Development of retinal synaptic arrays in the inner plexiform layer of dark-reared mice

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SUMMARY

In the central area of the retina of mouse the rate of synaptogenesis in the inner plexiform layer (IPL) drops precipitously at about the time the eyes open. To determine if the visual input at eye opening provides a signal for the neurons to stop adding synapses, mice were raised in darkness during the period of maximal synaptogenesis and through eye opening. Retinal synaptic arrays of dark-reared and normally reared animals were compared quantitatively. The rate of synaptogenesis after eye opening in dark-reared mice indicated that the onset of visual stimulation was not the cue to stop synaptogenesis. However, the synaptic arrays of the IPL of dark-reared mice consistently had more conventional synapses than those of normally reared mice. It is concluded that the number of conventional synapses in the central retina was increased by dark-rearing.

INTRODUCTION

The synaptic array of the inner plexiform layer (IPL) of the central retina exhibits three phases of development in mouse (Fisher, 1979). Phase I, from postnatal day 3 to day 10, is characterized by the formation of conventional synapses — those made by amacrine or interplexiform cells (Dowling & Boycott, 1966; Witkovsky & Dowling, 1969; Dowling, Ehinger & Hedden, 1976; Kolb & West, 1977), but not of ribbon synapses — those made by bipolar cells (Dowling & Boycott, 1966; Raviola & Raviola, 1967). Phase II, day 11 to day 15, is characterized by the simultaneous formation of conventional and ribbon synapses. During these first two phases the eyes are closed. Phase III, day 15 to adulthood, begins at eye opening and is characterized by a sharp reduction in the rate of formation of both conventional and ribbon synapses. During Phase III a constant value for each synaptic type is attained.

The striking coincidence of eye opening and the reduction of synapse formation suggests that some event related to eye opening might signal the retinal neurons that the synaptic array of the IPL is complete. An obvious possibility is that the increased sensory input to the IPL after eye opening supplies this hypothetical signal. If this were the case, depriving the neonatal pups of any photic stimulus during the transition from Phase II to Phase III should eliminate the

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signal to cease synaptogenesis and result in an increased number of synapses later in Phase III. This paper reports a test of the hypothesis that a photic stimulus is required at the time of eye opening to attain the normal synaptic complement of the retinal IPL during Phase III.

MATERIALS AND METHODS

Mice of the C57BL/6 strain were born (day 0) and raised in a light:dark cycle (LD 14:10) until day 9, 10 or 11, when they were transferred, along with their mothers, into continuous darkness. Since no input to the IPL via ribbon synapses is possible in the mouse retina until day 11 (Fisher, 1979) and since testing of the hypothesis required no light at the transition from Phase II to Phase III, the period of darkness was chosen to begin with Phase II. The pups were therefore deprived of photic stimuli while the input to the IPL formed. Cleaning the cages and supplying food and water were performed under infrared illumination by using an infrared imaging scope (Find-R-Scope, FJW Industries). Animals were killed on days 13, 16, 21 or 25 by breaking the neck. The eyes were sutured at the dorsal-most aspect for orientation, and prepared for light and electron microscopy using aldehyde fixation (Fisher, 1979). Sections of the retina were taken in the horizontal plane, and areas farther than 250 \( \mu \text{m} \) but within 750 \( \mu \text{m} \) medial or lateral to the optic disc were sampled. One retina from each of at least three animals was sampled for each data point. Thirty-four animals were included in the study.

Conventional (Fig. 1A) and ribbon (Fig. 1B) synapses were identified and their profiles measured on mosaics of electron micrographs according to established criteria (Dubin, 1970; Fisher, 1979). Magnifications were computed from a micrograph of a calibration grid (Fullam replica grating 1002), which was prepared along with each mosaic. Nuclei of the inner nuclear layer (INL) were counted on 1 \( \mu \text{m} \) plastic sections using a light microscope and drawing tube (magnification 450 \( \times \)). All synaptic and nuclear densities were calculated from samples taken through the entire thickness of the layer being examined. Nuclear counts were corrected for sampling errors by use of Abercrombie’s method (Abercrombie, 1946). Synaptic counts were corrected by the modified Abercrombie correction of Dubin (1970). The results were described using three measures: (1) numerical density, the number of synapses in one unit volume (1000 \( \mu \text{m}^3 \)) of IPL, (2) planimetric density, the number of synapses found in a column of IPL having a unit cross-section (1000 \( \mu \text{m}^2 \)) and extending from the ganglion cell layer to the INL, and (3) synapses per nucleus, the average number of ribbon synapses made by a bipolar cell or of conventional synapses made by an amacrine or interplexiform cell (for further details about each of these units see Fisher & Easter (1979) or Fisher (1979)). All synapses were counted under a blind procedure in which the age and treatment of the animals were unknown until the data were reduced.
Fig. 1. (A) A conventional synapse (arrow) from the mouse's inner plexiform layer (IPL). These synapses are formed by amacrine or interplexiform cells presynaptically on to ganglion, bipolar, amacrine or interplexiform cells postsynaptically. (B) A ribbon synapse (arrow) from the mouse's IPL. Ribbons are found exclusively in bipolar processes and are associated with synapses onto ganglion or amacrine cells postsynaptically.
Fig. 2. Comparison at days 13, 16, 21 and 25 of the numerical density of conventional synapses (A), planimetric density of conventional synapses (B), and conventional synapses per nucleus (amacrine plus interplexiform cell) (C), for mice raised in cyclic light (open symbols) or in darkness (filled symbols). Although the magnitude of the differences on each day did not achieve significance, the consistently higher values attained by the dark-reared mice over the four ages sampled differed significantly from the values for normally reared mice for all three measures ($P \approx 0.02$, $P \approx 0.05$, $P \approx 0.01$ for A, B, and C respectively; analysis of covariance). Error bars = 1 s.e.m.

