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Functional Postnatal Development of the Rat Primary Visual Cortex and the Role of Visual Experience: Dark Rearing and Monocular Deprivation

MICHELA FAGIOLINI,* TOMMASO PIZZORUSSO,* NICOLETTA BERARDI,† LUCIANO DOMENICI,† LAMBERTO MAFFEI*†

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Postnatal development of rat visual cortical functions was studied by recording extracellularly from the primary visual cortex of 22 animals ranging in age from postnatal day 17 (P17) to P45. We found that in the youngest animals (P17–P19) all visual cortical functions tested were immature. Selectivity for orientation and movement direction of visual stimuli was almost absent, most cells received binocular input and their mean receptive field size was 5–6 times the adult size. Visual acuity was half its adult value. These functional properties developed gradually during the following weeks and by P45 they were all adult-like. This functional development is affected by manipulations of the visual input such as dark rearing (DR) and monocular deprivation (MD). DR prevented the normal postnatal maturation of visual cortical functions: in P60 rats, dark reared from birth, their visual cortical functions resembled those of P19–P21 rats. MD from P15 to P45 resulted in a dramatic shift of the ocular dominance distribution (ODD) in favour of the open eye and in a loss of visual acuity for the deprived eye. To determine the sensitive period of rat visual cortex to MD (critical period) we evaluated the shift in ODD of visual cortical neurones in rats that were subjected to the progressive delay of the onset of fixed MD period (10 days). Our results show that the critical period begins around the end of the third postnatal week, peaks between the fourth and fifth week and starts to decline from the end of the fifth week.

Development Visual cortex Rat Dark rearing Critical period

INTRODUCTION

The visual cortex of many mammals is immature at birth, both anatomically and physiologically and develops gradually during the first weeks of postnatal life (Hubel & Wiesel, 1963; Blakemore & van Sluyters, 1975; Boothe, Dobson & Teller, 1985; Teller, Regal, Videen & Pulos, 1978; Albus & Wolf, 1984).

During this period there is a substantial anatomical reorganisation of the connectivity in the visual cortex, while visual neurones develop their adult functional properties in response to visual stimuli. Both anatomical and functional development depend at least in part upon visual experience during an early period of plasticity called "critical period". Visual experience acts by modulating the level and the patterning of neural activity within the visual pathways. Neural activity plays a crucial role in the strengthening, remodelling and elimination of synapses during development of the visual system (for a review see Shatz, 1990).

*Scuola Normale Superiore, P.zza Cavalieri, 56127 Pisa, Italy.
†Istituto Neurofisiologia CNR, via S. Zeno, 51 56127 Pisa, Italy.

The postnatal development of the visual system and in particular of the visual cortex is well known for the cat, monkey and partly for the human visual system, much less is known about the development of the rat visual system. This lack of knowledge for the rat is particularly unfortunate since most biochemical and molecular studies on CNS development have been performed on the rat. In the modern approach to the study of the CNS a correlation between biochemical and molecular data with functional properties has became a useful and, several times, necessary strategy. It became therefore necessary to perform physiological studies on the postnatal development of the rat visual cortex.

Previous electrophysiological studies on the visual system of adult rats found that cortical neurones have well defined functional properties (Parnavelas, Burne & Lin, 1981; Maffei, Berardi, Domenici, Parisi & Pizzorusso, 1992) and are distributed in different classes of ocular dominance, with a high proportion of binocular cells, comparable to that in cats and monkeys (Maffei *et al.*, 1992; Berardi, Domenici, Parisi, Pizzorusso, Cellerino & Maffei, 1993). We also found

that the rat visual cortex is sensitive to monocular deprivation during the early period of postnatal development, as in other mammals (Domenici, Berardi, Carmignoto, Vantini & Maffei, 1991; Maffei *et al.*, 1992; Berardi *et al.*, 1993).

We have confirmed and extended these results in a systematic electrophysiological study of the postnatal development of the rat visual cortex and of its sensitivity to manipulations of visual inputs. We focused our attention on the following points:

(1) development of functional properties of visual cortical neurones (cell responsiveness, ocular dominance, orientation and direction selectivity, receptive field size);

(2) development of visual acuity (visual evoked potential) in the primary visual cortex;

(3) effects of dark rearing on postnatal development;

(4) effects of monocular deprivation and definition of the critical period.

MATERIALS AND METHODS

Animal treatment

A total of 49 Long Evans hooded rats were used. 22 rats were normal. 13 rats were kept in total darkness from birth until P60 (DR rats). Extreme care was taken to ensure that the room where animals were housed was completely light-tight. The effectiveness of the light seal was checked by leaving pieces of fast photographic film in the room for a day; they failed to show any evidence of being fogged. Every two days feeding and cleaning of cages were performed using infra-red light.

Nine rats were monocularly deprived for 10 days starting from P14, P23 and P33 (MD rats). Five rats were monocularly deprived for 30 days starting from P45. Eyelids suture was performed under ether anaesthesia.

Single cell recording

Normal rats were recorded during the critical period (P17, $N = 3$; P19–23, $N = 8$; P26–30, $N = 6$; P45, $N = 5$); DR rats were recorded after P60. MD rats were recorded at the end of the deprivation period. Recording procedures followed the protocols of Maffei *et al.* (1992) (single unit recording) and Domenici *et al.* (1991) (VEP recording).

To record single cortical unit activity, animals were anaesthetized in urethane (6 cm^3/kg, 20% solution, Sigma) by intraperitoneal injection. A hole was drilled in the skull in correspondence with the binocular portion of the primary visual cortex (area OC1B). In the rat, OC1B corresponds to the lateral portion of the primary visual cortex (stereotaxic coordinates >4 mm from the central fissure), mapping the upper nasal visual field. After exposure of the brain surface, the dura was removed and a micropipette filled with NaCl (3 M) was inserted into the cortex. Cortical surface was protected from drying with Agar (1.5 g in saline, 60 ml). Body temperature was continuously monitored and maintained around 38°C by means of a thermostated electric blanket. Electrocardiogram was also continuously monitored.

To prevent sampling biases our penetrations were angled and for each animal at least two well spaced penetrations were performed. The last penetration was marked by lesions made by passing a small current (10 μA, 10 sec) every 250 μm during electrode withdrawal for subsequent track reconstruction. This confirmed that recordings had been performed in area OC1B. The range of cortical depths over which cells were found were identical in different age groups and spanned layers II to V and less frequently layer VI.

Both eyes were fixed and kept open by means of adjustable metal rings (Parnavelas *et al.*, 1981) surrounding the external portion of eye bulbs, and the cornea was protected with artificial tears (Lacrinorm, Farmigea, Pisa). To backproject the optic disk we dilated the pupils (Atropine sulphate, 0.1%) at the end of the experiment. The position of the optic disk was marked onto a tangent screen where all the cell receptive fields positions had been plotted (Lennie & Perry, 1981).

Visual stimuli were hand moved light bars projected on a reflecting screen or gratings or bars computer generated on a display (HP1300A, 28 × 22 cm, mean luminance 12 cd/m^2). Both the screen and the display were 20 or 30 cm from the rat eyes.

