

## Spatial responses to light in mice with severe retinal degeneration

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### Abstract

It is known that mice homozygous for the retinal degeneration (*rd*) mutation are able to synchronize their circadian rhythms to light-dark cycles. In the present experiments mice were given a choice of a dark or an illuminated living and nesting area. C3H, CBA and C57 *rd/rd* mice spent more time in the dark than in the illuminated area. Also, they spent as much time in the dark area as did wildtype controls. This shows that, despite advanced retinal degeneration, light can be used to control behaviour in space as well as in time. This was true of mutant mice over a year old, when retinal degeneration is very severe, and also of a transgenic strain of mice whose rods are destroyed as they begin to develop in the first few weeks after birth. © 1997 Elsevier Science Ireland Ltd.

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Mice carrying the retinal degeneration mutation (*rd/rd*) lose nearly all their photoreceptors [2], lack electroretinograms [15], and are unable to see patterns [13]. Nevertheless, they remain capable of synchronizing their circadian rhythms to cycles of light and dark [6–8]. Evidently, in addition to the classical visual system for detection of pattern and movement, mammals possess another system that can be used for processing information about overall levels of illumination, such as differences between night and day [3,8,15]. The present experiments explored the extent to which such an irradiance detection system is dedicated to synchronizing circadian rhythms or can be used to control other responses to light that do not depend on image formation.

Mice were placed in a black Plexiglas box divided into two compartments by a partition. A small opening permitted movement between the two sides. Light was prevented from reaching one side by an opaque cover. The box was 75 × 24.5 × 38 cm, divided into two compartments 37.5 cm long. There was an opening (8 × 5 cm) in the partition at floor level. Food and water were placed on cage lids 15 cm above the floor on each side of the apparatus. Transparent and opaque Plexiglas panels slotted

in above the lids. The two covers were interchangeable, enabling either the left or the right side to be illuminated. Illumination was provided by a 57 cm long fluorescent bulb (Sylvania cool white 20 W, F20T12 CW) attached to a light-tight lid over the apparatus. Illuminance, measured with an ISO-TECH ILM350 metre, averaged close to 300 lx on the floor of the light side, and was not detectable on the dark side. Tests were run at room temperature (approximately 22°C).

In order to rule out the influence of information other than illumination, the following precautions were taken. The boxes were thoroughly washed and fresh food, water, and sawdust bedding were added before each test. Males and females were not tested on the same day, even though each box had its own air exhaust fan. At the start of tests, mice were placed on the left side of the box which was dark on approximately half the trials. The animals had not been in this apparatus before, except for four of the C57+/+ and four of the C57 *rd/rd* mice which had been tested once before but with a different lighting schedule. The mice were taken from the colony room and placed in this box for a 22-h period. The percentage of time the animals were in the dark or light side was recorded by infra red photo-beams.

All but one out of 21 individuals homozygous for *rd/rd* spent more time in the dark side of the box (Fig. 1). Most

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of the wildtype mice also showed a preference for the dark. There were no significant differences in time spent in the dark between homozygous *rd/rd* mice and wildtype mice