The data from dark-reared animals were compared with data from animals raised in a 14:10 light:dark cycle. An analysis of covariance tested the null hypothesis that the means and slopes of linear regressions through the data points were equal.
RESULTS

Dark-rearing had no effect on the shape of the growth curve for conventional synapses (Fig. 2). A reduction in synaptogenic rate occurred by day 16 for both groups of mice and each measure reached a constant value in Phase III. The hypothesis that visual stimuli at eye opening were required to stop synaptogenesis must therefore be rejected for conventional synapses.
Dark-rearing did result in a change in the absolute number of conventional synapses, however. The values for every measure, numerical density, planimetric density, and synapses/nucleus, were greater in the dark-reared mice than in the normally reared mice ($P \geq 0.02$, numerical density; $P \geq 0.05$, planimetric density; $P \geq 0.01$, synapses/nucleus; analysis of covariance). It must be stressed that the analysis of covariance takes the data for all days into account. Thus, even though differences on a single day are too slight to reach significance by a Student’s $t$ test, the consistently higher values over time for dark-reared animals are significant.

Ribbons attained the same densities during Phase III in both dark-reared and normally reared mice (Fig. 3). The array of ribbon synapses thus reached characteristic values for density and numbers without having had sensory input. Since the same values were attained under both conditions of rearing, it is concluded that light was not required as a signal to stop ribbon synaptogenesis when the eyes opened.

The differences found for all measures on day 16 hint that ribbon synaptogenesis is somewhat retarded in the dark-reared mice and that maximal synaptogenesis by the bipolar cells takes place later in Phase III. This interpretation cannot be confirmed by the data since the within-day differences between dark-reared and normal are not statistically significant ($P > 0.05$ for all cases, Student’s $t$) and an analysis of covariance based upon quadratic regressions fails to reject null ($P > 0.45$ for all measures). By these two statistics, therefore, the differences between sets of curves are no greater than could be accounted for by chance alone. It is concluded that dark-rearing during Phase II did not affect ribbon synaptogenesis.

During Phase III, thicknesses of the IPL and INL, nuclear planimetric densities and mean profile lengths of ribbons and conventional synapses were unaffected by dark-rearing (Table 1, $P > 0.05$ for all within-day comparisons, Student’s $t$). On day 13, however, nuclear planimetric densities for amacrine cells, mean length of ribbon profiles, and mean length of conventional synapse profiles were greater in the dark-reared animals ($P < 0.01$, $P < 0.02$, $P < 0.01$, respectively; Student’s $t$). Since both ribbons and conventional synapses increase in size between days 13 and 15 in normally reared animals (Fisher, 1979) the larger sizes in dark-reared animals on day 13 suggest accelerated synaptic maturation in the dark.

DISCUSSION

Several investigators have found effects on IPL synaptic arrays of the retina resulting from light or form deprivation (Sosula & Glow, 1970; Fifkova, 1972; Chernenko & West, 1976). Both Fifkova, reporting on lid-sutured rats in cyclic light, and Sosula and Glow, reporting on adult rats kept in darkness, counted increased numbers of conventional synapses in the IPL of deprived animals. Using different conditions of darkness and lid-suturing Chernenko and West
Table 1

