Retinal functional development is sensitive to environmental enrichment: a role for BDNF

S. Landi,* A. Sale,* N. Berardi,†,‡ A. Viegi,* L. Maffei,*,† and M. C. Cenni†

*Laboratorio di Neurobiologia, Scuola Normale Superiore, Pisa, Italy; †Istituto di Neuroscienze del CNR, Pisa, Italy; and ‡Dipartimento di Psicologia, Università di Firenze, Florence, Italy

ABSTRACT **Retina has long been considered less plastic than cortex or hippocampus, the very sites of experience-dependent plasticity. Now, we show that retinal development is responsive to the experience provided by an enriched environment (EE): the maturation of retinal acuity, which is a sensitive index of retinal circuitry development, is strongly accelerated in EE rats. This effect is present also in rats exposed to EE up to P10, that is before eye opening, suggesting that factors sufficient to trigger retinal acuity development are affected by EE during the first days of life. Brain derived neurotrophic factor (BDNF) is precociously expressed in the ganglion cell layer of EE with respect to non-EE rats and reduction of BDNF expression in EE animals counteracts EE effects on retinal acuity. Thus, EE controls the development of retinal circuitry, and this action depends on retinal BDNF expression.—Landi, S., Sale, A., Berardi, N., Viegi, A., Maffei, L., Cenni, M. C. Retinal functional development is sensitive to environmental enrichment: a role for BDNF. *FASEB J.* 21, 130–139 (2007)**

Key Words: retina · visual acuity · pattern electroretinogram · brain derived neurotrophic factor

IT IS COMMONLY ASSUMED that retinal development is independent from sensory inputs, leading to the notion that retina is less plastic than cortex or hippocampus, the very site of experience-dependent plasticity. For instance, visual deprivations, such as monocular deprivation or dark rearing (DR), known to dramatically affect visual cortical acuity, are virtually ineffective on retinal acuity in cat, rat, and humans (1, 2, 3). However, it has been shown that DR alters inner retina development in mice (4, 5, 6), preventing the segregation of the ON and OFF pathways, both at electrophysiological and anatomical level.

We have recently found that environmental enrichment (EE) affects visual cortical development and plasticity (7) and prevents DR effects (8). We considered that EE, which so powerfully affects visual cortical development, was a paradigm suitable to probe the actual sensitivity of retinal development to experience and to gain insight on the factors involved.

To understand whether retinal functional development is a target of EE and to compare EE effects on cortical and retinal development, we have monitored the development of cortical responses with visual evoked potentials (VEPs) and retinal responses using pattern electroretinogram (P-ERG). P-ERG is a sensitive measure of the function of retinal ganglion cells (RGCs), the very output of retinal circuitry (9). Our results show that both retinal acuity and cortical acuity development are sensitive to EE on the same time scale. In particular, retinal acuity development is strongly accelerated in rats enriched from birth (EE rats) with respect to non-enriched (non-EE) rats, and this accelerated retinal development in EE animals is found also in rats exposed to EE only for the first 10 days of life, that is, before eye opening.

In the adult, EE is known to affect the expression of many factors, both peripherally and in the central nervous system (CNS), which could mediate its well-known effects on neural plasticity (10, 11, 12). Which factors are affected by EE during development are less known. We have recently found that EE increases the expression of brain derived neurotrophic factor (BDNF) in the developing visual cortex (7). BDNF is an important factor in retinal development, controlling the development of subtypes of amacrine cells (13, 14, 15) and regulating ganglion cell morphological maturation, both at the level of the dendrites and of the axonal terminal (16). We have investigated BDNF's possible involvement in retinal plasticity in response to EE. Our results show that BDNF protein is precociously increased in the RGC layer of developing EE rats. Blocking BDNF expression in the retina during this window of enhanced expression by means of antisense oligonucleotides counteracts the precocious development of retinal acuity in EE animals.

Our data show that EE accelerates the maturation of RGC functional properties and that this effect requires retinal BDNF action.

