

Protection against deprivation amblyopia depends on relative not absolute daily binocular exposure

Donald E. Mitchell

Department of Psychology, Dalhousie University,
Halifax, NS, Canada



Frank Sengpiel

School of Biosciences, Cardiff University,
Cardiff, UK



David C. Hamilton

Department of Mathematics and Statistics,
Dalhousie University, Halifax, NS, Canada



D. Samuel Schwarzkopf

School of Biosciences, Cardiff University,
Cardiff, UK, &
Wellcome Trust Centre for Neuroimaging at UCL,
London, UK



Jan Kennie

Department of Psychology, Dalhousie University,
Halifax, NS, Canada



Short daily periods of binocular exposure (BE) can offset longer single daily episodes of monocular exposure (ME) to prevent the development of deprivation amblyopia. To determine whether the outcome depended upon an absolute daily amount of BE or its proportion of the daily visual exposure, daily mixed visual input of 3 different durations (3.5, 7, or 12 h) was imposed on 3 cohorts of kittens. Measurements of the visual acuity of the deprived eye at the end of mixed daily visual input revealed that the acuity of the deprived eye developed to normal values so long as the proportion of the total exposure that was binocular was 30% or more. By contrast, the development of functional ocular dominance domains in V1 revealed by optical imaging suggests that normal domains emerge with a fixed *amount* of daily binocular exposure. The latter result is consistent with the effects of any daily period of ME, or BE, or both, effectively saturating with a small dose so that the effects of ME of any length can be offset by a short period of BE. The different result for vision may reflect neural events at higher and/or multiple levels in the visual pathway.

Keywords: visual acuity, visual development, visual cortex, plasticity

Citation: Mitchell, D. E., Sengpiel, F., Hamilton, D. C., Schwarzkopf, D. S., & Kennie, J. (2011). Protection against deprivation amblyopia depends on relative not absolute daily binocular exposure. *Journal of Vision*, 11(7):13, 1–11, <http://www.journalofvision.org/content/11/7/13>, doi:10.1167/11.7.13.

Introduction

Much of our understanding of experiential influences on the postnatal development of the central visual pathways of higher mammals has been derived from the study of the effects exclusively abnormal visual exposure imposed early in the animal's life (Daw, 2006; Kiorpes & McKee, 1999; Mitchell & Timney, 1984; Movshon & Kiorpes, 1990; Rauschecker, 1991). The authoritative experiments of Hubel, Wiesel, and Le Vay (1977; Le Vay, Wiesel, & Hubel, 1980; Wiesel & Hubel, 1963, 1965) that employed periods of monocular deprivation (eye closure) demonstrated that early abnormal visual input resulted in profound anatomical and physiological changes in the primary visual cortex (V1) of cats and monkeys. The physiological changes in V1 occur only if the selective

visual exposure (or deprivation) is imposed during a postnatal critical period that has a different temporal profile for cats and monkeys (Horton & Hocking, 1997; Hubel & Wiesel, 1970; Le Vay et al., 1980). In kittens, the critical period begins at about 2 weeks, peaks at 4–5 weeks of age, and then declines gradually over the next 8–9 months (Cynader, Timney, & Mitchell, 1980; Daw, Fox, Sato, & Czepita, 1992; Olson & Freeman, 1980b). Monocular deprivation in both cats and monkeys leads to large visual losses in the deprived eye, the severity of which depend on the length of deprivation and the age of onset (Giffin & Mitchell, 1978; Harwerth, Crawford, Smith, & Boltz, 1981; Mitchell, 1988; Von Noorden, Dowling, & Ferguson, 1970). The visual deficits resemble closely those experienced by humans with deprivation amblyopia as is observed in children following correction for congenital monocular cataracts (Maurer & Lewis, 1993).

Previously, we have shown in kittens (Mitchell, Kind, Sengpiel, & Murphy, 2003, 2006) that visual deficits are not obligatory following early monocular deprivation as they are reduced, or even prevented, if the animals receive a brief period of concordant binocular visual input each day either before or after a longer daily period of monocular exposure. In the original experiments (Mitchell et al., 2003, 2006), the kittens were restricted to 7 h of such mixed visual exposure each day from 4 to 8 weeks of age that consisted of a period of abnormal monocular exposure (ME) followed or preceded by an interval of binocular exposure (BE). For the remaining 17 h each day, the kittens were placed with their mother in a darkroom facility (Beaver, Mitchell, & Robertson, 1993). It was found that 2 h of daily BE, if both concordant and continuous (i.e., presented as a single episode), could outweigh or protect from a much longer (5 h) daily period of ME so that each eye developed normal visual acuity. Shorter daily periods of BE lead to deficits of visual acuity in the deprived eye. Because the total daily visual exposure was held constant (7 h) for all the animals, the original studies could not identify whether the beneficial outcome required a minimal daily *amount* of BE or whether it was determined by the *proportion* of the total visual exposure that was binocular. In principle, this issue can be addressed by changing the total daily amount of visual experience. Apart from trivial cases, where the total daily exposure is either quite brief or very long, the length of BE required to prevent deprivation amblyopia should approximately double with a twofold increase in the daily total visual exposure if the outcome was dictated by its proportion of the total exposure. On the other hand, such an increase in the total daily visual exposure should not alter the length of daily BE for prevention of amblyopia if it required an absolute amount of such exposure.

An experimental design that employed a twofold difference in total visual exposure was recently employed in a study (Schwarzkopf, Vorobyov, Mitchell, & Sengpiel, 2007) of the effects of mixed daily BE and ME on kitten V1 cortical architecture as assessed by optical imaging of intrinsic signals. Near-normal ocular dominance domains developed with only 30 min daily BE, regardless of the total daily duration of visual exposure (3.5 or 7 h), suggesting that this gross anatomical and physiological outcome in V1 was determined by an absolute amount of BE. That this absolute binocular exposure was shorter than the minimum exposure (2 h) found to prevent deprivation amblyopia in our earlier studies (Mitchell et al., 2003, 2006) raises the possibility that the requirements for vision may follow a different rule than for the functional development of ocular dominance domains in V1 and instead be better expressed as a proportion of the total exposure.

Because of its relevance to clinical amblyopia, it is important to find out how to best express the outcome of mixed daily monocular and binocular visual exposure on the visual acuity of the two eyes. To this end, we explored

systematically the effects of mixed visual exposure on two cohorts of kittens that received two different lengths of total visual exposure, namely 3.5 and 7 h. In addition, limited strategic data were obtained from 3 kittens that received 12-h daily visual exposure.

