Development of Adult-like Open-field Behaviors In Young Retinal-Degenerate C3H Mice

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Retinal degenerate C3H mice were tested in the open field under 2 illumination conditions before, during, and after the ages at which the early degenerate process occurs to determine whether the progressive destruction of the retina would result in the loss of brightness discrimination ability. From data collected on mice tested at 10, 14, 18, 22, 26, and 30 days of age, longitudinal and cross-sectional analyses were performed upon activity and and defecation scores to separate the effects of prior test experience and age at testing. Whereas illumination differences had no effect upon defecation scores, activity differences under the 2 illumination levels formed an inverted U-shaped function, with the greatest activity difference at 22 days of age. Prior experience was found to have no effect upon total activity scores at a particular age, although it did affect the pattern of activity over test days at the later ages. Naive animals tended to defecate less on their first day than did animals of the same age with prior test experience. The results of this experiment provide further evidence that retinal degeneration does not lead to complete blindness in this mouse strain, although it does lead to a loss of brightness discrimination ability following the degeneration period.

Mice afflicted with retinal degeneration (*rd*), an inherited abnormality, undergo spontaneous degeneration of the retina during the second postnatal week and appear to lose their visual capacities thereafter. Anatomical evidence reported by Tansley

Received for publication 10 August 1971
Developmental Psychobiology, 5(3): 249-258 (1972)

(1954) and Sidman and Green (1965), in 2 different homozygous *rd* mouse strains, indicated normal development of the retina from birth until 14 days of age. Rapid degeneration then begins in the central retina and outer nuclear layers, and, by 21 days of age, leads to the destruction of virtually the entire photoreceptor layer. The bipolar and ganglion cells may yet be intact at this age (Sidman & Green, 1965), with further degeneration occurring between 3 and 8 months of age (Karli, 1952).

Behavioral studies comparing normal and *rd* mice have reported superior performance by mice with normal retinas in a variety of tasks, including maze learning (Lindzey & Winston, 1962; McClearn, 1965), black-white discrimination (Wimer & Weller, 1965), and depth discrimination on the visual cliff (Fox, 1965; Frank & Kenton, 1966). Most of these studies tested mice at ages well beyond the early retinal degeneration period, and the *rd* strains' poor performance was generally attributed to their apparent blindness.

More recently, Nagy and Misanin (1970) have provided evidence for some visual capacities in the *rd* C3H strain at ages beyond the early degeneration period. Mice of this strain responded to brightness differences in the open field between 20 and 100 days of age although they were no longer capable of pattern discrimination on a visual cliff after 40 days of age. The same brightness differences had no effect on the open field activity of blinded control mice. Furthermore, blinded mice demonstrated increased activity over test days similar to increases in activity in non-*rd* rats following enucleation (Glickman, 1958; Lester, 1967; Zucker & Bindra, 1961), whereas the non-blinded mice showed decreases in activity over test days.

Since the retinas of *rd* mice develop normally until the degeneration process begins, brightness differences may be discriminated better at ages prior to the extensive retinal damage. The present study was conducted to determine whether the degenerative process would be reflected behaviorally in the open field. The behavior patterns of *rd* C3H mice from 10-33 days of age were examined under 2 illumination conditions with the anticipation that activity differences due to illumination would increase shortly after eye opening, reflecting the onset of visual functioning, but would then become progressively smaller as retinal degeneration became more advanced. Longitudinal and cross-sectional groups were included to assess the effects of both age and prior experience.

Method

Subjects

The subjects were 240 naive C3H mice (*Mus musculus*) comprising 6 age groups (beginning at 10, 14, 18, 22, 26, and 30 days of age) with 20 males and 20 females in each group. The mice were born and reared in 48.3 X 27.9 X 12.7 cm polyethylene cages with wire mesh tops and wood shavings on the floor, and permitted *ad libitum*

access to food and water. The mice in all age groups remained with their littermates and mothers at all times except for the test sessions, and were reared in the Psychology Mouse Colony at 23 ± 1°C, with 50 ± 2% humidity, and on a 12 hr light-12 hr dark cycle.