MATERIALS AND METHODS

Subjects and mating protocol

All experiments followed approved national and institutional guidelines for animal use.

[1] Correspondence: Scuola Normale Superiore c/o Istituto di Neuroscienze del CNR, Via G. Moruzzi, 1, 56100 Pisa, Italy. E-mail: landi@in.cnr.it

doi: 10.1096/fj.06-6083com

0892-6638/07/0021-0130 © FASEB

Long-Evans hooded rats lived in an animal house with a temperature of 21°C, 12:12-h light-dark cycle, and food and water were available *ad libitum*. Female subjects were put with males (one male for every mating cage) in standard cages for reproduction (60×40×20 cm). At least 7 days before delivery, pregnant females were transferred to an enriched or standard cage; with this procedure, both enriched and standard females received equivalent levels of stress deriving from cage transfer during pregnancy. No difference in gestation time was detected in the two experimental conditions (7).

EE consisted of a large wire mesh cage (at least 60×50×80 cm) with two to three floors containing several foodhoppers, two running wheels (one bigger for adults, the other for postweaning pups), and differently shaped objects (tunnels, shelters, stairs) that were completely substituted with others once a week. At least two or three pregnant mothers were put into the enriched cage with four or five filler females. Cages for standard environment (non-EE) were standard laboratory cages (30×40×20 cm) housing one dam with her pups as established by the Italian law for the care of laboratory animals. After birth, all of the litters were housed with their mother until postnatal day 45.

Eye-opening observations

From postnatal day 8, pups (n=45 pups for EE, n=33 pups for non-EE) were inspected for eye-opening twice a day at ~8 AM and 7 PM. Eye-opening was defined as the initial break in the membrane sealing the lids of both eyes. The eyes of all of the animals in this study were clear and without obvious optical anomalies. Optics were checked with an ophthalmoscope and were completely transparent from P19, age of beginning of our electrophysiological recordings.

In particular, we checked optics in rats with forced eye opening, finding it clear in all of them. That the application of this experimental paradigm did not produce a retinal damage in our animals is further supported by the fact that retinal acuity in rats with forced eye opening is the same as that of standard animals at the same age.

Electrophysiological assessment of retinal and cortical acuity

A total of 148 rats [animals non-enriched (non-EE): n = 63; enriched (EE): n = 57; rats with a forced eye-opening at P10 (EO-P10): n = 5; enriched and dark reared between P10 and P14 (EE(DR_P10–P14)): n = 5; enriched until P10 (EEuntilP10): n = 6; enriched treated with BDNF antisense oligonucleotides (EE-AS): n = 7; enriched treated with BDNF sense (EE-S): n = 5] was used for electrophysiology. Rats were anesthetized with an intraperitoneal (i.p.) injection of 20% urethane (0.7 ml/hg; Sigma, St. Louis, MO) and mounted on a stereotaxic apparatus allowing full viewing of the visual stimulus. Additional doses of urethane (0.03–0.05 ml/hg) were used to keep anesthesia level stable throughout the experiment.

During electrophysiology, the body temperature of rats was monitored with a rectal probe and maintained at 37.0°C with a heating pad. Visual stimuli were horizontal sinusoidal gratings of different spatial frequency and contrast generated by a VSG2/2 card (Cambridge Research System, Cheshire, UK) and presented on a computer display (mean luminance=10 candles/m^2; area, 24×26 cm) placed 20 cm in front of the animal. To analyze the contribution of the different phases of EE to the development of retinal acuity, one enriched mother with its offspring born in EE was transferred at P10 in a standard environment.

Recordings were always made in blind in relation to the animal's rearing condition to avoid subjective judgments of the experimenter.

