Daily mixed visual experience that prevents amblyopia in cats does not always allow the development of good binocular depth perception

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Kittens reared with mixed daily visual input that consists of episodes of normal (binocular) exposure followed by abnormal (monocular) exposure can develop normal visual acuity in both eyes if the length of the former exposure exceeds a critical amount. However, later studies of the tuning of cells in primary visual cortex of animals reared in this manner revealed that their responses to interocular differences in phase were not reliable suggesting that their binocular depth perception may not be normal. We examined this possibility in 3 kittens reared with mixed daily visual exposure (2 hrs binocular vision followed by 5 hrs monocular exposure) that allowed development of normal visual acuity in both eyes. Measurements made of the threshold differences in depth that could be perceived under monocular and binocular viewing revealed a 10-fold superiority of binocular over monocular depth thresholds in one animal while the depth thresholds of the other two animals were poor and there was no binocular superiority. Thus, there was evidence that only one animal possessed stereopsis while the other two were likely stereoblind. While 2 hrs of daily binocular vision protected against the development of amblyopia, the poor outcome with respect to stereopsis points to the need for additional measures to promote binocular vision.

Keywords: stereopsis, plasticity, visual deprivation, visual acuity, amblyopia, visual cortex


Introduction

Monocular deprivation of patterned vision to one eye of cats and primates, when applied continuously for a period in early postnatal life, results in a severe loss of form vision in the deprived eye reminiscent of the visual losses experienced by human deprivation amblyopes (Daw, 2006; Kiorpes, 2006; Kiorpes & McKee, 1999; Kiorpes & Movshon, 2004; Mitchell, 2004; Movshon & Kiorpes, 1990). The visual deficits that emerge following this form of early deprivation are accompanied by profound changes to the functional responses of neurons in the primary visual cortex (Daw, 2006; Kiorpes & Movshon, 2004; Mitchell & Timney, 1984). The ability to examine the nature and timing of the anatomical and physiological alterations in the central visual pathways have lead to the widespread use of monocular and other forms of early visual experiential restrictions to model human amblyopia for examination of its neural substrate.

Recent studies conducted on both cats and monkeys that received mixed daily periods of normal (i.e. binocular) and abnormal (i.e. monocular) visual exposure reveal that deprivation amblyopia may be prevented if monocular deprivation is not continuous. Animals for which the amount of concordant binocular visual exposure each day exceeds a critical threshold develop normal visual acuity and contrast sensitivity functions in both eyes (Mitchell, Kind, Sengpiel, & Murphy, 2003, 2006; Sakai et al., 2006; Wensveen et al., 2006). The critical amount of daily binocular exposure required to prevent amblyopia has still not been firmly established, although preliminary investigations for total daily visual exposure of 3.5 hrs or more suggest that it may be determined by the proportion of daily visual exposure that is binocular (Mitchell & Sengpiel, 2009). Interestingly, in both cats and monkeys the critical value appears to be 30% (Mitchell & Sengpiel, 2009;
Wensveen et al., 2006). On the other hand, the anatomical dimensions and layout of ocular dominance domains in cat V1 appear to require a fixed amount of daily visual exposure (1 hr) to acquire a normal topography (Schwarzkopf, Vorobyov, Mitchell, & Sengpiel, 2007).

In cats, daily episodes of concordant binocular exposure equal to 30% of total daily visual exposure allow not only the development of normal visual acuity and contrast sensitivity functions in both eyes, but also the development of normal alignment accuracy (Mitchell et al., 2006). However, it is not yet known whether such critical daily binocular exposures lead to the development of normal binocular functions, particularly stereoscopic vision. Investigations of the responses of cells in V1 in cats with mixed daily visual experience to interocular phase disparity revealed that the proportion of cells that were phase-selective was reduced from normal, while the responses of the phase-selective cells were less reliable across consecutive trials in comparison to those from normal animals (Vorobyov, Schwarzkopf, Mitchell, & Sengpiel, 2007). Mixed early visual input appears to disrupt the interocular phase selectivity of V1 neurons in monkeys to an even greater extent than that observed in cats as a possible consequence of the much finer disparity tuning of V1 neurons in the former species (Sakai et al., 2006). On the basis of the less reliable responses of V1 cells to interocular phase disparity, it might be thought that not all cats reared with mixed visual experience would acquire normal binocular vision (particularly stereopsis) despite having developed normal visual acuity in both eyes.