Apparatus

The apparatus consisted of a 91.4 X 91.4 X 30.5-cm square field constructed of unpainted Masonite. The floor was divided by black lines into 36 15.2-cm squares. Illumination was provided by a 150 W incandescent bulb placed 76.2 cm above the center of the field, with a 76.2-cm square silver foil covered reflector directly above the light source to provide even illumination throughout the field. A Type 116B Powerstat variable transformer provided either a high illumination level of 1076.4 lux (140 V) or a low illumination level of 10.76 lux (40 V). The air temperature was 22 ± .5°C in all parts of the field under both illumination conditions.

Procedure

At 5-6 days of age, the mice were toe-clipped for identification and replaced with their littermates. At the appropriate age, each mouse was removed from the home cage and placed in the center of the field in an arbitrary direction. The number of squares entered and the number of boluses deposited during a 2-min trial were recorded. Beginning at 10, 14, 18, 22, 26, or 30 days of age, each mouse received one trial on each consecutive day through 33 days of age. Thus, the age groups were tested for 24, 20, 16, 12, 8, and 4 consecutive days, respectively.

One-half of the 20 mice of each sex at each age was tested under the high illumination condition on all days, whereas the remaining half was tested under the low illumination condition on all days. The selection of mice for a particular group was determined by a modified split-litter design in which each litter was represented in at least 3 different age groups, and only 1 mouse of each sex from any litter was used for either illumination condition at a particular age.

Separate analyses of variance, each with one repeated measure, were conducted on the daily activity scores for each of the 6 groups beginning testing at different ages.

Results and Discussion

Activity

Figure 1 shows the 2-day mean activity levels for all age groups over test days as a function of the two illumination conditions. The mice were more active under low

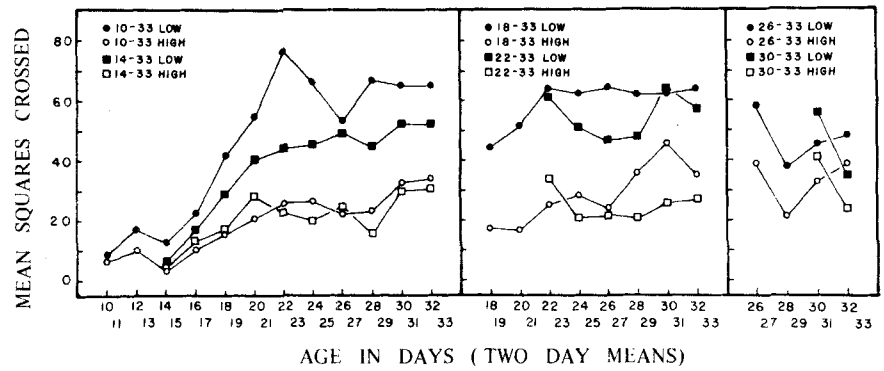

Fig. 1. Two-day mean activity levels over test days as a function of age and illumination level.

than high illumination at all ages tested although these differences appear to vary as a function of age and prior testing. Overall activity was greater under low than high illumination for all age groups (all F's > 4.15; $df = 1, 36$; all p's $< .05$), except the oldest, 30-33 days of age ($F = 3.11$; $df = 1, 36$; $p < .1$). Over days of testing, groups which began at 10, 14, and 18 days of age showed increases in activity (all p's $< .001$), whereas groups beginning at 22, 26, and 30 days of age showed decreases in activity (all p's $< .005$). Since the separate analyses revealed a reliable sex effect in only one age group, 18-33, and few reliable interactions of sex with other factors, sex was not considered a significant variable in activity levels at these ages and, therefore, not separately represented in this figure.

Illumination level had little effect upon activity on the early days of testing for the groups beginning at 10 and 14 days of age, but had a greater effect as the mice became older. This relation is reflected in significant Illumination X Test Day interactions for both the 10-33 ($F = 3.64$; $df = 23,828$; $p < .0005$) and 14-33 ($F = 1.69$; $df = 19, 684$; $p < .05$) groups. Individual comparisons within these interactions showed that activity differences due to illumination level became reliable by 16 days of age for the 10-33 group ($p < .01$) and by 20 days of age for the 14-33 group ($p < .025$), and remained reliable on all succeeding days. At all other ages, the Illumination X Test Day interactions were not significant, indicating that differences in activity due to illumination level were consistent over test days in agreement with Nagy and Misanin (1970).