Materials and methods

Animals and rearing procedures

Data were obtained from a total of 34 kittens that were born and raised in a closed breeding colony at Dalhousie University. The rearing, surgical, and behavioral testing procedures adhered to animal protocols approved by the Dalhousie University Committee on Laboratory Animals in accordance with standards and regulations established by the Canadian Council on Animal Care. Data from fifteen kittens were taken from two earlier studies on 21 kittens (Mitchell, Kennie, Schwarzkopf, & Sengpiel, 2009; Mitchell et al., 2006) that examined the effects on vision of mixed daily early visual input on animals that received 7-h visual experience each day. The excluded kittens from these studies were animals for which the BE was distributed in two episodes, or else received discordant visual input during the period of BE, or received just 0.5-h visual experience (BE only) each day. The rearing history of these 15 animals, including the mean hours of daily BE and ME and the order in which they were imposed together with the final visual acuity for each eye, is provided in Table 1.

The remaining 19 animals were reared specifically for this study to provide an additional cohort that received 3.5-h ($N = 16$) total visual experience each day as well as a limited set of data from 3 animals that received 12-h daily visual experience. As with our earlier studies, the mixed daily visual experience was begun when the kittens were 4 weeks old and lasted for 4 weeks; before that, they were housed with their mother in a colony room that was illuminated on a 12:12 L–D cycle. From 4 weeks of age and for the next 28 days, the animals that received just 3.5-h total visual exposure were housed with their mother in a darkroom facility (Beaver et al., 1993) for all but the period of visual exposure that was spent in an illuminated colony room with their littermates and several other adult cats. For logistic reasons, the rearing procedure for the 3 animals that received 12-h total visual experience was different in that they were housed in the colony room throughout each day; for them, the 12-h dark cycle of the colony room lighting provided the daily dark period rather than the darkroom facility. During the dark period, some faint light could be seen at the bottom of the entry door, but this did not permit a human to perceive any visual forms in the room.

Cat	Hours of visual exposure (mean \pm SD)			Order	Final acuity (cycles/deg)	
	ME	BE	Total		Deprived eye	Non-deprived eye
C889	7.00 \pm 0.16	0.03 \pm 0.14	7.03 \pm 0.06	ME	Blind	6.19
C903	6.99 \pm 0.05	0.00 \pm 0.00	6.99 \pm 0.05	ME	Blind	7.42
C887	6.54 \pm 0.05	0.52 \pm 0.02	7.05 \pm 0.05	ME–BE	1.40	6.19
C895	6.49 \pm 0.04	0.50 \pm 0.02	7.00 \pm 0.04	BE–ME	1.25	5.94
C896	5.99 \pm 0.02	1.00 \pm 0.01	6.99 \pm 0.02	BE–ME	1.87	5.94
C894	6.05 \pm 0.09	1.00 \pm 0.08	7.04 \pm 0.06	ME–BE	2.66	5.71
C905	5.51 \pm 0.03	1.49 \pm 0.04	7.00 \pm 0.04	ME–BE	3.49	6.19
C890	5.03 \pm 0.06	2.03 \pm 0.07	7.07 \pm 0.07	BE–ME	5.94	5.44
C888	5.03 \pm 0.09	2.01 \pm 0.04	7.04 \pm 0.09	ME–BE	5.70	6.19
C893	5.03 \pm 0.05	2.02 \pm 0.07	7.05 \pm 0.08	ME–BE	5.94	5.94
C904	5.00 \pm 0.13	2.01 \pm 0.06	7.01 \pm 0.10	ME–BE	5.71	5.94
C909	3.00 \pm 0.02	4.02 \pm 0.04	7.02 \pm 0.04	ME–BE	6.59	6.59
C086	4.97 \pm 0.22	2.04 \pm 0.20	7.01 \pm 0.04	BE–ME	5.94	5.94
C087	4.98 \pm 0.08	2.03 \pm 0.07	7.01 \pm 0.04	BE–ME	5.94	5.94
C088	4.98 \pm 0.07	2.03 \pm 0.06	7.01 \pm 0.04	BE–ME	5.94	5.94

Table 1. The visual history of 15 kittens reared for two previous studies (Mitchell et al., 2009, 2006) that received 7-h daily mixed visual experience for 4 weeks beginning at 4 weeks of age. The mean hours and standard deviation (SD) of daily monocular (ME), binocular (BE), and total exposure and their order are shown together with the visual acuities of, respectively, the deprived and non-deprived eyes of each animal. The latter acuity was usually measured on the last day of mixed visual exposure while the deprived eye acuity was measured on the day following, immediately after the kittens were removed from the darkroom.

The daily period of visual exposure was split into two intervals of varying lengths in which an interval of concordant binocular visual exposure (BE) was pitted against a (usually longer) period of exclusively monocular exposure (ME). The rearing history of the 19 new animals reared specifically for this study, including the mean hours of daily BE and ME and the order in which they were imposed, is provided in Table 2. As earlier work (Mitchell et al., 2006) had shown that the order of exposure was unimportant, the period of BE was imposed first for most animals. An opaque neoprene foam mask as used in our previous studies (Mitchell et al., 2003, 2006) was employed to occlude one eye to allow ME. During the time the mask was in place, a light cardboard ruff cut from a manila folder was worn to prevent kittens from pawing at the mask. For the kittens that received ME first, the mask was placed on the kitten's head in a dimly lit anteroom immediately following the removal of each kitten from the darkroom. For two kittens (C938, C965) that both received 1-h BE each day, the mask was modified so that one eye was occluded with 3-mm-thick translucent white Plexiglass that, by a visual match, reduced light transmission by 2.1 log units but allowed no transmission of form. The animals were monitored closely so that the few instances where the masks were dislodged were caught quickly and noted. The occlusion times displayed in Tables 1 and 2 incorporate these episodes as well as minor deviations in the timing of the exposure periods. At the end of the period of visual exposure each day, which for

most animals ended with the period of ME, the kittens were returned with their mother to the darkroom where the masks were removed.