Pattern electroretinogram

Pattern electroretinogram (P-ERG) was recorded as done previously (17, 18). The stereotaxic apparatus was oriented with an angle of ~40° as respect to the position of the screen; P-ERG electrodes were small silver rings positioned on the corneal surface by means of a microelectrode drive, so as to avoid occlusion of the pupil. Visual stimuli were sinusoidal gratings alternated in phase with a fixed temporal frequency of 4 Hz. Steady-state recorded signals were filtered (0.1–100 Hz) and amplified in a conventional manner, computer averaged, and analyzed; 15 packets of 20 sums each (300 events) were averaged for each stimulus spatial frequency, changing randomly the spatial frequency from one record to another. For each spatial frequency, the amplitude of the P-ERG signal was taken as the amplitude of the second harmonic in the averaged signal, calculated by a fast Fourier transform; the P-ERG amplitude decreases with increasing spatial frequency (17, 19). The noise level was estimated by measuring the amplitude of the second harmonic in records where the stimulus was a blank field. Retinal acuity was taken as the highest spatial frequency still evoking a response above noise level.

Visual evoked potentials

VEPs were recorded as done previously (20). Briefly, a large portion of the skull overlying the binocular visual cortex was drilled and removed taking away the dura. A glass micropipette (2–2.5 MΩ) was inserted into the binocular primary visual cortex (Oc1B; 21) in correspondence of the vertical meridian representation. Electrical signals were amplified, bandpass filtered (0.1–120 Hz), and averaged (at least 60 events in blocks of 10 events each) in synchrony with the stimulus contrast reversal. Transient VEPs in response to abrupt contrast reversal (0.5–1 Hz) were evaluated in the time domain by measuring the peak-to-baseline amplitude and peak latency of the major component. VEPs in response to a blank field were also frequently recorded to have an estimate of the noise. For each animal, VEP amplitude was plotted as a function of log spatial frequency, and visual acuity was determined by linearly extrapolating VEP amplitude to 0 V.

Retinal BDNF expression in EE vs. non-EE rats

BDNF immunohistochemistry

Four animals were used for each experimental group (P7, P10, P15) and the retinas were fixed in 4% paraformaldehyde in 0.1 PBS (pH=7.4) and cryoprotected in 30% sucrose. For BDNF coronal retinal sections (25 μm thick) were cut using a cryostat and then processed as follows. After a blocking step, sections were permeabilized in 0.01% Triton X-100 and incubated in 1:400 anti-human BDNF antibody (Ab) (Promega). Bound Ab was detected by incubating sections with byotinylated rabbit anti-chicken IgG (1:200, Promega) followed by fluorescein-conjugated extravidin (1:300, Sigma). Images of RGC portions were acquired at 20× magnification using a Zeiss HR Axiocam video camera connected to a Zeiss Axiophot microscope and digitized by Axiovision software. To compare different specimens, the time of exposure was optimized at the start and then held constant throughout image acquisition. Then, the collected images of the retina were imported to the image analysis system MetaMorph and used to evaluate pixel intensity of cellular immunofluores-

cence. All image analyses were done blind. The profile of cells into RGC layer was outlined, and the pixel intensity was measured within this area. BDNF immunoreactivity levels were calculated as the ratio between the pixel intensity of RGC profiles and the background level, measured in the outer nuclear layer (ONL). Values obtained from at least 8–10 retinal fields were used to calculate the average pixel intensity value per animal.

BDNF ELISA

We have attempted to measure BDNF protein content in the retina of EE and non-EE rats at P10, when a difference was evident from the immunohistochemical data. BDNF protein levels were assessed by means of ELISA (Promega, BDNF E_{max} Immunoassay System). We choose the ELISA technique in view of its high sensitivity, as it is known that endogenous BDNF amounts are around the range of few picograms in the postnatal rodent retina (22–24). Indeed, BDNF is produced only by cells in the ganglion cell layer, primarily by retinal ganglion cells (25, 26) and as such, the BDNF protein revealed by immunohistochemistry is diluted in the process of protein extraction, performed on the whole fresh retinas. We collected $n = 128$ retinas for non-EE animals and $n = 134$ retinas for EE animals and pooled them in five different samples for non-EE and for EE animals, respectively ($\approx$ 26–27 retinas per sample).