The purpose of the present study was to test this prediction by examination of the depth perception of 3 cats reared with mixed early visual input, who had developed normal visual acuity in the two eyes. A previously described (Mitchell, Kaye, & Timney, 1979) test of binocular depth perception was used to measure the smallest differences in depth between two stimuli that could be discriminated under both binocular and monocular viewing conditions. Previously it has been shown (Kaye, Mitchell, & Cynader, 1981; Mitchell et al., 1979; Mitchell, Ptito, & Lepore, 1994) that typically reared animals show a more than ten-fold superiority of binocular over monocular performance, a result consistent with the presence of a uniquely binocular cue to depth such as stereopsis. By contrast, cats that had been deprived early in life of concordant binocular vision, such as strabismus, perform no better with both eyes open than the monocular performance of typically reared cats.

**Methods**

**Animals**

From 4 to 8 weeks of age, 3 kittens born and raised in a closed laboratory animal colony at Dalhousie University, received a regimen of mixed visual input that had been shown previously (Mitchell et al., 2003, 2006), and also demonstrated here, to allow development of normal visual acuity in both eyes. Specifically, the kittens received a total of 7 hrs visual experience each day that consisted first of a 2 hr period of binocular vision (both eyes open) followed by a 5 hr period of monocular vision with one eye occluded by a neoprene foam mask. For the reminder of the day, the animals were placed with their mother in a darkroom. A detailed description of the rearing procedure is provided elsewhere (Mitchell et al., 2006). The research adhered to the ARVO Statement for the Use of Animals on Ophthalmic and Vision Research and followed animal protocols approved by Dalhousie University in accordance with standards and regulations established by the Canadian Council on Animal Care.

**Measurement of visual acuity**

When the animals were 5 weeks old, they received training on a 2-choice visual discrimination between adjacent vertical and horizontal square-wave gratings each weekday on a jumping stand. The training and subsequent measurement of visual acuity occurred during the daily period of binocular vision by use of procedures described in detail elsewhere (Mitchell, Giffin, & Timney, 1977). Measurements of the binocular visual acuity were made on the days immediately prior to termination of the period of daily mixed visual exposure.

Following the last day of such exposure the kittens were returned to the darkroom where they remained until the next morning when the vision of the deprived eye alone was measured for the first time. The acuity of the deprived eye was measured immediately after the animal was removed from the darkroom with the non-deprived eye occluded by an opaque hard contact lens. The acuity of the non-deprived eye was measured the next day; for all 3 animals the acuity of this eye was identical to the binocular acuity measured on the last day of mixed visual experience.

**Measurement of depth perception**

These measurements were begun when the animals were about 7 months old and continued for 1–2 months by use of a slight modification of the jumping stand and the procedure described much earlier (Mitchell et al., 1979). The main features of the jumping stand are shown in schematic form in Figure 1. The animal was required to jump from a platform toward the closer of two adjacent stimuli that were visible through a black mask placed on the transparent clear glass (5 mm thick) surface of the jumping stand that was located 67 cm below the jumping platform. The stimuli were two transparent plates upon
which black opaque circles of 3 different sizes (5, 14 & 20 mm) were displayed upon a virtual grid of 20 mm dia squares with a distribution density of 30% in a quasi-random fashion (i.e. the circles could not appear on adjacent squares of the grid). The rectangular masks (19 × 14.5 cm) restricted the angular dimensions of the displays to 15.8 × 12.2 deg. Because the arrangement of the patterns on the two stimuli were independently determined, they looked quite different, a feature that reduced the ability of the animals to employ density and size cues for their judgments. Once the discrimination was learned with a large (23 cm) depth difference between the two stimuli, measurements were made of the smallest separation in depth (ΔD) of the stimuli that could be discriminated with the closest stimulus always located 2 cm below the surface of the glass plate onto which the cat jumped. As in the past (Kaye et al., 1981; Mitchell et al., 1994) the animal received a minimum of 5 trials at each depth interval (ΔD). If an error was made, the animal was required to make 5 consecutively correct choices or be correct on at least 7 of the maximum of 10 trials provided for each depth interval which were changed in 2 cm steps. The threshold on any day was defined as the smallest depth interval for which the animal achieved this criterion (i.e. at least 70% correct). Typically, animals went from 100% correct performance to chance over only 3 depth intervals.