On isolating a cell, the following experimental protocol was followed:

- The location of monocular receptive field in visual space and the orientational and directional properties were determined with hand held stimuli. The preference for moving and/or stationary bars was determined on the basis of cell firing rates read on a counter. In the pigmented rat, the binocular portion of each visual hemifield extends approx. 40 deg from the vertical meridian in the upper visual field and vertical meridian is 55–58 deg from the optic disk (Montero, Brugge & Beitel, 1968; Hughes, 1979; Reese & Jeffery, 1983; Reese, 1988). Only cells with receptive fields farther than 30° nasal from the optic disk and in the upper visual field were included in our sample. Care was taken that cells were sampled at comparable eccentricities in the different age groups.
- Cell responsiveness was assessed with bars or gratings of optimal orientation according to standard criteria (Hubel & Wiesel, 1962; Burne, Parnavelas & Lin, 1984). It was evaluated in terms of amplitude of modulation of the cell discharge in response to an optimal stimulus. The marked sluggishness and extreme response fatigue of cells in the cortex of the youngest animals (P17) and of DR rats made it necessary to adopt interstimulus intervals of up to 15 sec between stimulus passages over the receptive field to elicit reliable responses. In particular visual cortical neurones were classified as *Hab* + if the response became indistinguishable from spontaneous activity after only one stimulus passage, as *Hab* if the response disappeared after 3–4 passages, as *No Hab* if the response remained of the same strength after repeated stimulus presentations.

• Neurones in ocular dominance class 1 were neurones driven only by the stimulation of the contralateral eye; neurones in ocular dominance classes 2–3 were binocular and preferentially driven by the contralateral eye; neurones in ocular dominance class 4 were equally driven by the two eyes; neurones in ocular dominance classes 5–6 were binocular and preferentially driven by the ipsilateral eye and neurones in ocular dominance class 7 were driven only by the ipsilateral eye. The category NR contains those neurones which could not be driven by visual stimulation (Hubel & Wiesel, 1962).

Binocular index is

$$[1/2N(2\text{–}3) + N(4) + 1/2N(5\text{–}6)]/N_{tot};$$

Ipsilateral index is

$$[1/2N(5\text{–}6) + N(7)]/N_{tot};$$

with N $(i–j)$ = number of cells in classes $i–j$ and N_{tot} = total number of responsive cells. Indices range from 0 to 1 with high values corresponding to high proportions of binocular or ipsilaterally-driven neurones (Paradiso, Bear & Daniels, 1983; Kasamatsu & Pettigrew, 1979).

• Receptive field (RF) size and type were assessed with bars or gratings of optimal orientation according to standard criteria (Hubel & Wiesel, 1962; Burne *et al.*, 1984). Receptive field size was determined both by the use of PSTHs, as reported in Fig. 5, or by mapping the minimal response field.

• Orientation selectivity was determined with bars or gratings (contrast 30–40%). Neurones were classified as **orientation-selective** if the cell response was maximal for a given stimulus orientation (preferred orientation) and indistinguishable from spontaneous activity for at least the orthogonal stimulus orientation; cells were classified as **orientation-biased** if the response was present at all orientations but was clearly greater (>2×) for certain orientations than for others; cells were classified as **non-orientation-selective** if the response was of comparable strength on six orientations (0°, ±30°, ±60°, ±90°). In some cells orientation selectivity was judged qualitatively on the basis of the cell firing rate, read from a counter. In some cells orientation tuning curves were measured from computer averaged responses. In Fig. 1 we report examples of orientation tuning curves for each category [Fig. 1(A)]. For each orientation the cell response was computer averaged over at least 20 stimulus cycles. The response amplitude was taken as the peak amplitude minus spontaneous discharge (response to bars) or as the amplitude of modulation (response to gratings). Examples of cell responses to the best and worst stimulus orientation are reported in Fig. 1(B) for each category.

Orientation index is

[Number of *orientation selective* cells

+ 1/2 Number of *Orientation Biased* cells]/ .

(Total number of responsive cells).

To choose the optimal spatial frequency of the grating used to evaluate cell orientation selectivity and responsiveness, spatial frequency-response curves were evaluated in animals older than P19. However, given the size of our display we could not present gratings of spatial frequency lower than 0.03 c/deg. For this reason and for the condition of eye optics of P17 rats it was not possible to evaluate spatial frequency-response relationship at P17. In these animals cell response was evaluated with drifting bars of optimal size and velocity.

Visual evoked potentials

The visual stimuli were vertical gratings of different contrast and spatial frequencies generated on a display (HP1300A, 28 × 22 cm, mean luminance 12 cd/m^2) positioned 20 cm from the rat eyes and centred on the previously determined receptive fields. The gratings were alternated in phase with a fixed temporal frequency, from the 2–4 Hz range. The signals were filtered and amplified in a conventional manner, computer averaged and analysed. For each condition (viewing eye, spatial frequency, contrast) at least 400 responses were averaged. For each record the amplitude, phase and relative power of the first 12 harmonics were measured. For the temporal frequencies used, signals consisted mainly of the second harmonic (relative power >70%). For this reason, amplitude of the second harmonic in each record (1/2 peak-to-trough amplitude) was taken as the VEP amplitude for that condition. Noise level for a given condition was considered to be the amplitude of the second harmonic in records where the stimulus was covered with a translucent screen (blank field). To assess the spatial resolution value (visual acuity), gratings of maximum available contrast were used (70%); spatial frequency was progressively increased until signal was indistinguishable from noise. Visual acuity was taken as the highest spatial frequency still evoking a response above noise level. To test whether younger animals needed any optical correction, recordings were made with and without lenses of different dioptric power placed in front of the rat eyes.

At the end of the experiment, animals were perfused with normal saline followed by 4% paraformaldehyde (Riedle, D) in 0.1 M phosphate buffer. The brains were coronally sectioned (vibratome, series 1000) and stained with cresyl violet.

Statistical analysis

The following types of statistical analysis have been performed to evaluate the significance of differences between data obtained for different groups: χ^2 test (4 d.f.) for the difference between ocular dominance distributions (ODD); t-test for the difference between means, and the variance of binomial distribution for differences between percentages. A difference was considered significant if its probability p was less than 0.05.