Behavioral testing: Grating acuity

The kittens were trained and tested on a discrimination task between horizontal and vertical square-wave gratings by means of a jumping stand (Mitchell, Giffin, & Timney, 1977) and by use of the same procedures as those employed extensively in the past (Murphy & Mitchell, 1987) and in our earlier studies on the effects of mixed early visual experience (Mitchell et al., 2003, 2006). A detailed description of the procedures is provided in these earlier papers; here, we provide only the important details. The laser-printed gratings were 19-cm squares with a space-average luminance of 55 cd/m² and had a Michelson contrast of 1.0. Initial training of the kittens was initiated as early as possible at about 5 weeks of age during the daily periods of BE. Binocular measurements of visual acuity were made on a daily basis during the last week of mixed visual experience in order to allow accurate measurement of the acuity of the non-deprived eye at the conclusion of the 4-week period of mixed rearing. For all but 5 animals (C937, C940, C074, C103, and C104), only binocular measurements of acuity were made prior to the end of the period of mixed rearing. On the basis of the results obtained in our earlier studies (Mitchell et al., 2003, 2006), the grating acuity of the

Cat	Hours of visual experience (mean \pm SD)			Order	Final acuity (cycles/deg)	
	ME	BE	Total		Deprived eye	Non-deprived eye
<i>3.5-h cohort</i>						
C002	3.22 \pm 0.07	0.28 \pm 0.07	3.50 \pm 0.01	BE–ME	0.62	5.71
C003	3.23 \pm 0.06	0.28 \pm 0.06	3.50 \pm 0.02	BE–ME	0.52	5.71
C005	2.99 \pm 0.04	0.52 \pm 0.04	3.51 \pm 0.02	BE–ME	1.44	5.71
C006	3.00 \pm 0.06	0.51 \pm 0.07	3.51 \pm 0.02	BE–ME	1.04	5.94
C938*	2.50 \pm 0.03	1.01 \pm 0.02	3.51 \pm 0.03	BE–ME	6.19	6.59
C939	2.49 \pm 0.05	1.02 \pm 0.04	3.51 \pm 0.03	BE–ME	6.19	6.59
C940	2.51 \pm 0.04	1.01 \pm 0.02	3.51 \pm 0.04	BE–ME	6.19	6.59
C965*	2.49 \pm 0.02	1.00 \pm 0.01	3.50 \pm 0.01	BE–ME	6.19	6.19
C968	2.50 \pm 0.01	1.00 \pm 0.01	3.50 \pm 0.01	ME–BE	6.19	6.19
C969	2.49 \pm 0.07	1.00 \pm 0.01	3.51 \pm 0.04	BE–ME	6.19	6.19
C990	2.49 \pm 0.03	1.02 \pm 0.06	3.51 \pm 0.05	ME–BE	6.19	6.19
C966	1.50 \pm 0.01	2.00 \pm 0.07	3.50 \pm 0.01	ME–BE	5.94	5.94
C936	1.52 \pm 0.05	2.00 \pm 0.03	3.52 \pm 0.04	BE–ME	6.59	6.59
C937	1.52 \pm 0.05	2.00 \pm 0.02	3.51 \pm 0.04	BE–ME	6.59	6.59
C967	1.49 \pm 0.03	2.01 \pm 0.02	3.50 \pm 0.02	BE–ME	6.19	6.19
C987	3.49 \pm 0.05	0.02 \pm 0.01	3.50 \pm 0.01	ME	Blind	6.19
<i>12-h cohort</i>						
C103	10.14 \pm 0.58	1.86 \pm 0.58	12.00 \pm 0.02	ME–BE	2.51	5.98
C104	10.14 \pm 0.58	1.86 \pm 0.58	12.00 \pm 0.02	ME–BE	2.66	5.98
C074	8.39 \pm 0.28	3.62 \pm 0.28	12.02 \pm 0.00	ME–BE	6.19	6.19

Table 2. The visual history of 19 kittens from two cohorts prior to and during the 4-week period of daily mixed visual input initiated at 4 weeks of age. Whereas the 16 kittens from the 3.5-h cohort were kept in a darkroom outside of the 3.5 h of daily visual experience, the 3 animals from the 12-h cohort remained in their colony room with the lights turned off after the 12 h of daily visual exposure. The mean hours and standard deviation (SD) of daily monocular (ME), binocular (BE), and total exposure and their order are shown together with the final visual acuities of, respectively, the deprived and non-deprived eyes of each animal. For two animals (C938 and C965, asterisks), the occluder worn during the period of ME was translucent. The latter acuity was usually measured on the last day of mixed visual exposure while the deprived eye acuity was measured on the day following, immediately after the kittens were removed from the darkroom.

non-deprived eye was assumed to be the same as that measured binocularly, an assumption confirmed by monocular measurements of the acuity of the non-deprived eye of C937 and C940. On the next day, the animals were removed from the darkroom for monocular measurements of the acuity of the deprived eye made with an opaque contact lens occluder placed over the cornea of the other eye. The grating acuity of the deprived eye measured the day following the 28-day period of mixed visual exposure and the acuity of the non-deprived eye measured on the last day of mixed visual exposure are provided in [Tables 1](#) and [2](#).

Results

3.5 h cohort

The visual acuity of the deprived eye (DE) of the 16 kittens of this cohort are plotted in [Figure 1](#) as a function

of the length of this exposure. With the exception of C987 that received exclusively monocular exposure and that exhibited no signs of vision with its deprived eye, all the animals possessed measurable visual acuity with the DE at the end of the period of mixed daily visual input. The acuity of the DE increased with the length of daily BE to achieve values closely similar (mean 6.24 cycles/deg) to that of the fellow non-deprived eye (mean 6.35 cycles/deg) with one or more hours of daily BE. The mean acuities of the two eyes were not statistically different using the Wilcoxon Rank Sum test ($P = 0.25$). Moreover, the acuity of the deprived eye of animals that received 1 or more hours of BE each day appeared to be no different from that of normal animals of comparable age (Giffin & Mitchell, 1978). Although most animals received the period of BE first each day, the pattern of results from the 3 animals (C966, C968, and C990) for which ME preceded BE appeared no different. This finding complements our earlier results from animals that received 7 h of mixed visual experience each day where the order of ME with respect to BE did not influence the results (Mitchell et al., 2003, 2006). In addition, the results from 2 of