The present study indicates that the pattern of decreasing activity over test days (Nagy & Misanin, 1970) is a function of both age and prior experience. For example, over the first 4 days of testing, mice beginning at 10 and 14 days of age showed increases in activity (both p's $< .05$), those beginning at 18 days of age showed a nonsignificant increase, whereas groups beginning at 22, 26, and 30 days of age showed decreases in activity (all p's $< .05$). In these comparisons, test experience of 4 days was held constant while age varied. Examination of the groups with varying amounts of prior testing at a particular age, however reveals that this decrease in

activity is not simply an age effect, but depends upon whether the mice were experimentally naive or had prior test experience. Separate 3-way analyses of variance were conducted upon activity scores at 14-17, 18-21, 22-25, 26-29, and 30-33 days of age for all groups tested at those ages, with Prior Experience, Sex, Illumination Level, and Test Days as the main factors of each analysis. The results indicated that activity was greater under low illumination in all cases (all p's $< .005$) and that neither the amount of prior experience nor the Illumination Level X Prior Experience factor was reliable at any of the ages tested. These findings do not agree with Dixon and DeFries (1968), who reported that prior experience in Balb/cJ and C57BL/6J strains resulted in less activity than naive controls. The discrepant results may be due to strain differences, to the differing patterns of prior test experience in the longitudinal groups, to the presence of the *rd* abnormality in the C3H mice, or to interactions among these differences. Clearly, general statements regarding the effects of prior experience in mice may not yet be possible.

Reliable Prior Experience X Test Day interactions indicated that although prior experience did not affect total activity scores over the 4-day test period, it did have an effect upon the pattern of activity over those days. Groups beginning testing at 22, 26, and 30 days of age demonstrated decreases in activity over days (all p's $< .0005$), whereas all groups tested at those ages with prior experience in the open field demonstrated either nonsignificant changes or, in several instances, increases in activity. Thus, the pattern of decreasing activity over 4 test days appears to develop after 18 days of age, and typifies the activity patterns of naive C3H mice through 100 days of age (Nagy & Misanin, 1970).

Figure 2 illustrates the mean daily activity levels of groups 10-33 and 14-33 from their first test day through 20 days of age under both illumination levels. Eye-opening generally occurred at 12-13 days of age in this strain, and this appears to be reflected in the increase in activity differences due to illumination from Day 11 ($F < 1.0$; $df = 5, 180$) to Day 12 ($F = 3.22$; $df = 5, 180$; $p < .01$). A second point of interest is that both illumination groups beginning testing at 10 days showed a sharp decrease in activity between 13-15 days of age (both p's $< .025$), and then a reliable increase over the remaining days. Both groups that began testing at 14 days showed similar but smaller decreases at 15 days of age and then reliable increases in activity levels. Thiessen (1965) has reported a similar diminution of activity a few days after eye-opening in several other mouse strains with histologically normal retinas, and has suggested that this decrease in activity may correspond with the onset of visual functioning and other sensory systems in the young mouse. Whatever the underlying cause, the *rd* C3H mouse at these ages exhibits activity patterns similar to several non-*rd* strains.

Figure 3 presents the 2-day mean activity difference scores between low and high illumination conditions for each group. All groups beginning testing before 22 days of age showed increasing activity differences between illumination conditions until 22 days of age, and then generally remained at about the same levels through the last days