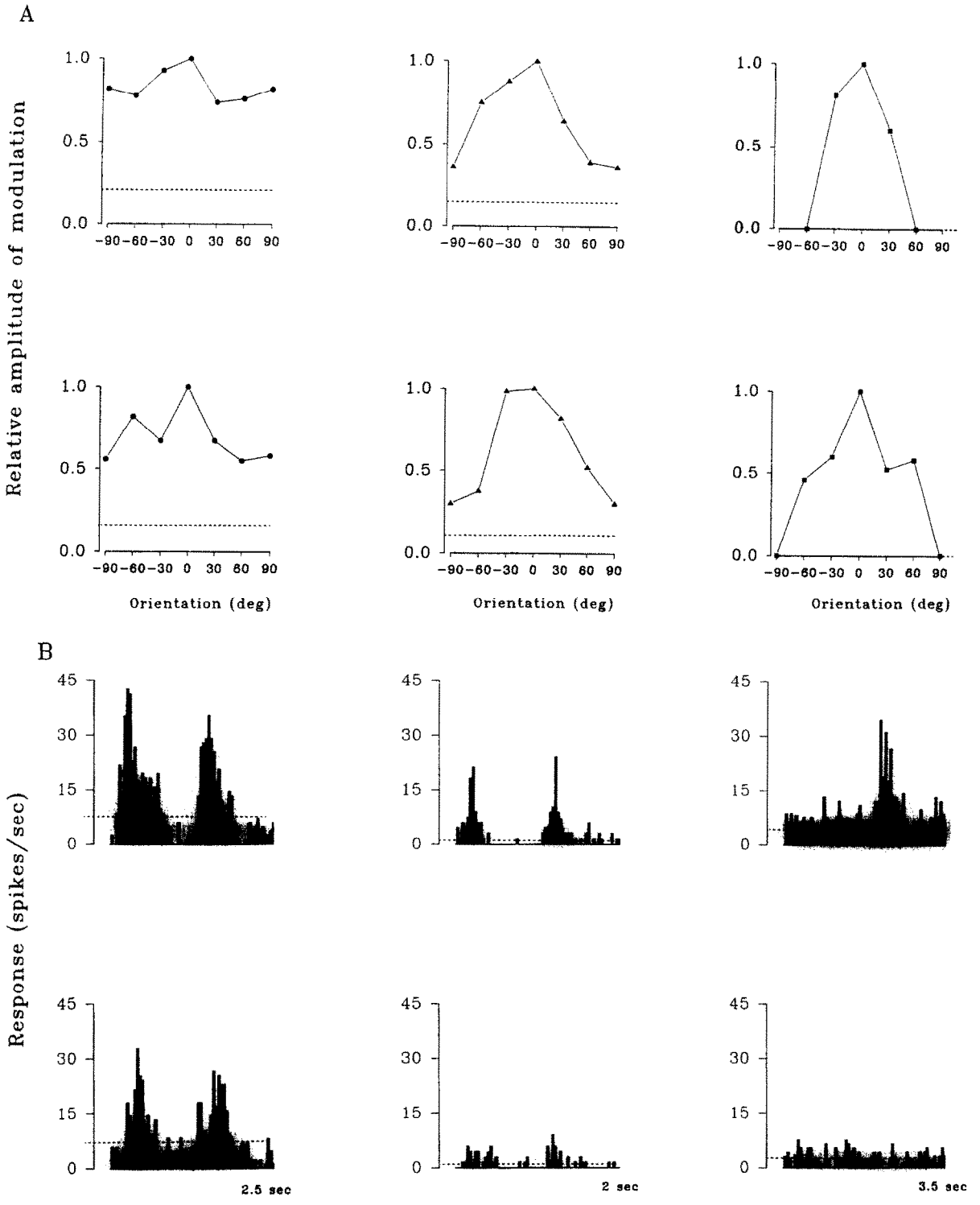

FIGURE 1. (A) Examples of orientational tuning curves for non-orientation selective (left), orientation biased (middle) and orientation selective (right) cells (data from a P45 animal). The cell relative response amplitude (1 = strongest response) is plotted as a function of stimulus orientation. For non-orientation selective and orientation biased cells dotted line corresponds to the spontaneous discharge. For the orientation selective cells, spontaneous discharge corresponds to the abscissae axis. 0 is the preferred orientation for O and B cells and is the vertical orientation for NO cells. Clockwise and anticlockwise rotations from 0 are represented as positive and negative values respectively, in steps of 30 deg. For each orientation, the cell response was computer averaged over at least 20 stimulus cycles. Spontaneous discharges are: left, top, 8.4 spikes/sec; bottom, 8 spikes/sec; middle, top, 1 spike/sec; bottom, 5 spikes/sec; right, top, 4.5 spikes/sec; bottom, 2 spikes/sec. Peak responses are: left, top, 12 spikes/sec (amplitude of 2nd harmonic), bottom, 30 spikes/sec (response to bar); middle, top, 5 spikes/sec (amplitude of 2nd harmonic), bottom 15 spikes/sec (amplitude of 2nd harmonic); right, top, 30 spikes/sec (response to bar), bottom, 15 spikes/sec (response to bar). (B) This figure shows cell response (spikes/sec) for one cell in each category of orientation selectivity. The cells shown are those plotted in the upper row in (A). For each cell, response to the best (upper) and worst (lower) stimulus orientation are shown. The abscissa corresponds to 1 stimulus cycle (stimulus period indicated below abscissa in figure). Visual stimuli for the non-orientation selective (left) and the orientation biased (middle) cells were phase reversed gratings (contrast 30%, spatial frequency 0.5 c/deg); the visual stimulus for the orientation selective cell was a drifting bar, contrast 35%, velocity 12 deg/sec, bin size 54 msec. Dotted line represents spontaneous activity.

RESULTS

To test whether visual cortical functions develop gradually in rats as in other mammals, we have recorded from 22 rats at different stages of postnatal development between postnatal day 17 (P17) and P45. We made extracellular recordings from single units in area 17, within 30 deg from the vertical meridian and in the upper visual field, which corresponds to the binocular part of rat primary visual cortex. For each cell we assessed ocular dominance, orientation selectivity, direction selectivity and receptive field size (Maffei *et al.*, 1992). We also tested spatial resolution (visual acuity) of the primary visual cortex by means of visual evoked potentials (VEP) (Domenici *et al.*, 1991).

The transparency of the optic media, as tested ophthalmoscopically, is very poor at the time of eye opening (P14), as in the cat. Registrations began at P17. At this age the optic of the eyes still presents a few patches of opacity. Eye optics is completely clear by P19.

Development of cell responsiveness

At P17 responses were sluggish and variable both to moving and stationary stimuli. In particular it was necessary to leave intervals of up to 15–20 sec between successive bar passages over the receptive field to avoid progressive decrease of cell responses (habituation). Only 22% (8/36) of responsive cells showed no habituation (see Table 1). This marked sluggishness and response fatigue to visual stimuli of cells in the cortex of very young rats is similar to that found in cats (Hubel & Wiesel, 1963; Pettigrew, 1974; Blakemore & van Sluyters, 1975; Buisseret & Imbert, 1976; Bond & Freeman, 1979; Albus & Wolf, 1984). In the youngest rats, visual cells generally preferred moving stimuli rather than flashed bars: 55% of P17 cells gave stronger response to moving than to flashing bars. During the following days the proportion of neurones responsive to both moving and flashed stimuli increased and became the same as the adult by P23. At this age, 78% of responsive cells did not show habituation (59/76) and only 8% strongly habituated (6/76).