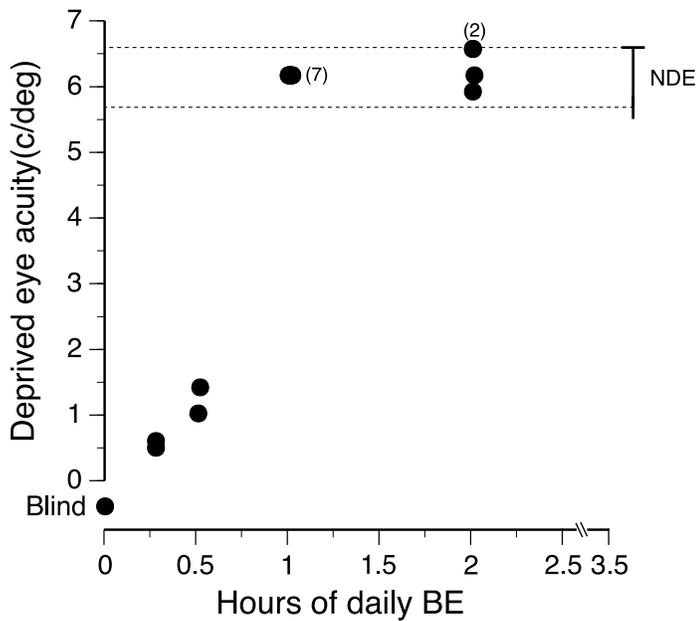


Figure 1. The visual acuity (cycles/degree) of the deprived eye of 16 kittens as a function of the number of hours of binocular visual exposure received each day. Each kitten received 3.5 h of mixed visual exposure each day for 4 weeks starting at 4 weeks of age. The numbers beside some data points represent the number of animals with identical acuities. The dashed horizontal lines indicate the range of values for the fellow non-deprived eye.

7 animals (C938 and C965) that received 1-h BE each day but wore a translucent instead of the opaque occluder were no different from the 5 other animals (Table 1). However, by contrast with our earlier studies on animals that received 7-h visual experience each day (Mitchell et al., 2003, 2006), the acuity of the deprived eye achieved normal levels with a shorter daily period of BE (1 versus 2 h), a finding that suggests that the outcome of mixed daily visual experience may depend upon the *proportion* of daily visual exposure that is binocular rather than by the *absolute* amount of such exposure.

The three cohorts

In order to examine the issue further, the acuity data for all the animals from this and our earlier study (Mitchell et al., 2006) are shown plotted together in Figure 2A as a function of the proportion of the total visual experience that was BE. The acuity of the deprived eye has been plotted relative to the acuity of the fellow eye as it provides a within-animal control with regard to variables such as motivation as the two measurements were made in close temporal proximity (usually within a day). To explore formally the qualitative impressions of substantial overlap of the data, separate logistic functions were fitted to the data from the two large cohorts (3.5 and 7 h) and

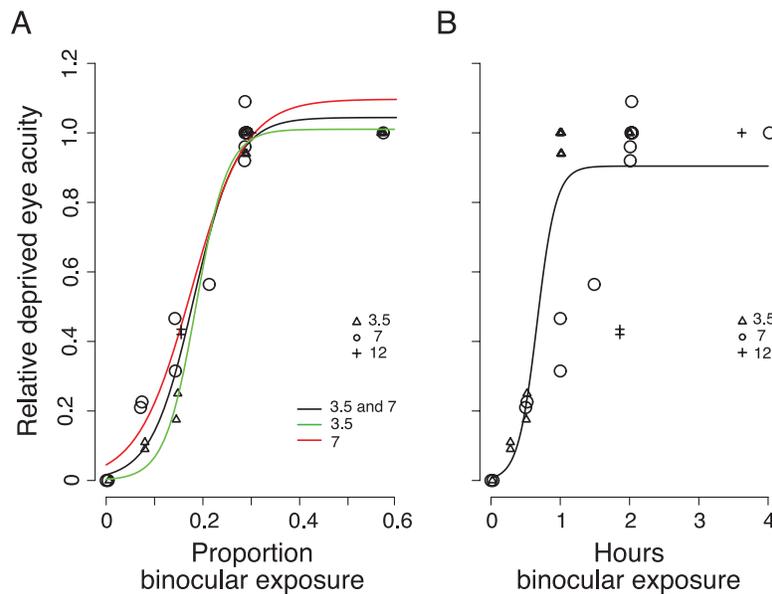


Figure 2. (A) The acuity of the deprived eye, plotted relative to that of the fellow eye, as a function of the proportion of the daily visual exposure that was binocular. Separate logistic functions have been fitted to the data for the 3.5- (green line) and 7-h (red line) cohorts as well as the combined data for both cohorts (black line). Data are also shown for the 3 animals that received 12 h of daily visual exposure. (B) The acuity of the deprived eye, plotted relative to that of the fellow eye, as a function of the hours of daily visual exposure that was binocular. A single logistic function fitted to the combined data from the 3.5- and 7-h cohorts shows a poor fit. Data are also shown for the 3 animals that received 12 h of daily visual exposure.

<i>a</i>	<i>b</i>	<i>c</i>	<i>s</i>	R^2
1.04 (0.03)	−4.13 (0.41)	22.83 (2.33)	0.07	0.97
1.01 (0.02)	−5.73 (0.51)	30.77 (3.13)	0.03	0.99
1.10 (0.06)	−3.15 (0.42)	17.88 (2.53)	0.08	0.97

Table 3. Fitted parameters with standard errors in parentheses for the logistic curves of Figure 2A that describe the relationship between the relative acuities of the deprived eye as a function of the proportional binocular exposure for the 3.5- and 7-h cohorts as well as for the combined data from both. The parameter *a* is the maximum relative deprived eye acuity, and *b* and *c* are shift and scale parameters for the abscissa. Measures of fit are R^2 , the proportion of variation in acuity explained, and *s*, the standard error of the fit.

compared statistically to a single logistic function fitted to the combined data from both cohorts. The data from the two cohorts were well fit by logistic functions of the form

$$DA_{rel} = a/[1 + \exp(-b - cBE_p)], \quad (1)$$

where DA_{rel} is the acuity of the DE relative to the fellow NDE, BE_p is the proportion of the total daily exposure that is binocular, and *a*, *b*, and *c* are coefficients that represent, respectively, the computed maximum DA_{rel} (*a*) and shift (*b*) or scale (*c*) parameters for the BE_p axis. The excellence of the fit of the separate curves fitted to the data from the two cohorts as well as to the combined data from both is revealed by the high values for R^2 that were in excess of 0.96. The various coefficients for the three data sets (3.5, 7, and combined) are displayed in Table 3. The curve fitted to the combined data from the 3.5- and 7-h cohorts displayed as the black curve in Figure 2A:

$$DA_{rel} = 1.04/[1 + \exp(4.13 - 22.83BE_p)], \quad (2)$$

permits calculation of the value of BE_p in order for the acuity of the DE to attain key levels such as 50% (0.18) or 95% (0.28) of that for the fellow eye. Although this curve was derived from the combined data from animals in the 3.5- and 7-h cohorts, the data from the 3 animals from the 12-h cohort appeared to conform well to the curve fitted to the other two cohorts.