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>13</th>
<th>16</th>
<th>21</th>
<th>25</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>N*</td>
<td>D†</td>
<td>N*</td>
<td>D†</td>
</tr>
<tr>
<td>INL thickness ± 1 S.E.M. (μm)</td>
<td>58 ± 4</td>
<td>65 ± 1</td>
<td>62 ± 3</td>
<td>67 ± 13</td>
</tr>
<tr>
<td>IPL thickness ± 1 S.E.M. (μm)</td>
<td>58 ± 3</td>
<td>57 ± 2</td>
<td>59 ± 2</td>
<td>61 ± 10</td>
</tr>
<tr>
<td>Ribbon length ± 1 S.E.M. (Å)</td>
<td>945 ± 92</td>
<td>1235 ± 64</td>
<td>1281 ± 77</td>
<td>1402 ± 100</td>
</tr>
<tr>
<td>Length of synapse ± 1 S.E.M. (Å)</td>
<td>2322 ± 95</td>
<td>2977 ± 49</td>
<td>2697 ± 48</td>
<td>2668 ± 39</td>
</tr>
<tr>
<td>Nuclear planimetric density of bipolar cells ± 1 S.E.M.</td>
<td>39 ± 2</td>
<td>52 ± 5</td>
<td>50 ± 4</td>
<td>50 ± 6</td>
</tr>
<tr>
<td>Nuclear planimetric density of amacrine and interplexiform cells ± 1 S.E.M.</td>
<td>36 ± 1</td>
<td>54 ± 4</td>
<td>49 ± 5</td>
<td>49 ± 9</td>
</tr>
</tbody>
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*N, Normally reared. † D, Dark reared.
reported a rearrangement but no increase in conventional synapses. Although the time course for retinal synaptogenesis is not known for the rat, it presumably is similar to that of the mouse. If such a similarity exists, the periods of deprivation in the rat experiments followed the period of maximal synaptogenesis. Thus, unlike the deprived animals of the present study, the retinas of the rats were probably exposed to light through the closed lids while the synapses formed.

Numerical densities of conventional synapses and ribbons in the IPL of *Xenopus* larvae raised in the dark were higher than in light-reared controls (Tucker & Hollyfield, 1977). Since the retina of *Xenopus* grows and adds synapses continually (Fisher, 1976), the larvae were deprived of light during a period of normally occurring synaptogenesis. By being raised in darkness during Phase II, the mouse pups in the present study were also deprived of light during retinal growth and synaptogenesis. The similarities between *Xenopus* and mouse suggest that a sensitivity to lighting exists during the period of synaptogenesis.

Light is not necessary for the decrease in synaptogenesis observed at eye opening. This is demonstrated by comparing the descriptive measures from normally reared and dark-reared animals. In normally reared animals the rate of production of conventional synapses during Phase II was 1·15 synapses/1000 µm³/h or 1·4 synapses/nucleus/h. Had this rate of production continued into Phase III in the dark-reared animals, such as would happen if a sensory signal required to stop synapse formation was missing, there would have been 509 conventional synapses/1000 µm³ or 588 synapses/neuron on day 25. These values are 3·7 and 2·8 standard deviations, respectively, above the values found. A similar calculation for ribbon synapses predicts 136 ribbons/1000 µm³ and 147 ribbons/bipolar cell by day 25, 2·2 and 3·9 standard deviations, respectively, greater than the values found. Therefore, darkness at the time of eye opening did not extend the period of rapid synaptogenesis.

The synaptic array of the retina is altered by dark-rearing, but not in the way predicted by the original hypothesis which linked photic stimuli at eye opening to the inhibition of synapse formation. Rather, the number of conventional synapses was elevated under conditions of dark-rearing. Increased numbers of synapses/nucleus demonstrate that each amacrine or interplexiform cell in the INL of dark-reared mice contributed more synapses to the neuropil, on the average, than did those cells of normally reared mice. Increased planimetric density shows that there were more synapses in the IPL of the central retina, while increased numerical density indicates that these synapses were packed closer together. These slight but consistent increases were noted only for conventional synapses.

The data suggest that during Phase II the rates of production and maturation of conventional synapses are increased by dark-rearing. Since in normally reared animals light reaches the eye only through the closed lids, before the eyes open, the elevated number of synapses in the dark must be the result of the absence of light and not of the absence of contrast in the retinal image.
The final number of conventional synapses in the IPL of any individual mouse would be determined by the combined effects of the rate of synaptogenesis and the duration of the period of synaptogenesis. In mice the rate, but not the duration of synaptogenesis, during Phase II is affected by exposure to light (or darkness). Thus, at least in part, the light-history of the pup during the time of maximal synaptogenesis determines the number of conventional synapses in the IPL of the adult mouse.

I thank Stephen S. Easter Jr for his helpful suggestions on a preliminary manuscript, Sharon Burke for her excellent technical assistance, Bonita Johnson for typing the manuscript, and Mary White for her critical reading of the manuscript. This study was funded by Research Grant EY-01281 from the National Eye Institute.

REFERENCES


(Received 27 April 1979, revised 5 July 1979)