Cell responsiveness, evaluated in terms of amplitude of modulation of the cell discharge in response to an optimal stimulus, increased from P19–23 (median value 6 spikes/sec, inter quartile range 2–16 spikes/sec, $n = 32$) to adult value (median value 12 spikes/sec, inter quartile range 4–32 spikes/sec, $n = 57$). At P19–P23 69% of the cells responses had amplitude of modulation less than 8 spikes/sec, while in adult animals this figure fell to 46%. Spontaneous discharge was almost absent at P17, increased slightly at P19–23 (median value 1.5 spikes/sec, inter quartile range 0.5–3 spikes/sec, $n = 77$) and was near adult value at P30 (median value 3.5 spikes/sec, inter quartile range 0.5–7 spikes/sec, $n = 70$, at P30, median value 4 spikes/sec, inter quartile range 1.5–10 spikes/sec, $n = 65$ in adult).

Development of ocular dominance and orientation selectivity

The ocular dominance distribution in adult rats is illustrated in Fig. 2(A) (bottom, left): There is an evident dominance of the contralateral eye (class 1 and classes 2–3 cells) which reflects predominance of crossed vs uncrossed optic nerve fibres in rats, where crossed fibres are estimated to be 90–95% of all optic nerve fibres (Polyak, 1957). Despite the small size of uncrossed fibres contingent, the percentage of binocular cells (80% in the total distribution) is quite high, comparable to that found in other mammals (Hubel & Wiesel, 1962; Baker, Grigg & Noorden, 1974).

TABLE 1. Summary of properties of visual cortical neurones of dark reared and normal animals

	Dark reared animals P60	P17 animals	P19–23 animals	Adult animals P45
Mean binocular index	0.70	0.625	0.6	0.52
SD	0.11	0.06	0.05	0.03
N	13	3	8	5
Mean orientation index	0.12	0.14	0.3	0.65
SD	0.05	0.006	0.09	0.14
N	13	3	8	5
Mean receptive field size	19.1 deg		31 deg	6.7 deg
SD	3.9 deg		8.5 deg	2.5 deg
Cells	$n = 35$		$n = 36$	$n = 32$
Visual acuity	0.54 c/deg		0.52 c/deg	1.1 c/deg
SD	0.12 c/deg		0.06 c/deg	0.1 c/deg
N	4		9	6
Habituation				
Hab +	31%	18.2%	8.6%	0%
Hab	46.6%	63.6%	18.5%	0%
No Hab	22.4%	18.2%	72.8%	100%
Cells	$n = 164$	$n = 44$	$n = 81$	$n = 116$

Binocular index $= [1/2N(2–3) + N(4) + 1/2N(5–6)]/N_{tot}$; orientation index = (*orientation selective* cells + 1/2 *orientation biased* cells)/(total number of responsive cells). N = number of animals, SD = standard deviation. Significance of differences between dark reared vs normal rats was binocular index (two-tailed t test) $p < 0.01$, orientation index (two-tailed t test) $p \ll 0.001$, RF size (two-tailed t test) $p < 0.001$, visual acuity (two-tailed t *test*) $p \ll 0.001$, (*Hab* +) (test for binomial distribution) $p \ll 0.001$.