In contrast to the excellent overlap of the deprived eye acuity data from the various cohorts when the BE is expressed as a proportion of the total daily visual exposure, the same acuity data when plotted as a function of the absolute daily BE show considerable scatter among the different cohorts (Figure 2B). The logistic function fitted to the combined data shows a poorer fit to the data ($R^2 = 0.76$; Table 4). Inspection of Figure 2B shows that the poorer fit to the curve arises largely from an apparent lateral shift of the data from the 7- and 12-h cohorts with respect to the data from the 3.5-h cohort. Taken together with the overall poorer fit, the apparent lateral displacement

of the data from the two longer exposure cohorts from the 3.5-h cohort when plotted as a function of absolute daily BE is consistent with a dependence in terms of the proportion of total daily exposure that is binocular as opposed to the absolute amount of such exposure.

As a contrast to the behavioral findings, Figure 3 shows a replot of the previously published (Schwarzkopf et al., 2007) optical imaging data on the cortical territory devoted to the deprived eye in V1 of cats following mixed daily visual experience. The dimensions are displayed in Figure 3A with BE expressed as a proportion of the total daily exposure and in Figure 3B with respect to the absolute daily BE. To ease comparison with the acuity results, the data have been fitted with logistic functions. The data expressed as a proportion of the total daily exposure (Figure 3A) were fit best by separate logistic functions for the two cohorts and poorly by a function fitted to the combined data ($R^2 = 0.60$). By contrast, and in agreement with the previous analysis (Schwarzkopf et al., 2007), the data plotted with respect to the absolute binocular exposure (Figure 3B) were better fit ($R^2 = 0.73$) by a logistic function calculated for the combined data. There was no evidence that curves fitted to the separate data (3.5 and 7 h) provided a better fit than the curve fitted to the combined data ($F = 0.85$; 23 *df*; $P = 0.47$).

Discussion

As with our earlier studies of animals that received 7 h of daily mixed visual exposure (Mitchell et al., 2003, 2006), short daily periods of binocular vision offset much longer periods of monocular visual exposure to prevent the development of deprivation amblyopia. A daily period of BE as short as 15 min was sufficient to allow the development of measurable visual acuity in the deprived eye while 1 h of such exposure permitted this eye to achieve visual acuity in the normal range (Figure 1). By contrast, our earlier studies (Mitchell et al., 2003, 2006) indicated that such an equivalent beneficial outcome

Cohort	<i>a</i>	<i>b</i>	<i>c</i>	<i>s</i>	R^2
3.5 and 7	0.904 (0.05)	−4.84 (1.69)	7.28 (2.71)	0.201	0.759

Table 4. Fitted parameters with standard errors in parentheses for the logistic curves of Figure 2B that describe the relationship between the relative acuities of the deprived eye as a function of the absolute binocular exposure for the combined data from both the 3.5- and 7-h cohorts. The parameter *a* is the maximum relative deprived eye acuity, and *b* and *c* are shift and scale parameters for the abscissa. Measures of fit are R^2 , the proportion of variation in acuity explained, and *s*, the standard error of the fit.

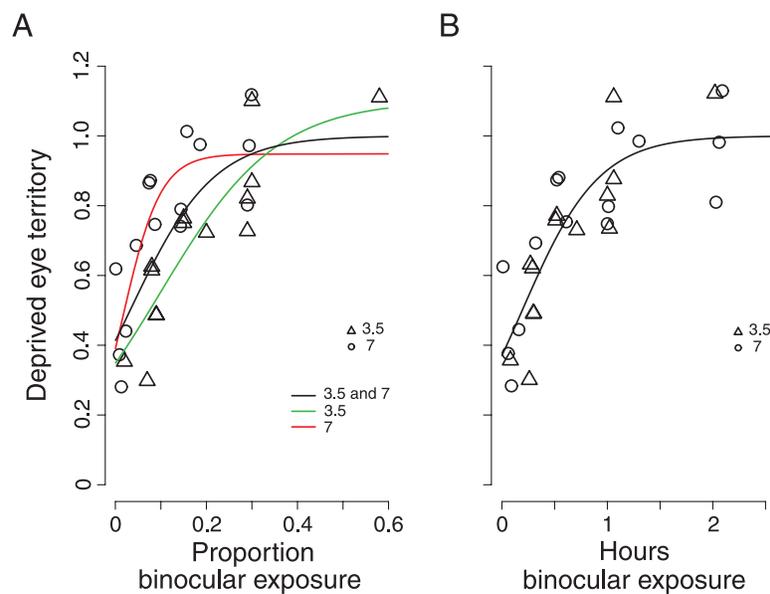


Figure 3. A replot of previously published (22) data on the dimensions of ocular dominance domains in the visual cortex of kittens as a function of the (A) proportion or (B) absolute amount of daily binocular exposure. Data are shown for two cohorts that received either 3.5- (triangles, green line) or 7-h (circles, red line) daily visual exposure. Whereas a single logistic function (black line) provides a good fit to the data from both cohorts when the daily binocular exposure (BE) is expressed in absolute terms (B), separate functions were necessary to fit the data for the different cohorts when BE is expressed as a proportion of the total daily visual exposure (A). The black line in (A) shows the logistic curve fit to the combined data.

required 2 h of daily BE. By itself, comparison of the minimum daily BE required to completely offset ME in terms of the acuity of the deprived eye suggests that the outcome is dictated by the proportion of the total visual exposure that is binocular rather than by an absolute amount of such exposure.