Figure 1. A schematic view of the jumping stand employed to measure depth perception. Cats jumped from the open ended box, B, to the closer of two stimuli visible through the glass surface of the jumping stand. The distance, ΔD, between the two stimuli could be varied in ~2 cm steps. Threshold performance under monocular or binocular viewing conditions was expressed as ΔD/D where D was the distance to the more distant stimulus.

Figure 2. A photograph of the stimuli from the jumping platform located 69 cm above them. The stimuli were separated in depth by 1.75 cm (left closest) and illustrate the static depth information available to a cat when viewing monocularly.

Figure 3. Histograms that show the acuity of each eye of the 3 animals immediately after 4 weeks of mixed visual exposure from 4 to 8 weeks of age. In order, from left to right, the data show the binocular acuity (white) measured the day before the end of mixed visual exposure, the acuity of the deprived eye (DE—black) and the acuity of non-deprived eye (NDE—hatched). The vertical bracket on the right shows the range of values encountered for the acuity of the NDE measured on animals reared in a similar fashion in the past. Note the absence of amblyopia in terms of the grating acuity of the DE. The 24 hr clock at the top depicts the daily visual experience of each animal with a 2 hr episode of binocular vision (BE—white) preceding a 5 hr period of monocular exposure (ME—gray). For the remaining 17 hrs each day the animal was kept in complete darkness (black).
intervals. When performance stabilized, measurements were made each day for 10 days of first the binocular depth threshold followed immediately by a monocular threshold with one eye occluded (the occluded eye alternated on successive days) with an opaque hard contact lens. This procedure highlighted any difference between binocular and monocular performance as the two measurements were made daily. For typically reared animals, the monocular thresholds were a factor of 10 times larger than the binocular values, a difference that suggests that monocular depth cues were sparse. The paucity of monocular cues can be seen by the photograph of Figure 2 which shows the appearance of the stimuli when separated by 1.75 cm, the threshold value for binocular viewing for the best animal of this study at an observation distance of 69 cm.

Results

Visual acuity

The visual acuity of the deprived eye of the 3 kittens measured at 8 wks of age immediately following the 4 wk period of daily mixed visual experience is shown by the histograms of Figure 3. Shown for comparison for each animal is the binocular acuity measured the day before, and the acuity of the nondeprived eye measured the day after. The three acuity measures were identical for all 3 animals thereby confirming our previous finding (Mitchell et al., 2003, 2006) that 2 hrs of binocular exposure protected against 5 hrs of subsequent monocular deprivation each day. Measurements made over the next 2–3 months revealed a further parallel improvement of acuity in both eyes commensurate with the pattern observed in typically reared animals.

Monocular and binocular depth discrimination

The measurements of depth perception completed when the animals were 8–9 months old provided no evidence of a binocular superiority in two animals but a 10-fold improvement in the third (C087). The different results are made apparent in Figure 4 which displays for the 3 animals the results of the binocular (filled circle symbols) and monocular threshold (open triangle symbols) measurements made over 10 consecutive days. As in the past (Kaye et al., 1981; Mitchell et al., 1994), the data shown are the threshold depth intervals (ΔD) expressed as a percentage of the distance (D) to the more distant of the two stimulus plates.