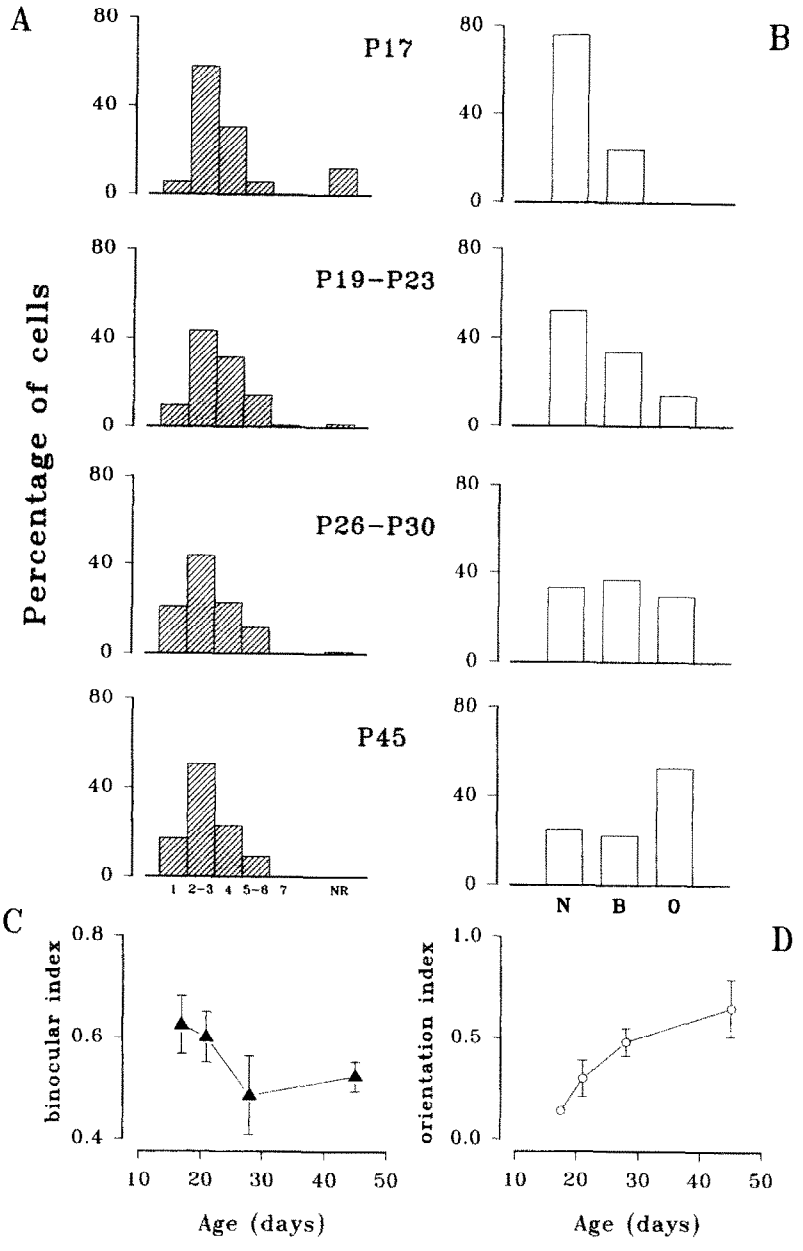

FIGURE 2. (A) Ocular dominance distributions of visual cortical cells recorded from the binocular portion of rat primary visual cortex, area 17, at different postnatal ages from postnatal day 17(P17) to P45. P17: $N = 3$ animals, $n = 41$ cells; P19–23: $N = 8$ animals, $n = 152$ cells; P26–30: $N = 6$ animals, $n = 109$ cells; P45: $N = 5$ animals, $n = 134$ cells. P17 distribution is not significantly different from P19–23 ($p > 0.05$ χ^2 test 4 d.f.) but is significantly different from the others ($p < 0.01$). P19–23 distribution is significantly different from P26–30 and P45 distribution ($p < 0.01$). P26–30 is not significantly different from P45 distribution ($p > 0.05$). Significance of differences between percentage of monocular cells (test for binomial distribution) is given below: P17 vs P19–23, n.s.; P17 vs P26–30, s., $p < 0.05$; P17 vs P45, s., $p < 0.05$; P19–23 vs P26–30, s., $p < 0.001$; P19–23 vs P45, s., $p < 0.05$; P26–30 vs P45, n.s. (B) Distributions of visual cortical neurones recorded from binocular area 17 at different postnatal ages from P17 to P45 and classified according to their orientation selectivity. P17: $N = 3$ animals, $n = 41$ cells; P19–23: $N = 8$ animals, $n = 160$ cells; P26–30: $N = 6$ animals, $n = 111$ cells; P45: $N = 5$ animals, $n = 134$ cells. Each distribution is significantly different from the others ($p > 0.001$, χ^2 test, 2 d.f.). Significance of differences between percentage of non-orientation selective cells (test for binomial distribution) is given below: P17 vs P19–23, s., $p < 0.003$; P17 vs P26–30, s., $p < 0.001$; P17 vs P45, s., $p < 0.001$; P19–23 vs P26–30, s., $p < 0.001$; P19–23 vs P45, s., $p < 0.001$; P26–30 vs P45, n.s. (C) Mean binocular index for each age group [for each data point, animal and cell number as for (A)] plotted as a function of age to show the age dependent decline of binocularity. Vertical bars represent SD. Significance of differences between groups (two-tailed t test) were: P17 vs P19–23, n.s.; P17 vs P26–30, s., $p < 0.05$; P17 vs P45, s., $p < 0.05$; P19–P23 vs P26–30, n.s.; P19–23 vs P45, s., $p < 0.05$; P26–30 vs P45, n.s. (D) Mean orientation index for each age group [for each data point, animal and cell number as for (B)] plotted as a function of age to show the age dependent increase of orientation selectivity of visual cortical neurones. Vertical bars represent SD; where not shown, SD is smaller than the symbol size. Significance of differences between groups (two-tailed t test) were: P17 vs P19–23, s., $p < 0.01$; P17 vs P26–30, s., $p < 0.001$; P17 vs P45, s., $p < 0.001$; P19–23 vs P26–30, s., $p < 0.05$; P19–23 vs P45, s., $p < 0.01$; P26–30 vs P45, n.s.

Ocular dominance distributions from rats at four different ages (P17, P19–P23, P26–P30 and P45) are reported in Fig. 2(A). At P17 ($N = 3$) almost all visually responsive cells were binocular, most of them being dominated by contralateral eye (classes 2–3). Out of the 41 recorded neurones five were not activated by visual stimuli (NR). The proportion of unresponsive cells rapidly declined in the following days and reached zero in the adult. At P19–23, the percentage of binocular cells was smaller (90.2%) and monocular cells (9.8%) were present, most of them dominated by the contralateral eye (15/16). By P26–30 the ocular dominance distribution was not significantly different from the adult. The percentage of binocular cells was 79% and monocular cells were 21%. At P45 the ocular dominance distribution was the same as in adult rats. The major component of the age-dependent change in ocular dominance distribution is the increase in monocular, contralaterally driven cells.

In Fig. 2(C) the mean binocular index is displayed as a function of developmental age to summarise ocular dominance development.

The major component of the age-dependent change in the orientation selectivity is the increase in orientation selective cells [from 0% at P17 to 53% at P45, Fig. 2(B)] and the decrease in non-orientation selective neurones (from 76% at P17 to 25% at P45). In Fig. 2(D) the development of orientation selectivity is summarised by the mean orientation index. Orientation tuning curves were not systematically investigated in the youngest animal. At P30 orientation selective cells had tuning width similar to the adult value (40–45 deg, Maffei *et al.*, 1992).

In addition to ocular dominance and orientation specificity, we also tested cell selectivity for the direction of movement of visual stimuli. This property is absent at early postnatal ages (P17, direction selective cells, $d = 0/36$ and P19–23, $d = 0/152$) and reaches the adult value (10%) by P27–30 ($d = 11/109$).

Development of receptive fields and visual acuity

Receptive fields were mapped with stationary and moving stimuli. In adult rats receptive fields are small and well defined (Shaw, Yinon & Auerbach, 1975; Wiesenfeld & Kornel, 1975; Parnavelas *et al.*, 1981). At P17 receptive field size almost extended through the whole binocular hemifield and their definition was difficult because responses were weak and tended to habituate to repeated stimulations.

At P19–21 the average RF size was 34 deg (standard deviation SD = 6 deg, $n = 21$). Receptive field size decreased to adult value (6.7 deg, SD = 2.5 deg, $n = 32$) during the following weeks [shown in Fig. 3(a)]. Examples of receptive fields (PSTH) in P23 and adult animals are shown in Fig. 5.

Visual acuity

Earlier works on young kittens and monkeys have shown that there is a systematic increase in visual acuity with age (Freeman & Marg, 1975; Mitchell, Giffin,

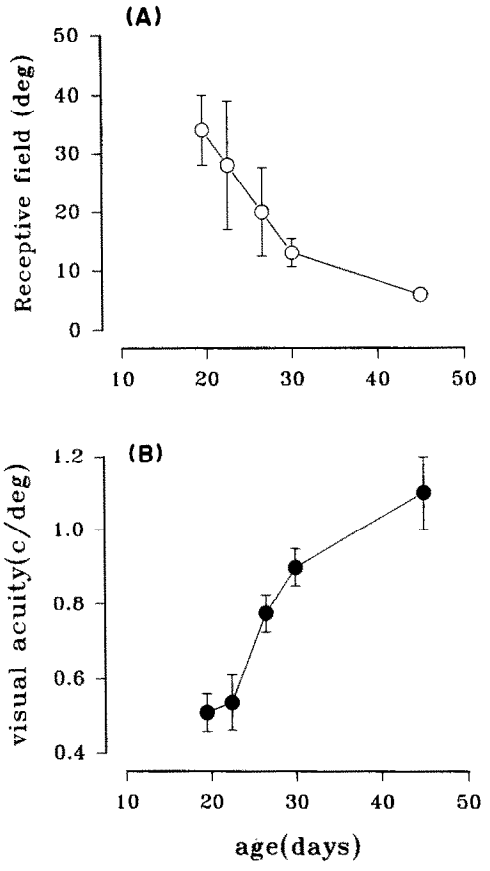

FIGURE 3. Mean receptive field size of visual cortical neurones at different ages. P19–20, $N = 3$ animals, $n = 21$ cells; P22–23, $N = 3$ animals, $n = 15$ cells; P26–27, $N = 3$ animals, $n = 17$ cells; P30, $N = 2$ animals, $n = 15$ cells; P45, $N = 3$ animals, $n = 20$ cells [other conventions as for Fig. 1(D)]. Each experimental point is not significantly different from its first neighbour while it is different from the second. (B) Mean visual acuity (contralateral eye) evaluated by visual evoked potentials at different ages. P19–20, $N = 5$ animals; P22–P23, $N = 4$ animals; P26–27, $N = 3$ animals; P30, $N = 3$ animals; P45, $N = 3$ animals [other conventions as for Fig. 2(D)]. Each experimental point is significantly different from its first neighbour except for P19–20 vs P22–P23.