Although manipulation of the period of total visual exposure may intuitively be thought of as a direct way to address this issue, it is important to recognize that this approach is limited by certain constraints. First, the total daily visual exposure must exceed a certain critical length as a minimum daily exposure must be necessary to effect improvement in the vision in either eye over time. In our previous study (Mitchell et al., 2006), we demonstrated for a single animal that a daily (binocular) exposure of only 30 min was sufficient for it to develop visual acuity in the normal range. In addition, in the course of our imaging study (Schwarzkopf et al., 2007), we found that 15 min of daily binocular exposure is sufficient for normal ocular dominance maps in V1 to develop. Second, as the length of daily total visual exposure is increased, the inability to control or document the amount of sleep during the two exposure conditions correspondingly increases as well, thereby introducing potential variability to the data. Third, the amount of ME associated with a fixed proportion of the total visual exposure changes with the length of the latter. Consequently, with decrease in the length of total visual exposure, the anatomical and functional *sequelae* of a period of ME that represents a

constant proportion of the total exposure may change. Finally, the period the animal spends in the darkroom each day represents a time when neurons in the central visual pathways may receive some balanced non-visually driven (spontaneous) activity from the two eyes. Depending on its magnitude with respect to the activity generated during the daily period of visual exposure, the former may effectively contribute to the effectiveness of the daily period of BE. The potential contribution of this factor will also change with the length of total daily visual exposure. The deterioration of the functional properties of visual cortical neurons that occurs in animals placed in complete darkness (Crair, Gillespie, & Stryker, 1998; Freeman, Mallach, & Hartley, 1981), even following periods of monocular deprivation (Freeman & Olson, 1982), may potentially offset any benefit from balanced spontaneous activity. On the other hand, the fact that kittens that receive, respectively, only 15 or 30 min visual exposure per day can develop both normal orientation and ocular dominance maps in V1 (Schwarzkopf et al., 2007) and visual acuity (Mitchell et al., 2006) suggests that the supposed deleterious effects of extended daily periods of darkness may be negligible.

With these limitations in mind, we selected periods of total daily visual exposure that were at least a factor of 2 apart and sufficiently long to ensure the development of normal visual acuity as well as allowing for frequent monitoring of the animals when they were in a lighted environment. The exposures would, on the basis of

existing data, ensure that the daily periods of ME would, by themselves (i.e., in the absence of a daily period of BE), produce substantial cortical anatomical and functional changes over the 4-week period of selected visual exposure. A single 4-h period of ME is known to produce a substantial shift of ocular dominance in the visual cortex, while the electrophysiological effects of 24-h ME can be as extensive as those observed after weeks or months of deprivation (Freeman & Olson, 1982). The cumulative effects on cortical ocular dominance of cells in the visual cortex of daily 4-h periods of ME in otherwise dark-reared kittens after a month have also been shown to be extremely profound (Olson & Freeman, 1980a). On the basis of this existing data, it is reasonable to conclude that the daily periods of ME experienced by our kittens would lead to extensive cortical alterations. Certainly, the cumulative behavioral effect of 3.5 h of daily ME in an animal that received no BE (C987) was maximal as it appeared blind on the jumping stand with its deprived eye, and optical imaging revealed profound deprivation effects resulting from the same exposure regimen (see also Figure 4 of Schwarzkopf et al., 2007).

The picture that emerged from comparison of the results from the two large cohorts is that the outcome with respect to the vision of the deprived eye is determined by the proportion of the total exposure that is binocular rather than by an absolute amount of such exposure. This conclusion was supported by the limited set of data from a third cohort that received 12-h daily visual exposure. Remarkable support was also provided by the results of a study on monkeys that employed a 12-h period of total visual exposure each day (Sakai et al., 2006; Wensveen et al., 2006). The monkeys were maintained in a colony room on a 12-h light–dark cycle from approximately 3 to 21 weeks of age during which time they received mixed visual input on a daily basis. Four hours of BE each day offset 8 h of ME to permit the development of near-normal spatial contrast sensitivity in both eyes. This critical amount of BE represented approximately 33% of the total daily visual exposure, a value close to the proportion (28%) calculated from the curve fit to the combined data for our kittens.

As with kittens, there was evidence from monkeys that BE may have different protective effects at the level of the visual cortex than for vision. Two hours of daily BE was sufficient to allow for the development of normal ocular dominance distributions, while 1 h of BE reduced the proportion of binocularly suppressive units in V1 to normal levels. On the other hand, the interocular phase (disparity) responses of cells in V1 were abnormal even with 4 h of daily BE suggesting that although the monkeys may not be amblyopic, they may lack stereoscopic vision. Interestingly, daily periods of BE imposed on kittens that were sufficient to prevent amblyopia in all animals so far tested did not always protect against the development of

stereoblindness (Mitchell et al., 2009). Binocular cortical circuits of infant monkeys and particularly the long-range horizontal connections in the superficial layers of V1 are known to be very fragile and easily disrupted by abnormal early visual exposure (Zhang et al., 2005). A similar fragility of binocular connections in kitten V1 (Trachtenberg & Stryker, 2001) combined with the possibility that they may be also less susceptible to protection from daily binocular exposure in both species may lead to differences in the protective effects of BE on visual acuity and stereoscopic vision.

From the limited number of studies of the consequences of mixed daily visual input that have been published to date, it is apparent that daily binocular exposure may not be equally effective in terms of its protective effects for different visual functions or indeed for functional or anatomical effects at the level of visual cortex. Moreover, the results reported here point to an additional dimension along which the protective effects of daily BE may be examined. Our results indicate that for kitten visual acuity, the critical amount of daily BE required to protect against disruption due to monocular occlusion is best described in terms of a proportion of the total daily visual exposure as opposed to a critical absolute exposure. This result stands in contrast to preliminary data for the dimensions of ocular dominance domains in cat V1, which appear to be protected by a critical absolute duration of BE each day.

The possible existence of different outcomes for normal physiological development in V1 or visual development with mixed daily visual exposure may reflect their dependence on neural processing at different or even multiple levels in the visual pathway. A related factor that may contribute to the different outcomes is a potential difference between the neural populations that are probed by the two techniques. Whereas the ocular dominance domains revealed by optical imaging predominantly reflect the responses of neurons tuned to low to medium spatial frequencies, behavioral visual acuity is a test of the highest spatial frequency that can be perceived. It is possible that the neural populations that underlie the processing of high spatial frequencies may be more susceptible to deprivation and, consequently, require more daily offsetting BE to develop normally than the neural populations stimulated during optical imaging. The requirement for a binocular exposure of a critical absolute duration as opposed to a critical proportion is consistent either with, BE exerting a protective or offsetting effect that saturates with a short exposure so that it can overcome a period of ME of any duration, or else with the effects of a daily period of ME effectively saturating with a small dose so that it can be offset by a short period of BE. Such situations might be anticipated to rule for particular neuronal functions mediated at a relatively low level in the visual pathway. On the other hand, for visual functions that may be dependent upon processing at multiple levels

in the pathway or else reflect the activity of specific neural populations such as those tuned to high spatial frequencies, a different situation may prevail where neither the effects of daily BE or ME may saturate with short exposures.

