td>332-0 (3)</td>
<td>775-0 (4)</td>
<td>1-39</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>310-0 (7)</td>
<td>540-0 (5)</td>
<td>748-0 (3)</td>
<td>2-02</td>
</tr>
<tr>
<td>Post-ejaculatory interval (sec)</td>
<td>1</td>
<td>329-5 (8)</td>
<td>387-0 (3)</td>
<td>362-5 (2)</td>
<td>0-27</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>285-0 (7)</td>
<td>380-0 (5)</td>
<td>475-0 (2)</td>
<td>3-47</td>
</tr>
<tr>
<td>Mount frequency (1st ejac.)</td>
<td>1</td>
<td>16-0 (8)</td>
<td>25-0 (3)</td>
<td>26-5 (4)</td>
<td>0-17</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>19-0 (7)</td>
<td>27-0 (5)</td>
<td>28-0 (3)</td>
<td>1-41</td>
</tr>
<tr>
<td>Intromission frequency (1st ejac.)</td>
<td>1</td>
<td>11-0 (8)</td>
<td>14-0 (3)</td>
<td>14-0 (4)</td>
<td>0-30</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>15-0 (7)</td>
<td>16-0 (5)</td>
<td>13-0 (3)</td>
<td>0-97</td>
</tr>
</tbody>
</table>

*\( P = <0.05 \).

Table 4. Mean (± s.e.m., no. of rats in parentheses) serum concentrations of androgen, prolactin, LH and FSH for Groups LD, LL and DD.

<table>
<thead>
<tr>
<th>Group</th>
<th>Androgen (ng/ml)</th>
<th>Prolactin (ng/ml)</th>
<th>LH (ng/ml)</th>
<th>FSH (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD</td>
<td>3.10 ± 0.36 (11)</td>
<td>33.09 ± 5.36 (11)</td>
<td>103.02 ± 10.8 (8)</td>
<td>312.11 ± 34.25 (8)</td>
</tr>
<tr>
<td>LL</td>
<td>2.88 ± 0.46 (12)</td>
<td>56.00 ± 4.99 (11)</td>
<td>93.70 ± 15.6 (8)</td>
<td>364.29 ± 17.19 (11)</td>
</tr>
<tr>
<td>DD</td>
<td>7.13 ± 0.57 (11)</td>
<td>39.28 ± 7.94 (10)</td>
<td>91.73 ± 20.5 (7)</td>
<td>272.22 ± 29.14 (9)</td>
</tr>
</tbody>
</table>

Sex organ weights

There were no significant differences amongst the 3 groups of males in the weights of any of these organs (one-way ANOVAs with d.f. = 2,24).

Activity rhythms

There was a lack of coherent circadian organization of the activity rhythms of 6 of the 12 males in Group LL while only one of the males in Group DD showed similar disruption (Fisher exact test, \( P = 0.045 \)). None of the 3 LL males that ejaculated had a disrupted rhythm.

Discussion

These data support the conclusion that constant illumination, whether white (Group LL) or red (Group DD), disrupts copulatory behaviour in sexually naive adult male rats. This disruption is
most pronounced when tests occur during the active (dark) phase. The results of a pilot study suggest that 6 months of housing in constant light are more detrimental to male sexual behaviour than 3 months of housing in constant light and that 5 weeks in 12 h light : 12 h dark reverses this effect.

Group differences in sexual behaviour may be due to experimental variables other than the lighting conditions, such as the availability of running wheels, the use of a red light in the test room, or the novelty of the test situation, but this is unlikely. Although the rats in Group LD did not have access to running wheels, it is unlikely that the effects of exercise may have significantly contributed to the observed differences between the groups. Two pilot studies and one subsequent replication, in which none of the animals had access to a running wheel, produced behavioural results consistent with those reported in the present study. In addition, the hormonal effects were different between Groups LL and DD even though both were able to exercise.