We performed protein extraction according to Promega protocol. Unfortunately, at this developmental age, BDNF retinal quantities resulted outside the reliable range of BDNF ELISA Promega kit.

Intraocular injections of oligonucleotides

For the study of the role of the neurotrophin BDNF on the maturation of retinal acuity in EE animals, EE rats received intraocular injections of BDNF antisense or sense phosphorothioate oligonucleotides (Eurogentec, 500 μM; injection vol: at P6, 500 nl and at P9 1,000 nl, according the size of the eye camera; estimated oligo concentration in the eye: 25 μM, (27)).

Antisense oligonucleotides have been previously used in various tissues, including retinal tissue, as therapeutic agents, and as research tools for down-regulating certain genes (28–31). Antisense oligonucleotides can be successfully introduced into the retina intravitreally (28, 32, 30); they appear to penetrate in the entire thickness of the retina and, in particular, in RGC layer and are found intracellularly in retinal ganglion cells (32, 30). Moreover, recent findings from our laboratory (27, 23) have demonstrated that intraocular injection of BDNF antisense oligos reduces retinal expression of BDNF in P9 and P26-P27 rats.

Injections were performed under ether anesthesia at P6 and P9, that is, in correspondence with the period of enhanced BDNF protein levels in EE animals (see Fig. 4).

Intraocular injections of BDNF sense and antisense oligos were performed using a glass micropipette inserted at the ora serrata connected to an Hamilton syringe. Sequences of the BDNF antisense—to reduce BDNF mRNA translation—and sense oligonucleotides (targeted to the BDNF translation initiation codon) were 5′-CATCACTCTTCTCACCTGGTGGAAC-3′ and 5′-GTTCCACCAGGTGAGAAGAGTGATG-3′, which correspond to nucleotides 51–75 of the BDNF mRNA and are the same used by (27) and (23) to effectively reduce BDNF levels in the developing rat retina.

Fully phosphorothioate oligonucleotides were dissolved in saline with stock solutions of 1 mM. Stock solutions were preserved at −80°C and diluted in saline at the desired concentration at the time of the injection. To document whether intraocular oligo injections decreased BDNF expression, we adopted the same strategy used by (27), performing immunohistochemistry on retinas from untreated (n=3), sense (n=3) and antisense (n=4) injected rats 48 h after the last injection.

P-ERG recordings were made at P25 and P26, and retinal acuity was determined for each oligo-injected EE animal, as described previously. Seven EE rats were intraocularly injected with antisense oligos in one eye, while the other eye was left untreated. In these animals, P-ERG recordings from both eyes were made at P25-P26 in order to compare in the same EE animal the retinal acuity of the normal eye and of the eye with decreased BDNF levels. Five EE rats were intraocularly injected with sense oligos and P-ERG recorded from that eye at P25-P26 allow us to control for the aspecific effects of oligo injections.

RESULTS

Development of retinal acuity is accelerated by EE

In observations made at P16, we have found that the disappearance of opacities in the eye optics was complete in EE rats but not in non-EE rats (data not shown). To avoid confounding effects due to this problem, we have begun a systematic study of P-ERG responses starting from P19–P20 (**Fig. 1**).

Retinal acuity in adult rats is 0.87 ± 0.02 cycles per degree (c/deg) (18). At P19–20, retinal acuity is around 0.49 c/deg in non-EE rats and rapidly increases with age; the adult value is reached at P44–45: at this age, P-ERG acuity is 0.84 ± 0.02 c/deg and does not differ from the value obtained in P60 rats, (0.83±0.03 c/deg, n=4).