The possible impact of form deprivation myopia (FDM)

A refractive error induced by deprivation, if uncorrected, could result in an underestimation of the behaviorally measured visual acuity. However, for various reasons, it is extremely unlikely that the acuities reported here are contaminated by uncorrected refractive errors. First, whereas form deprivation induces myopia reliably in many species including primates (Daw, 2006), the outcome for cats is inconsistent and far less marked. At one extreme, two studies report that image degradation, even when prolonged, does not produce myopia at all in kittens (Nathan, Crewther, Crewther, & Kiely, 1984; Smith, 1981), while other studies report either only mild myopia in a very small proportion (17.6%) of animals (Yinon, Koslowe, & Rassin, 1984) or else non-systematic changes (Gollender, Thorn, & Erickson, 1979). Even when myopia was reported reliably (Smith, Maguire, & Watson, 1980; Wilson & Sherman, 1977), its magnitude was small and much less than that observed in monkeys. Our own experience mirrors these observations; refractions performed by retinoscopy on lid-sutured animals revealed very small and inconsistent refractive errors with a tendency toward hypermetropia rather than myopia (Giffin & Mitchell, 1978). In addition, we found no significant refractive errors in the animals reared with mixed daily visual experience for the imaging studies (Schwarzkopf & Sengpiel, unpublished observations). Second, any small uncorrected myopia would be very unlikely to degrade the retinal image at the observation distance (typically 70 cm) employed for the behavioral measurements. Finally, daily periods of normal visual input have been shown to prevent the development of FDM in both chickens (Napper et al., 1995) and monkeys (Smith, Hung, Lee, & Qiao, 2002) making it even more unlikely that our kittens exposed similarly to mixed daily visual input would have developed myopia.

The fact that, for both cats and monkeys, daily episodes of binocular vision can prevent the development of amblyopia even when the visual input for two eyes is discordant for the majority of the day suggests that temporary manipulations that allow balanced input to the two eyes may permit normal visual development until more permanent solutions (such as surgery or refractive correction) can be provided (Mitchell, 2008; Wensveen et al., 2006). The finding that the critical duration of binocular exposure is a proportion of the total daily exposure suggests that in situations where short-term provision of concordant binocular exposure is difficult (such as temporary lifting of a drooping eyelid in ptosis),

the manipulation could be combined with a daily period where both eyes are covered with translucent material to effectively shorten the daily period of patterned visual exposure.

Acknowledgments

This work was supported by grants to DEM from the CPR network of the McDonnell Foundation, Grant 7660 from NSERC and CIHR (Canada), and Grant G0500186 to FS from the MRC (UK). DCH acknowledges the support of a Discovery Grant from the Natural Sciences and Engineering Research Council of Canada.

Commercial relationships: none.

Corresponding author: Dr. Donald E. Mitchell.

Email: d.e.mitchell@dal.ca.

Address: Department of Psychology, Dalhousie University, Halifax, NS B3H 4R2, Canada.

References

- Beaver, C. J., Mitchell, D. E., & Robertson, H. A. (1993). Immunohistochemical study of the pattern of rapid expression of c-Fos protein in the visual cortex of dark-reared kittens following initial exposure to light. *Journal of Comparative Neurology*, *333*, 469–484.
- Crair, M. C., Gillespie, D. C., & Stryker, M. P. (1998). The role of visual experience in the development of columns in the cat visual cortex. *Science*, *279*, 566–570.
- Cynader, M., Timney, B. N., & Mitchell, D. E. (1980). Period of susceptibility of kitten visual cortex to the effects of monocular deprivation extends beyond six months of age. *Brain Research*, *191*, 545–550.
- Daw, N. W. (2006). *Visual development* (2nd ed). New York: Springer.
- Daw, N. W., Fox, K. D., Sato, H., & Czepita, H. (1992). Critical period for monocular deprivation in the cat visual cortex. *Journal of Neurophysiology*, *67*, 197–202.
- Freeman, R. D., Mallach, R., & Hartley, S. (1981). Responsivity of normal visual cortex deteriorates after brief binocular deprivation. *Journal of Neurophysiology*, *45*, 1074–1084.
- Freeman, R. D., & Olson, C. (1982). Brief periods of monocular deprivation in kittens: Effects of delay prior to physiological study. *Journal of Neurophysiology*, *47*, 139–150.
- Giffin, F., & Mitchell, D. E. (1978). The rate of recovery of vision after early monocular deprivation in kittens. *The Journal of Physiology*, *274*, 511–537.