Wilkinson, Anderson & Smith, 1976; Teller, Regal, Videen & Pulos, 1978). Here we extended these results to the rat. By means of visual evoked potential (VEP) visual acuity was evaluated in 18 rats between P19 and P45, beginning at P19, when optic media were clear. Visual acuity [Fig. 3(b)] increased from 0.51 c/deg (SD = 0.05 c/deg) to 0.9 c/deg within the first month of life and then reached the adult value (1.1 c/deg, SD = 0.1 c/deg and 1.0 c/deg, SD = 0.1 c/deg, contralateral and ipsilateral eye respectively) at P40–45.

It has to be noted that the development of visual acuity is correlated with the decrease of mean receptive field size.

Dark reared rats

13 rats were kept in total darkness from birth to P60. The visual cortex of dark-reared adult rats resembled that of a young rat with the vast majority of cortical cells (103/108) visually responsive, but typically unselective for orientation and direction of moving stimulus. Visual responses were weak and variable, combined with

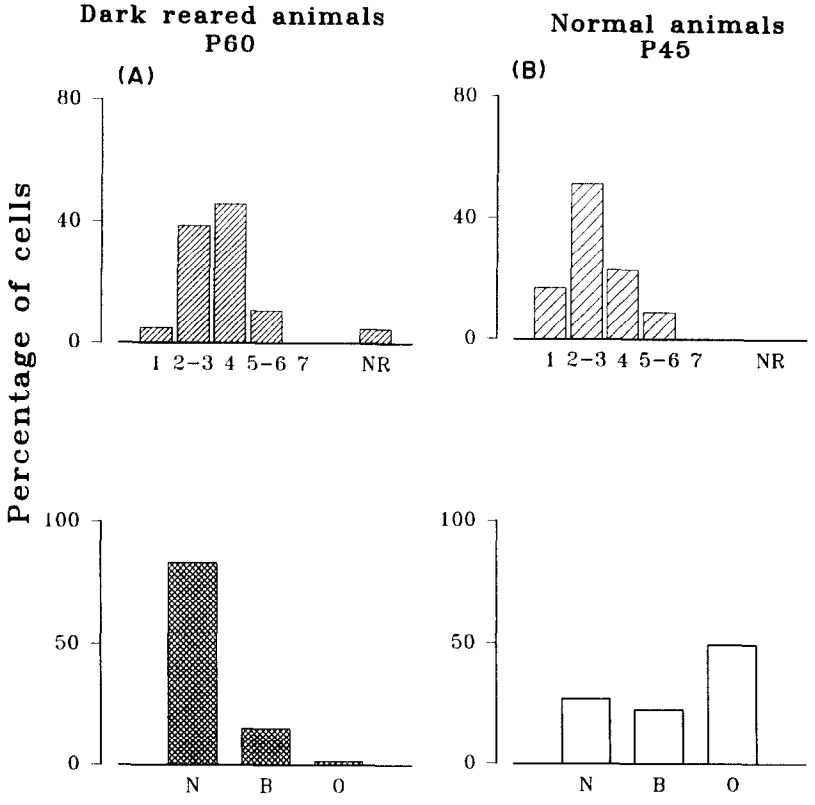

FIGURE 4. (A) Ocular dominance distribution (ODD, top) in animals dark reared from birth to P60. $N = 13$ animals, $n = 103$ cells. Orientation selectivity distribution (bottom) in dark reared animals. $N = 13$ animals, $n = 124$ cells. (B) ODD (top) and orientation selectivity (bottom) distribution for normal adult animals, replotted from Fig. 2(A, B) for comparison. The distributions for dark reared animals are significantly different from the corresponding distributions for normal animals (χ^2 test, $p < 0.001$).

a strong tendency for rapid habituation (Table 1). Spontaneous discharge was lower than normal: most cells had spontaneous activity less than 1 spike/sec (median value 0.5 spike/sec). Cell responsiveness was also lower than normal and comparable to that of P19–23 animals (see also Fig. 5): 74% of cell responses had an amplitude of modulation less than 8 spikes/sec (median value 6 spikes/sec, inter quartile range 2–26 spikes/sec, $n = 23$).

Figure 4(A) (top) shows the ocular dominance distribution of cortical cells in the binocular portion of the visual cortex for DR animals. The percentage of binocular cells (95%) is significantly higher than that of normal adult rats (80%).

In Fig. 4(A) (bottom) we reported the percentage of cells according to their orientation selectivity. The orientation selectivity for cells recorded in DR rats was dramatically reduced: orientation selective cells resulted in only 1.6% (vs 50% in normal rat), biased cells were 15.3% while the vast majority of visual neurones were non-orientation selective (83%). The orientation index was 0.09, significantly different from normal ($p < 0.001$).

Receptive field size and visual acuity development was also affected by dark rearing. Mean receptive field size was bigger (19.1 ± 3.9 deg, $n = 35$) while visual acuity was lower (0.53 ± 0.13 c/deg, $N = 4$) than that of normal adult rats (see Table 1). Examples of RF (PSTH) in DR animals are shown in Fig. 5, together with P23 and adult rats RFs for comparison.

Our results are in agreement with those found by other authors in dark reared cats (Pettigrew & Garey, 1974; Blakemore & van Sluyters, 1975) suggesting that the effect of dark-rearing is to maintain the visual system in an immature state.

Monocular deprivation and critical period

Monocular deprivation performed during the first months of postnatal life affects the normal postnatal development of the visual system of mammals (Wiesel & Hubel, 1963). The visual system is susceptible to alteration by anomalous visual experience only in an early period of life, called Critical Period.

In rats monocularly deprived for 1 month (from P14 to P45), the ocular dominance distribution (ODD) of visual cortical neurones is altered. Figure 6(A) shows the ODD in the binocular visual cortex contralateral to the deprived eye (data from five MD rats, 121 cells recorded, seven non responsive (6%), 114 responsive). The percentage of binocular cells was reduced from 80 to 44.8%, the contralateral deprived eye was dominant in 1.7% of cortical neurones and the ipsilateral non deprived eye was dominant, exclusively or predominantly, in 90.3% of the cells. The mean visual acuity for the deprived eye was 0.4 c/deg (SD = 0.05 c/deg, $N = 5$ animals).