This expression represents the magnitude of the size difference between corresponding black circles on the two stimulus plates. Whereas the binocular thresholds for C087 were consistently 2.5%, with either eye alone the threshold depth interval was between 22 and 24%. The large magnitude of the superiority of binocular over monocular performance of this animal that was evident over the 10 days of consecutive threshold measurements (Figure 4), suggests very strongly that this animal possesses stereoscopic vision.

However, by contrast, the binocular thresholds of the two other animals (C086, C088) were identical to their respective monocular thresholds and to the monocular thresholds for C087. Thus for C086 and C088 there was no evidence of the presence of a uniquely binocular cue to depth such as stereopsis to allow better performance when they could use both eyes together.

Figure 4. Binocular (filled circles) and monocular depth thresholds (open triangles; upright—left; inverted—right) for the three animals (C087, C088, C086) over 10 successive sessions. Only C087 showed superior performance with binocular viewing consistent with the presence of stereoscopic vision. The other two animals performed poorly and their depth thresholds were no better with two eyes than one; hence they were likely stereoblind.
Refraction

The refractive status of the animals was assessed by retinoscopy at the conclusion of all testing. All three animals appeared emmetropic in both eyes to within 0.25D.

Discussion

In terms of the effects on visual acuity, 2 hrs of daily concordant binocular exposure during the period of mixed early visual experience prevented the development of amblyopia and permitted acquisition of normal visual acuity in both eyes of all 3 animals. The same result was observed for all 4 animals reared in the same fashion in an earlier study (Mitchell et al., 2006) as well as all 8 animals reared subsequently with mixed visual experience for which 30% of the daily visual input was binocular. In contrast to the uniformity of the findings with respect to visual acuity, daily binocular exposure amounting to 30% of total visual exposure was apparently insufficient to allow development of good binocular depth perception in 2 of 3 animals. For only one animal was there a substantial binocular superiority of depth thresholds consistent with the acquisition of good local stereoscopic vision.

The substantial superiority of binocular over monocular depth thresholds does not by itself prove that this animal possesses stereoscopic vision. The ultimate proof of stereopsis requires that the animal demonstrate the ability to make depth judgments on the basis of retinal disparity information alone in the absence of any monocular cues.

However, the close similarity of the monocular performance of this animal to the poor binocular performance of the other two suggest that for only C087 was an additional cue available for depth judgments with binocular viewing.

The likelihood that the additional cue available to just C087 was stereopsis receives support from the magnitude of the retinal disparity corresponding to the threshold depth interval. This can be calculated (in radians) from the expression (Mitchell et al., 1979),

\[ \frac{2a \times \Delta D}{D(D - \Delta D)} \]

where 2a is the interpupillary distance and D and \( \Delta D \) are as defined earlier. The interpupillary distance for C087 as determined from photographs was 40 mm. For the purpose of this calculation the proportion of correct responses for each depth interval from the 10 consecutive binocular testing sessions were combined. The 70% threshold as determined by Probit analysis was 4.35 mins, a value similar to that calculated in the past (Blake & Hirsch, 1975; Mitchell et al., 1979) from comparable judgments of differences in real depth as opposed to virtual depth in typically reared animals.

Although the data suggest that C087 possesses at least local stereopsis, there is no evidence that the other two animals have acquired stereoscopic vision despite having normal visual acuity in both eyes. This result is consistent with the results of electrophysiological investigations of the reliability of the responses of such cells suggests that the stereoscopic acuity of these animals may be reduced. The results presented here indicate that the outcome in terms of stereopsis from mixed daily early visual experience is very variable with only one of three animals showing evidence of good stereopsis while the other two appeared stereoblind. It could be argued that the stereoblindness observed in two animals was secondary to the presence of a mild strabismus that arose as a consequence of the early mixed visual experience. However, all three animals appeared to have normal eye alignment on the basis of the relative disposition of their optical and pupillary axes as judged from photographs. Even if a strabismus of a magnitude smaller than the sensitivity of this criterion did exist, it still begs the question as to its origin and whether it preceded, followed, or developed in concert with the previously observed (Vorobyov et al., 2007) changes in disparity tuning of V1 neurons. The absence of any measurable refractive error or anisometropia by retinoscopy in all three animals precludes any simple optical explanation for the seeming lack of stereopsis in two of them. Moreover, an optical explanation for the results would require a substantial refractive difference between the eyes in view of the size of the stimuli and the observation distance employed.