The hypothesis that Group LD rats perform better in sexual tests because this is the only group in which dim red lighting (used in the tests) signals the onset of the activity period is not compelling. There is a dark-associated advantage in male sexual behaviour in rats characterized by lower initial latencies to mount, shorter post-ejaculation refractory periods, greater frequency of mounts and intromissions with shorter latencies to ejaculation, a higher ratio of intromissions to mounts, and a higher number of ejaculations (Beach & Levinson, 1949; Larsson, 1956, 1958; Harlan, Shivers & Moss, 1979). Males in Group LD in our experiment showed these dark-phase advantages with significantly more mounts and shorter ejaculation latencies, shorter post-ejaculatory intervals, and shorter inter-intromission intervals in the dark phase than during the light phase (Wilcoxon matched-pairs signed-ranks test, \( P < 0.05 \); Siegel, 1956). Nevertheless, male rats copulate during both the active and inactive phases of their activity cycles when presented with a receptive, soliciting female. In addition, the active period-associated advantage is less pronounced immediately after the transition from light to dark but increases as the exposure to darkness continues (Dewsbury, 1968; Sodersten & Eneroth, 1980).

The novelty of the testing situation (e.g. new chamber, presence of a female) should have been equally disruptive to all groups. It seems unlikely that the dim red light would cause any additional difficulties for Group LL males since they had been housed in 12 h light : 12 h dark before the 60–90-day exposure to constant light. One would also expect that, if the deficit in Group LL was the result of the novelty of the testing situation, then the initiation of copulation, rather than ejaculation, should be the aspect of sexual behaviour most disrupted.

Males in Group LD differed from those in Groups LL and DD in 3 ways: they were more likely to intromit, and, when they did intromit, they had more intromissions over the test and had these intromissions more rapidly, thus resulting in more ejaculations. Males in Groups LL and DD that failed to ejaculate often pursued the female but only touched, sniffed and licked her genital area without attempting to initiate coitus. Although anogenital exploration can occur for many reasons, its persistence and topography lead us to suspect that the initial 'arousal' or motivational process appears to be intact and the deficit seems to lie with the mechanism which is responsible for transforming this prelude into a complete, normal attempt to initiate coitus (Beach, 1956; Brown, Freeman & McFarland, 1974; Toates, 1980).

Male rats and mice that have been rendered hyperprolactinaemic through surgical transplantation of ectopic pituitary grafts manifest a sexual deficit similar to that which we observed in rats held in constant illumination. While plasma testosterone levels are not altered in these animals, graft recipients mount, intromit and ejaculate less than unoperated controls (Svare et al., 1979). The mice were reported to engage in excessive grooming of the stimulus female. This behaviour may be analogous to the increased sniffing and licking of the stimulus female exhibited by the rats in constant light which failed to ejaculate in this study. Therefore, the increased prolactin levels of such males may result from constant light and might mediate the reduction in sexual behaviour in these males.

Elevated prolactin levels in human males are typically associated with decreased sexual desire.
and potency which rapidly increase when prolactin release is suppressed (Thorner & Besser, 1978). The value of surgically-produced hyperprolactinaemia in male rats as an animal analogue for human male sexual dysfunction is not without controversy, although such animals have been used to study the effect of prolactin on male rat copulatory behaviour (Svare et al., 1979; Bailey & Herbert, 1982; Weber, Ooms & Vreeburg, 1982; Kalra, Simpkins, Lutgge & Kalra, 1983).

Constant lighting and darkness may cause a deficit in copulation by disrupting hormonal rhythms and/or dissociating hormonal and behavioural rhythms in the absence of the general synchronizing influence provided by a light–dark cycle. Since constant light produces greater disorder in the activity rhythms than does constant dark, constant light might disrupt sexual behaviour by disorganizing general activity rhythms. This, however, would not explain the similar detrimental effect of constant dark.

Constant light and dark alter hormone secretion, but have different effects and may, therefore, affect sexual behaviour by different neuroendocrine mechanisms. Since constant darkness results in elevated melatonin levels in rodents (Wurtman, 1967) and melatonin will inhibit reproductive behaviour in female rats (Chu, Wurtman & Axelrod, 1964), the reduced sexual behaviour seen in the males in constant darkness may be the result of elevated melatonin levels.

Melatonin, however, has anti-gonadal properties (Martin, McKeel & Sattler, 1982), and the elevated level of androgen in the males in constant dark appears to contradict the melatonin hypothesis. Nevertheless, melatonin will inhibit sexual behaviour in female rats independently of its effects on the gonads (de Catanzaro & Stein, 1984), and the melatonin hypothesis should be tested in male rats.

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References


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