Monocular deprivation performed after P45 did not bring about any alteration in visual cortical physiology. Ocular dominance distribution ($N = 3$ animals,

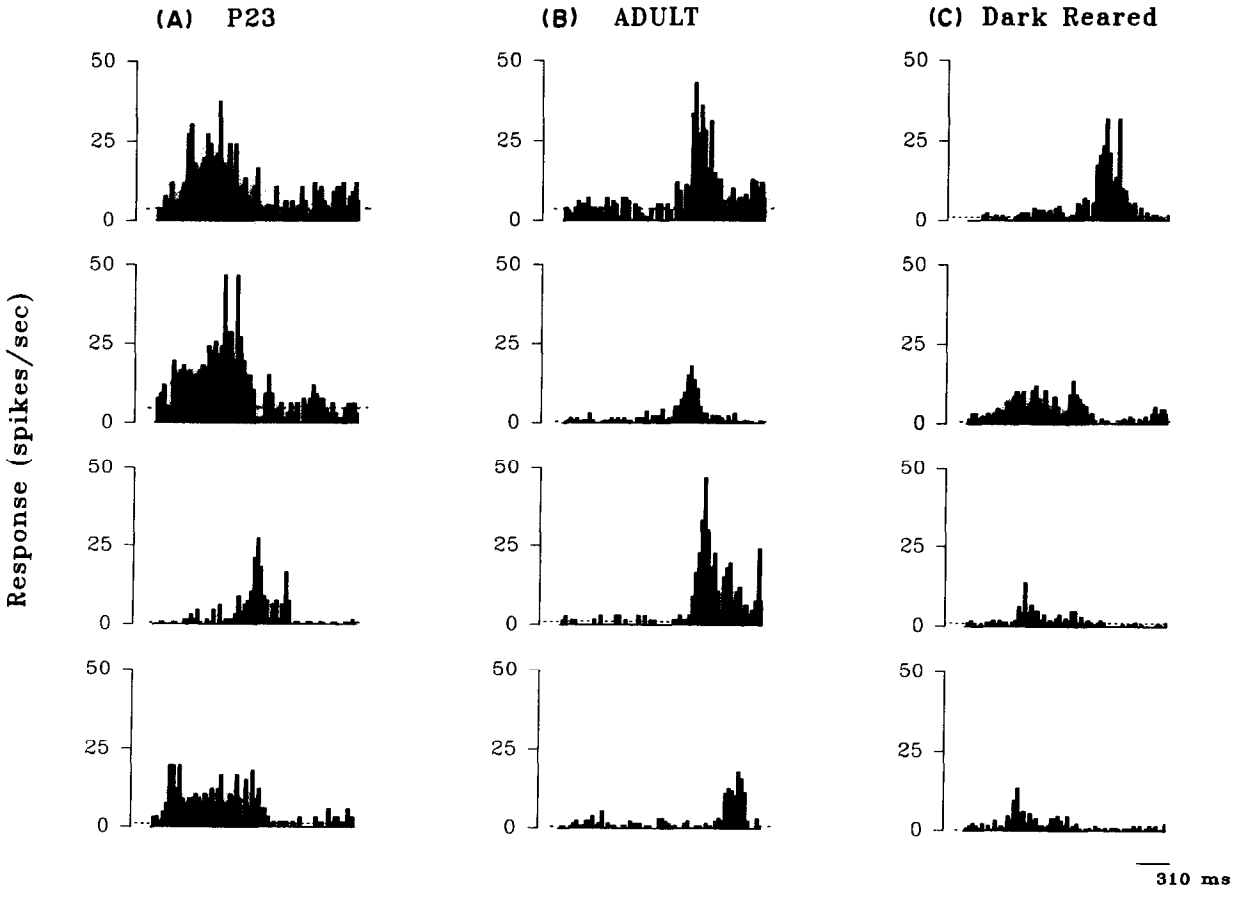

FIGURE 5. Examples of receptive fields determined with PSTH in P23 (A), adult (B) and dark reared (C) animals. Bar velocity: 15 deg/sec. Dotted line represents spontaneous discharge. Time calibration for abscissae axis is given in the bottom right corner of the figure. Each bin is 31 msec. Cell responses were averaged over 20 stimulus cycles (stimulus period 0.5 Hz).

$n = 104$ cells) and visual acuity ($N = 2$ animals) were normal.

In order to examine the time course of susceptibility of rat visual cortex to monocular deprivation (Critical Period) we monocularly deprived nine rats for a short time period (10 days) beginning at three different ages of development (P14, P24, P33). We determined the ODD of cortical neurones at the end of the deprivation periods. The results are presented in Fig. 6(B) where the mean ipsilateral index is plotted as a function of developmental age (end of MD period) to summarise the shift in ODD. It is evident that the rat visual cortex is maximally susceptible to monocular deprivation between the fourth and fifth week. The last short period of monocular deprivation performed (P33–43) did not affect the physiology of visual cortical cells (Fig. 6).

We conclude that the critical period of the rat visual cortex begins during the third postnatal week, peaks between P24–34 and starts to decline after the fifth week. This is in accordance with earlier observations (Rothblat, Schwartz & Kasdan, 1978).

DISCUSSION

We studied the postnatal development of some functional properties of rat visual cortical neurones beginning from P17, three days later than eye opening (P14). Our results indicate that the rat visual cortex is immature at P17 and that all functional properties of cortical neurones develop gradually during the first month of postnatal development.

We exclude that such a developmental trend is an artefact due to cloudiness of optic media in the youngest animals. We took also particular care, even in the younger rats, to record from cells belonging to all cortical laminae.

The responsiveness of visual cortical neurones improved markedly with age over the third postnatal week; in particular the fatigue, the extreme sluggishness and the tendency to habituation of neurone responses disappeared by P23.

In the youngest rats (P17) almost all cortical cells were binocular as in other mammals (Hubel & Wiesel, 1963); then, gradually, cortical neurones distributed in the ocular dominance classes and the process was complete around P30. In cats and monkeys, the functional development of the ODD in area 17 is well correlated with the anatomical segregation of LGN terminals into ocular dominance columns. Such a correlation is not possible in rats, where a clear ocular dominance pattern seems lacking (Thurlow & Cooper, 1988).

Most striate cortical cells in young rats before the fourth week of age were non selective for the orientation and direction of the moving stimulus. Orientation selectivity improved gradually from the time of eye opening to about 6 weeks of age. Our findings agree with the