The testing apparatus was designed so that static monocular depth cues were reduced to a minimum leaving texture density as potentially the most salient cue. However, it is possible that the animals could employ dynamic cues such as motion parallax to discern differences in the distances of the two stimuli. But, because motion parallax is a monocular cue, performance would be expected to be similar under both monocular and binocular viewing conditions. While the performance of two of the animals conformed with this prediction, we believe it most unlikely that any of the animals performed the task by use of this cue. Certainly the substantially superior binocular performance of C087 was inconsistent with the use of motion parallax. None of the animals made head movements suggestive of the use of motion parallax cues and their monocular performance was remarkably and consistently poor over 10 consecutive testing sessions, suggesting that they employed the same monocular depth cue(s) throughout and were unable to take advantage of the potentially more precise cue of motion parallax (Rogers & Graham, 1982) during this period. The latter
observation is important in the context of reports (Gonzalez, Steinbach, Ono, & Wolf, 1989; Steeves, Gonzalez, & Steinbach, 2008) that children enucleated at an early age fail for many years to use motion parallax to assist their depth judgments so that initially their precision is poor.

The apparent stereoblindness of two of the animals of this study indicates that daily episodes of binocular vision, a procedure that prevents the development of deprivation amblyopia does not, pari passu, reliably permit the acquisition of stereoscopic vision. This result leads naturally to the need for future investigations of the necessary requirements for the development of normal stereopsis. It is possible that the development of local stereopsis and normal stereoeacuity in animals with mixed monocular and binocular vision each day may require a higher proportion of daily concordant binocular input than that necessary for typical values for visual acuity. Whether or not the critical proportion of binocular vision that allows development of normal local stereopsis also permits global stereopsis, is also an open issue. Because dark rearing by itself leaves deficits of depth perception even after recovery of visual acuity (Kaye et al., 1981), additional experiments are required to determine the minimum amount of daily binocular vision required for local stereopsis to develop in animals otherwise deprived of all visual input. Previously we have shown that kittens require only respectively, 0.5 hrs or 0.25 hrs binocular visual experience each day for normal visual acuity (Mitchell et al., 2006) and ocular dominance domains in V1 to develop (Schwarzkopf et al., 2007).

Insofar as the rehabilitative effects of the daily episodes of binocular vision were concerned, their protective effects against monocular deprivation did not appear to extend reliably beyond their ability to prevent a loss of visual acuity indicating that the requirements for development of other aspects of vision such as good stereopsis may be stricter. The results point to the need for incorporation of additional measures to ensure binocular co-operation in order to enhance the protective effects of binocular visual input beyond prevention of amblyopia to permit the acquisition of good stereoscopic vision. Long-standing clinical language as well as findings from animal models point to the origins of amblyopia in terms of neural changes induced by imbalanced early binocular input. Because amblyopia is in essence a monocular manifestation of changes in binocular processing, therapies that target the preservation of binocular interactions or their restoration at an early stage of treatment when amblyopia develops, have a certain face validity. With respect to amblyopia, knowledge of the status of binocular vision has been shown to permit distinctions to be drawn between different subtypes (McKee, Levi, & Movshon, 2003). In turn, either knowledge or presumptions of the status of binocularity has prompted formal investigations of therapies for amblyopia that focus upon exploitation and/or preservation of binocular connections. For example, remarkable success has been reported in patients with anisometropic amblyopia that were treated with spectacles alone without recourse to patching of the non-amblyopic eye that may have compromised binocular vision (Chen et al., 2007; Moseley et al., 2002; Steele et al., 2006). Beyond this passive approach there is a recent trend to incorporate active treatment strategies designed to improve binocular co-operation as part of conventional therapy for a pre-existing amblyopia (Mansouri, Thompson, & Hess, 2008; Waddingham et al., 2006).

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References