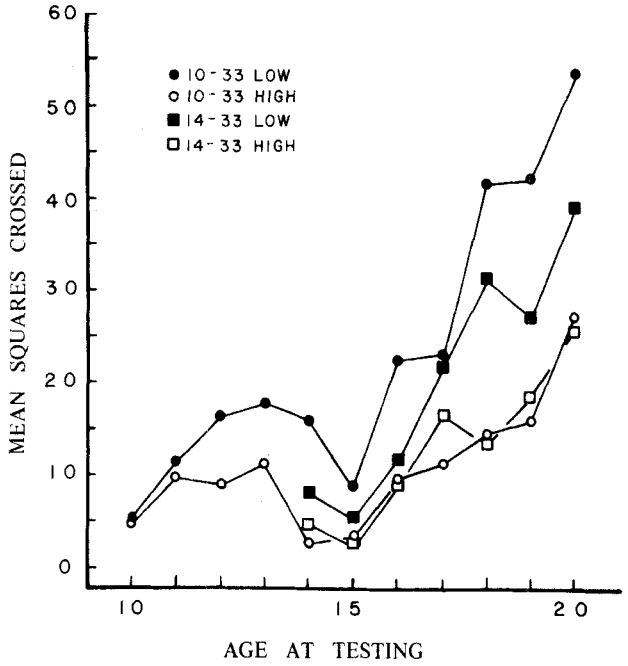

Fig. 2. Mean daily activity scores of groups 10-33 and 14-33 through 20 days of age under two illumination levels.

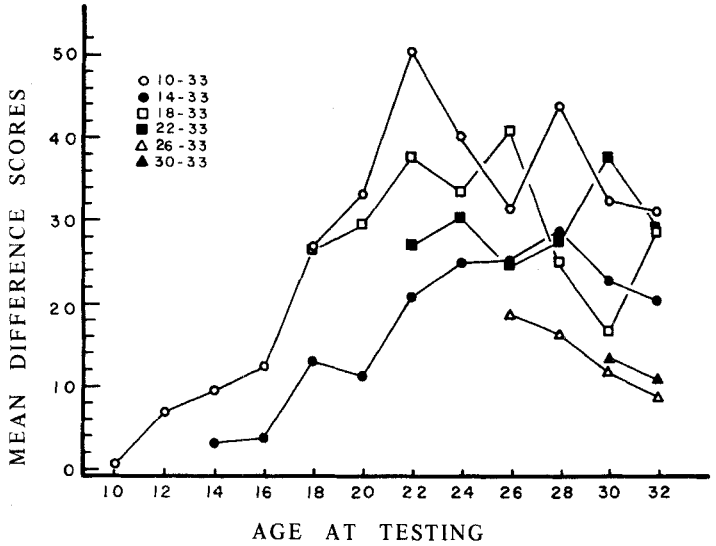

Fig. 3. Two-day mean activity difference scores (activity under low illumination minus activity under high illumination) as a function of days of testing.

of testing. These results were not anticipated, as the activity differences due to illumination should become less as the mice become older, reflecting the ongoing degeneration of the retina and subsequent loss of some brightness discrimination capacity. Furthermore, activity differences due to illumination level should decrease with repeated testing. On the assumption that the lower activity levels obtained under the high illumination condition were due to increased fear or "emotionality", repeated exposure to the fear situation was expected to lead to a decrease in "emotionality", possibly reflected by an increase in activity under the high illumination condition. Groups beginning testing at 26 and 30 days of age, however, did show smaller differences in activity under the 2 illumination conditions. Although not clear in these results, the extra handling at earlier ages in the younger groups may have been an important factor in these patterns of activity.

A 4-way analysis of variance of the activity scores of the first 4 test days for all groups, with Age, Illumination, Sex, and Test Days as the main factors, revealed that Age, Illumination, and Test Days were reliable (all p's $< .0005$). Of particular interest in this analysis was the effect of illumination level upon activity as a function of age, or the Age X Illumination interaction. This interaction, seen in Fig. 3, was reliable ($F = 3.40$; $df = 5,216$; $p < .01$). Individual comparisons within the Age X Illumination interaction supported the hypothesis that the activity differences due to illumination would form an inverted U-shaped function as the animals became older. Illumination level had little effect at 10-13 and 14-17 days of age (F's < 1.0), a large effect at 18-21 and 22-25 days of age (F's = 21.35 and 22.49; p's $< .0005$), and smaller, but still reliable effect at 26-29 and 30-33 (F's = 8.17 and 4.06; p's $< .005$) days of age (for all comparisons, $df = 5,216$).