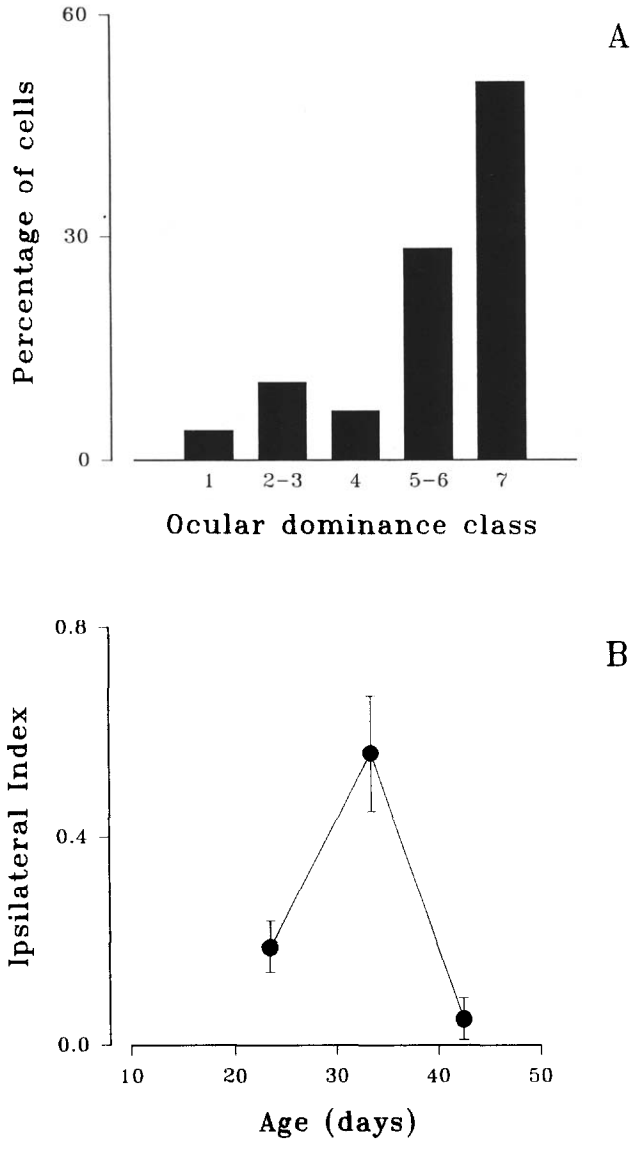

FIGURE 6. (A) Ocular dominance distribution of visual cortical cells recorded in animals monocularly deprived from P15 to P45. $N = 5$ animals, $n = 98$ cells. The distribution is significantly different from that of normal animals (χ^2 test, 4 d.f., $p < 0.001$). (B) Effect of 10 days of MD with progressively delayed onset. The mean ipsilateral index computed for groups of three animals is plotted as a function of the developmental age corresponding to the end of the deprivation period. (P14–23, $n = 74$; P23–33, $n = 59$; P33–43, $n = 74$. Vertical bars represent SD.)

conclusion from earlier studies performed on cats and ferrets, that there is a small number of orientation-selective cells in the visual cortex of visually inexperienced mammals and that orientation selectivity greatly improves with age. Recently, however, Freeman and Ohzawa (1992) demonstrated that cells in area 17 of very young kittens (cells with large receptive fields and low visual acuity) are driven most efficiently by low frequency gratings and were able to demonstrate a high orientation selectivity in very young kittens. We did not use gratings of low spatial frequency for estimating orientation selectivity in P17 rats. Therefore the possibility exists that we overestimated the number of non-orientation selective cells in P17 rats.

Orientation selectivity of cortical cells depends on intracortical inhibitory (GABAergic) circuitry (Sillito, Kemp, Milson & Beradi, 1980; Wolf, Hicks & Albus, 1986; Worgotter & Koch, 1991). According to recent findings, input from LGN neurones with receptive fields covering an elongated region of visual space (Chapman, Zahs & Stryker, 1991; Chapman & Stryker, 1992) and corticofugal feedback to dLGN cells (Sillito *et al.*, 1991) may also be important. Intracortical GABAergic circuitry is known to develop, at least in part, postnatally (Winfield, 1983; Wolff, Balcar, Zetzsche, Bottcher, Schmechel & Chronwall, 1984).

The progressive decrease in receptive field size is consistent with the time course of visual acuity development. In other mammals, these processes have been demonstrated to depend both on neural factors (decrease in photoreceptors size and interdistance, pruning of afferents and reduction of convergence, development of inhibitory processes sharpening the antagonistic organisation of receptive field and of lateral inhibition) and on geometric factors (the increase in retinal image size due to eye growth). We estimate that this second factor might contribute for 25% to development of spatial resolution. Indeed, eye growth in rats within this period is around a factor of 1.3. Little is known about the development of photoreceptor mosaic at these postnatal ages: however convergence rather than photoreceptor mosaic seems to be the major retinal limit of visual acuity in rat (Hughes, 1979). Elimination of LGN afferents is likely to occur during this developmental stage and the sharpening of antagonistic organisation of cortical RFs probably reflects the development of inhibitory processes, as known for the cat (Wolf *et al.*, 1986).

In order to discriminate the role of visual experience in the postnatal development of functional properties of visual cortical neurones from that of a simple age-dependent maturational process, the comparison between the results obtained from light-reared rat and those obtained in animals deprived of visual experience from birth was required.

Our results are in agreement with a number of studies examining the effect of dark-rearing on development of the cat visual cortex (Timmey, Mitchell & Giffin, 1978). We found that dark rearing prevents normal maturation of all functional properties of visual cortical neurones. At P60 when the postnatal development of the rat visual cortex is normally over, all functional properties in DR rats are less mature than in P19–23 animals, with the exception of receptive field size, and comparable to those in P17 animals.

The knowledge of the exact time course of visual cortical vulnerability to eye closure is an important aid to understand normal visual development. We found that the susceptibility to monocular occlusion begins around the end of the third postnatal week, peaks during the fourth and fifth week and sharply declines after the end of the fifth week.

The effect of dark rearing is not only to slow down the development of the visual cortex but also to prolong the sensitive period of monocular deprivation, at least in the cat (Cynader & Mitchell, 1980; Mower,

Caplan, Duffy & Duffy, 1985). Preliminary results obtained in our laboratory from two DR animals suggest that this is also the case for the rat.

The duration of the postnatal development of the rat visual cortex and of its critical period is comparable to that of the cat if we think of the gestation period of thetwo mammals, 22 day for the rat and 65 for the cat (caecal period, Dreher & Robinson, 1989).

It is interesting to note that the development of biochemical and electrophysiological properties reported in the literature strongly correlate with the critical period described by us. Notably mRNA and protein of neurotrophic factors, like BDNF and NGF, increase in the rat visual cortex during this period of postnatal life reaching maximal level of expression around P20 (Large, Bodary, Clegg, Weskamp, Otten & Reichardt, 1986; Bozzi, Pizzorusso, Cremisi, Comelli, Berardi & Maffei, 1993; Thoenen, 1991) and are modulated by visual experience (Bozzi *et al.*, 1993; Castren *et al.*, 1992). NGF is known to play a crucial role in cortical plasticity processes (Maffei *et al.*, 1992; Domenici *et al.*, 1991; Berardi *et al.*, 1992; Carmignoto & Vicini, 1992). In addition there is an age-dependent increase in ibotenate-stimulated phosphoinositide turnover (metabotropic glutamate receptor) that does not occur in the visual cortex of dark reared animals (Dudek & Bear, 1989). Furthermore it has been recently reported that the time constant t of NMDA-mediated excitatory postsynaptic currents (EPSC) in rat visual cortical cells of layer 4 progressively decreases during the critical period reaching adult level around P30 (Carmignoto & Vicini, 1992). This decrease is strongly delayed by dark rearing (Carmignoto & Vicini, 1992). A long lasting NMDA-EPSC, accompanied by a strong Ca^{2+} influx, has been correlated with activity dependent cortical plasticity (see Shatz, 1990 for a review). The change in NMDA-EPSC duration may regulate the decrease in cortical plasticity and might explain the results obtained in DR animals.

We conclude that the functional properties of rat visual cortex develop during the first month of postnatal life and that visual experience plays a role in this process, as it is known for other mammals. It is noteworthy emphasising that the short duration of the critical period and the knowledge of the biochemical and electrophysiological development of the visual cortex may make the rat visual system a suitable model for studies of cortical plasticity.

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