- Gollender, M., Thorn, F., & Erickson, P. (1979). Development of axial ocular dimensions following eyelid suture in the cat. *Vision Research*, *19*, 221–223.
- Harwerth, R. S., Crawford, M. L. J., Smith, E. L., III, & Boltz, R. L. (1981). Behavioral studies of stimulus deprivation amblyopia in monkeys. *Vision Research*, *21*, 779–789.
- Horton, J. C., & Hocking, D. R. (1997). Timing of the critical period for plasticity of ocular dominance columns in macaque striate cortex. *Journal of Neuroscience*, *17*, 3684–3709.
- Hubel, D. H., & Wiesel, T. N. (1970). The period of susceptibility to the physiological effects of unilateral eye closure in kittens. *The Journal of Physiology*, *206*, 419–436.
- Hubel, D. H., Wiesel, T. N., & Le Vay, S. (1977). Plasticity of ocular dominance columns in monkey striate cortex. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, *278*, 377–409.
- Kiorpes, L., & McKee, S. P. (1999). Neural mechanisms underlying amblyopia. *Current Opinions in Neurobiology*, *9*, 480–486.
- Le Vay, S., Wiesel, T. N., & Hubel, D. H. (1980). The development of ocular dominance columns in normal and visually deprived monkeys. *Journal of Comparative Neurology*, *191*, 1–51.
- Maurer, D., & Lewis, T. L. (1993). Visual outcomes after infantile cataract. In K. Simons (Ed.), *Early visual development: Normal and abnormal* (pp. 454–484). New York: Oxford University Press.
- Mitchell, D. E. (1988). The extent of visual recovery from early monocular or binocular visual deprivation in kittens. *The Journal of Physiology*, *395*, 639–660.
- Mitchell, D. E. (2008). A special role for binocular visual input during development and as a component of occlusion therapy for treatment of amblyopia. *Restorative Neurology and Neuroscience*, *26*, 425–434.
- Mitchell, D. E., Giffin, F., & Timney, B. (1977). A behavioural technique for the rapid assessment of the visual capabilities of kittens. *Perception*, *6*, 181–193.
- Mitchell, D. E., Kennie, J., Schwarzkopf, D. S., & Sengpiel, F. (2009). Daily mixed visual experience that prevents amblyopia in cats does not always allow the development of good binocular depth perception. *Journal of Vision*, *9*(5):22, 1–7, <http://www.journalofvision.org/content/9/5/22>, doi:10.1167/9.5.22. [PubMed] [Article]
- Mitchell, D. E., Kind, P. C., Sengpiel, F., & Murphy, K. (2003). Brief daily periods of binocular vision prevent deprivation-induced acuity loss. *Current Biology*, *13*, 1704–1708.
- Mitchell, D. E., Kind, P. C., Sengpiel, F., & Murphy, K. (2006). Short periods of concordant binocular vision prevent the development of deprivation amblyopia. *European Journal of Neuroscience*, *23*, 2458–2466.
- Mitchell, D. E., & Timney, B. (1984). Postnatal development of function in the mammalian visual system. In I. Darian-Smith (Ed.), *Handbook of physiology: Section I. The nervous system* (pp. 507–555). Bethesda, MD: American Physiological Society.
- Movshon, J. A., & Kiorpes, L. (1990). The role of experience in visual development. In J. R. Coleman (Ed.), *Development of sensory systems in mammals* (pp. 155–202). New York: Wiley.
- Murphy, K. M., & Mitchell, D. E. (1987). Reduced visual acuity in both eyes of monocularly deprived kittens following a short or a long period of reverse occlusion. *Journal of Neuroscience*, *7*, 1526–1536.
- Napper, G. A., Brennan, N. A., Barrington, M., Squires, M. A., Vessey, G. A., & Vingrys, A. J. (1995). The duration of normal visual exposure necessary to prevent form deprivation myopia in chicks. *Vision Research*, *35*, 1337–1344.
- Nathan, J., Crewther, S. G., Crewther, D. C., & Kiely, P. M. (1984). Effects of retinal image degradation on ocular growth in cats. *Investigative Ophthalmology & Visual Science*, *25*, 1300–1306.
- Olson, C. R., & Freeman, R. D. (1980a). Cumulative effect of brief daily periods of monocular vision on kitten striate cortex. *Experimental Brain Research*, *38*, 53–56.
- Olson, C. R., & Freeman, R. D. (1980b). Profile of the sensitive period for monocular deprivation in kittens. *Experimental Brain Research*, *39*, 17–21.
- Rauschecker, J. P. (1991). Mechanisms of visual plasticity: Hebb synapses, NMDA receptors, and beyond. *Physiological Reviews*, *71*, 587–615.
- Sakai, E., Bi, H., Maruko, I., Zhang, B., Zheng, J., Wensveen, J., et al. (2006). Cortical effects of brief daily periods of unrestricted vision during early monocular form deprivation. *Journal of Neurophysiology*, *95*, 2856–2865.
- Schwarzkopf, D. S., Vorobyov, V., Mitchell, D. E., & Sengpiel, F. (2007). Brief daily binocular vision prevents monocular deprivation effects in visual cortex. *European Journal of Neuroscience*, *25*, 270–280.
- Smith, D. C. (1981). Functional restoration of vision in the cat after long-term monocular deprivation. *Science*, *213*, 1137–1139.
- Smith, E. L., III, Hung, L. F., Kee, C. S., & Qiao, Y. (2002). Effects of brief periods of unrestricted vision on the development of form-deprivation myopia in

- monkeys. *Investigative Ophthalmology & Visual Science*, 43, 291–299.
- Smith, E. L., III, Maguire, G. W., & Watson, J. T. (1980). Axial lengths and refractive errors in kittens reared with an optically induced anisometropia. *Investigative Ophthalmology & Visual Science*, 19, 1250–1255.
- Trachtenberg, J. T., & Stryker, M. P. (2001). Rapid anatomical plasticity of horizontal connections in the developing visual cortex. *Journal of Neuroscience*, 21, 3476–3482.
- Von Noorden, G. K., Dowling, J. E., & Ferguson, D. C. (1970). Experimental amblyopia in monkeys: I. Behavioral studies of stimulus deprivation amblyopia. *Archives of Ophthalmology*, 84, 206–214.
- Wensveen, J. M., Harwerth, R. S., Hung, L.-F., Ramamirtha, R., Kee, C., & Smith, E. L., III (2006). Brief daily periods of unrestricted vision can prevent form-deprivation amblyopia. *Investigative Ophthalmology & Visual Science*, 47, 2468–2477.
- Wiesel, T. N., & Hubel, D. H. (1963). Single-cell responses in striate cortex of kittens deprived of vision in one eye. *Journal of Neurophysiology*, 26, 1003–1017.
- Wiesel, T. N., & Hubel, D. H. (1965). Comparison of the effects of unilateral and bilateral eye closure on cortical unit responses in kittens. *Journal of Neurophysiology*, 28, 1029–1040.
- Wilson, J. R., & Sherman, S. M. (1977). Differential effects of early monocular deprivation on binocular and monocular segment of cat striate cortex. *Journal of Neurophysiology*, 40, 891–903.
- Yinon, U., Koslowe, K. C., & Rassin, M. I. (1984). The optical effects of eyelid closure on the eyes of kittens reared in light and dark. *Current Eye Research*, 3, 431–439.
- Zhang, B., Bi, H., Sakai, E., Maruko, I., Zheng, J., Smith, E. L., III, et al. (2005). Rapid plasticity of binocular connections in developing monkey visual cortex (V1). *Proceedings of the National Academy of Sciences of the United States of America*, 102, 9026–9031.