Defecation

The number of boluses deposited each day by each mouse was transformed to the form $\sqrt{X + 0.5}$ to conform better to assumptions underlying the analysis of variance. Separate analyses were conducted for each age group over all test days, with Illumination Level, Sex, and Test Days as the main factors. The results showed that neither illumination level nor sex produced reliable differences in defecation in any of the age groups. However, all age groups demonstrated increases in defecation over days of testing (all p's $< .001$). Figure 4 illustrates the mean transformed defecation scores for all groups on the first day of testing of each age group and on the final test day for all groups. The overall pattern of increasing defecation is obvious for all age groups. It should be noted that with one exception (10-33 group), every age group defecated less on its first test day than the age group immediately preceding it defecated on that day. For example, group 18-33 defecated less on Day 18 than did group 14-33, and group 22-33 defecated less than group 18-33 on Day 22. This pattern of low defecation on the first test day and increasing defecation with repeated testing, which has been

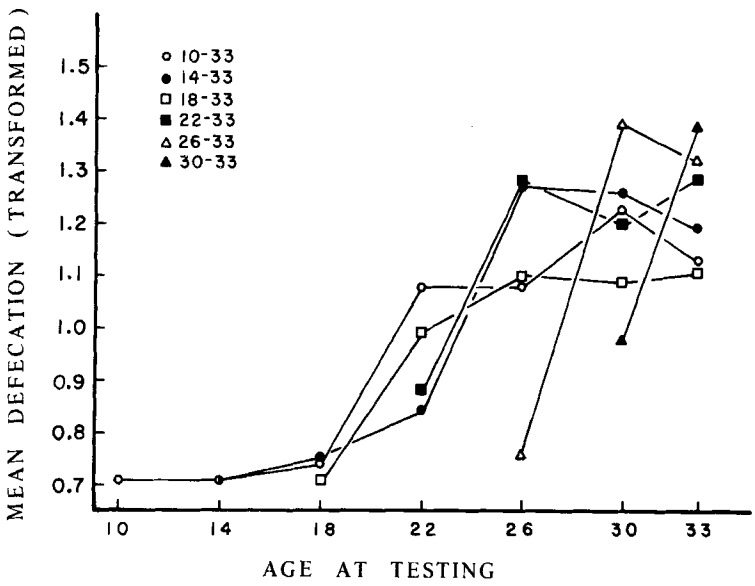

Fig. 4. Mean defecation scores (transformed) on the first test day of each age group and the final test day for all groups.

reported in adult C3H mice (Candland & Nagy, 1969; Nagy & Forrest, 1970), develops by 18 days of age, but does not become statistically reliable until 26 days of age ($p < .05$). The fact that the 10-33 and 14-33 groups did not show this pattern initially is indicative of the lack of maturation of the mechanisms responsible for defecation at these ages (Tobach & Schneirla, 1962). Mice in the 10-33 group did not begin defecating in the field until 17 days of age, whereas those in the 14-33 group began defecating at 18 days of age. These data again raise the question of whether high defecation represents high "emotionality" in the mouse, as has been reported for the rat (Broadhurst, 1957), or a process other than "emotionality", such as a territory marking response (Bruell, 1963), or both (Nagy & Forrest, 1970).

In conclusion, the present study provides further evidence that although retinal degeneration during the second postnatal week renders these mice relatively insensitive to certain kinds of visual cues, such as utilized in the visual cliff discrimination, it does not produce total blindness. The finding that activity differences under the 2 illumination levels over the first 4 days of testing became smaller between 18-30 days of age appears to reflect some loss of brightness discrimination ability during the early degeneration period. However, the fact that mice at all ages in this study demonstrated activity differences due to illumination is in contrast to results expected if degeneration of the retina were complete and the mice subsequently blind. Experimentally blinded mice of this strain are not able to discriminate between the illumination levels used in this study (Nagy & Misanin, 1970). Whether the later degeneration period occurring between 3 and 8 months of age (Karli, 1952) results in complete loss of brightness discrimination in this strain is not yet known. Research

comparing the performance of normal and *rd* strains to determine the presence of visual and nonvisual cues used in discrimination learning (e.g., Wimer & Weller, 1965) must be tempered by the fact of some remaining visual capacities in this strain and its possibility in other *rd* strains.

Note

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