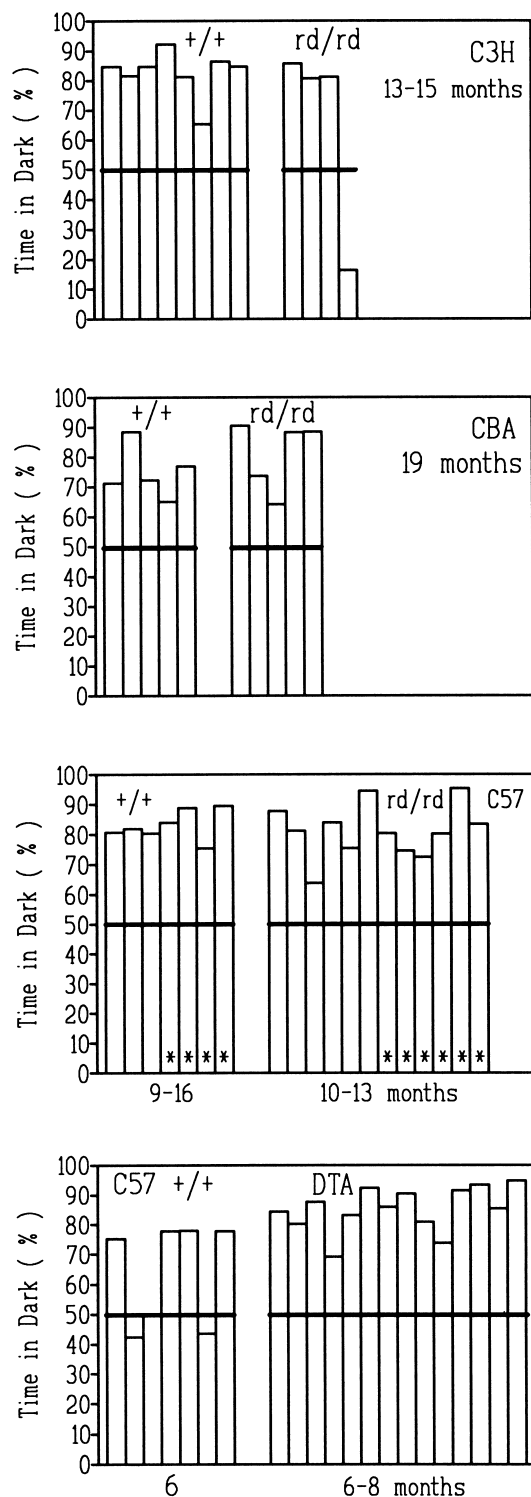


Fig. 1. Percentage of time (22 h) spent in the dark side of a two compartment box. Each bar represents data for one animal. All mice were males except those marked with an asterisk (\*). rd, Gene for retinal degeneration; DTA, transgenic mice with rods destroyed by an attenuated diphtheria toxin [11].

of the same background, and housed and tested in the same conditions ( $P > 0.05$ , two-tailed *t*-tests). To increase statistical power, we also compared the responses of all the mice with retinal degeneration in Fig. 1 ( $n = 35$ ) with all the wildtypes ( $n = 27$ ), regardless of background. There was still no statistically significant difference ( $P > 0.05$ , two-tailed *t*-test). Tests with enucleated C57 *rd/rd* mice (mean time in the dark,  $45.1\% \pm 5.4$  SEM;  $n = 15$ ) showed that preferences in this apparatus are based on information coming through the eyes, as has also been demonstrated in *rd/rd* mice for the resetting of circadian rhythms by light [8].

The ability of *rd/rd* mice to choose the darker side of the box is remarkable because of the ages of the mice tested. The idea that *rd/rd* mice retain some ability to react to light has been raised before [4,5], but most reports of residual responses to light have concerned mice of only a few months old when loss of rods may not be complete. Other than the persistence of phase shifting of circadian rhythms in a manner indistinguishable from that in wildtypes [15], responses to light have not been demonstrated in *rd/rd* mice over a year old. In the present tests, we found unimpaired behaviour on the mouse-house test in *rd/rd* mice over 1 year old; some were as old as 19 months (Fig. 1).

We have also tested transgenic mice whose rods begin to die a few days after birth, and are all gone within a few weeks after birth, because an attenuated diphtheria toxin has been fused to a rodopsin promoter gene [11]. Our tests were made at an age of 6 months or more. All 14 such transgenics tested preferred the dark side of the box (Fig. 1). In fact, they did so significantly more ( $P < 0.01$ ) than wildtype controls raised in the same conditions. Possibly some wildtype mice with normal vision are stimulated to explore areas where they can see pattern and form.

The results from these simple tests are noteworthy for a number of reasons. First, irradiance detection can be used not only to adjust the timing of behaviour to cycles of light and dark, but also to adjust location of behaviour to areas of light and dark. This suggests that whatever is spared when rods degenerate is not specialized for synchronizing circadian rhythms but mediates other responses based on irradiance detection. In this context it is interesting to recall some other reports of responses to light in *rd/rd* mice. Pupillary reflexes exist [14], although abnormal latencies have been reported [10]. Suppression of plasma melatonin levels after a light pulse can occur [9]. And inhibition of wheel running during a light pulse (i.e. negative masking) can occur [12]. Age of the animals is not given in all of these reports, and further work is needed to assess possible differences in these responses in *rd/rd* mice. Nevertheless, it is noteworthy that pupillary contraction, melatonin suppression and inhibition of activity require information only about overall light levels, not about pattern and distribution of light.

Second, the simplicity of the present mouse-house

choice test could make it a valuable tool for investigating irradiance detection. The procedure used here takes only 22 h and could probably be abbreviated: this contrasts with the 2 weeks or so, and the more complex analysis, that are needed to measure a phase shift of a free-running circadian rhythm. However, it is conceivable that the circadian response and the spatial response depend on different types of receptors, both of which are spared in *rd/rd* mutants.

Third, the ability to use light to influence behaviour is present even in animals old enough for retinal degeneration to become very severe. The outer nuclear layer of the mouse retina is composed almost entirely of rods. When these degenerate as a result of the *rd/rd* mutation, a very few cones survive, but as the cones make up only about 3% of the mouse's photoreceptors to start with, the reduction in total photoreceptor number is drastic. Based on information provided by Carter-Dawson et al. [1,2], it has been calculated that only about 0.09% of the total photoreceptor complement survives to a year [7,15]. Moreover, the surviving cones lack outer segments [2]. Yet there is no evidence even of a quantitative impairment in the present tests. This fits with the emerging realization that sensory channels and neural mechanisms for irradiance detection have to differ from those used in image formation [3,8,15]. Whatever these channels and mechanisms are, evidently they are spared in *rd/rd* mice.

Persistence of responses to light, as found here, despite severe retinal degeneration, should encourage studies of residual capacities in people with certain types of retinitis pigmentosa or other forms of retinal degeneration. Much attention has been given to studying the sensory and input aspects of these disorders, but perhaps more should be devoted to looking for residual capacities on the output side, especially those that require information only about overall light levels/irradiance.

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