In EE animals, P-ERG acuity at P19–20 does not differ from that in non-EE animals; however, as evident in Fig. 1*C*, EE clearly accelerates P-ERG acuity developmental time course. Starting from P25–26 up to P34–35, retinal acuity is significantly higher in EE than in non-EE rats and becomes no longer significant from the final adult value in EE rats at P34–35 (P60 EE rats, 0.89±0.02 c/deg, n=5). Thus, the final acuity level is reached almost 10 days before in EE than in non-EE rats. From P44–45 onward P-ERG acuity does not differ between EE rats and non-EE rats, indicating that EE affects the developmental time course of retinal acuity but not its final level (two-way ANOVA, housing per age, $P < 0.001$, post hoc Tukey's test). The acceleration in the development of retinal acuity produced by EE is particularly evident, normalizing mean retinal acuity for each age group to the respective mean final value (Fig. 1*D*).

Thus, retinal development is sensitive to the experience provided by an enriched environment.

We then performed an analysis of cortical acuity development in EE and non-EE rats by means of visual evoked potentials (VEPs). We found that environmental enrichment strongly accelerates cortical development, with VEP acuity in EE rats overtaking that of

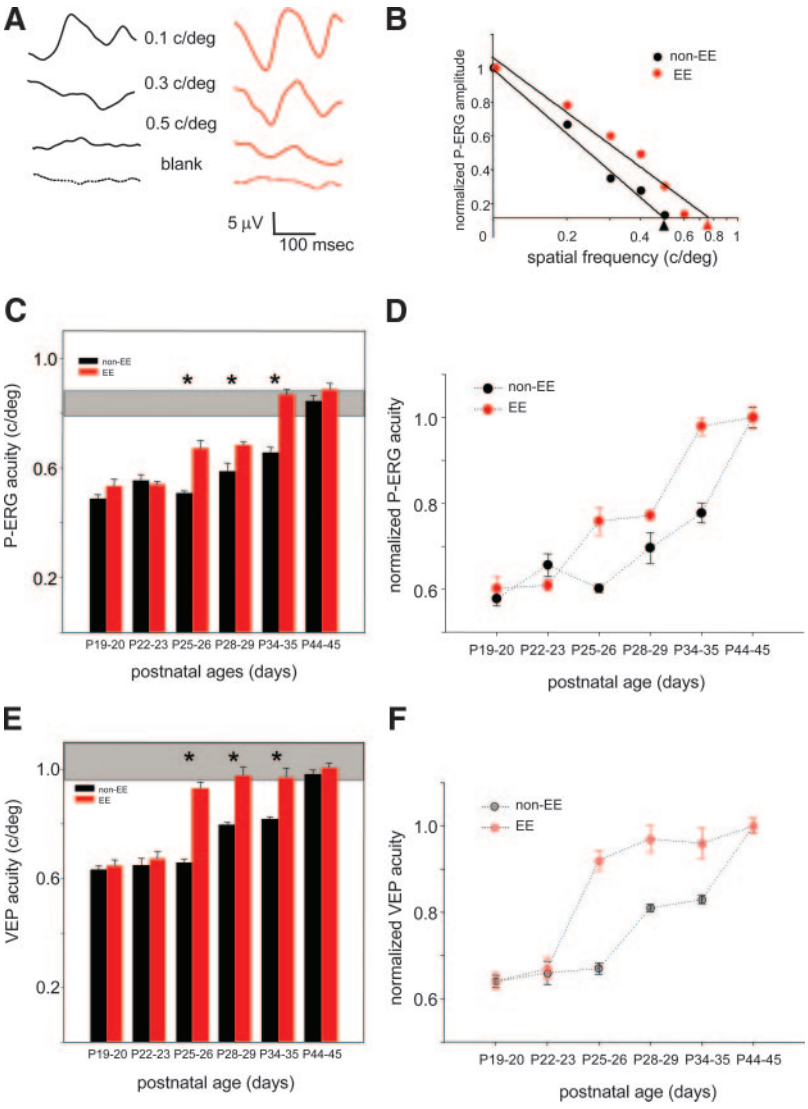

Figure 1. Development of retinal responses is sensitive to environmental enrichment. *A)* Examples of steady-state pattern electroretinogram (P-ERG) signals recorded at P25 in response to visual stimulation with gratings of three different spatial frequencies in one non-EE (black traces) and one EE (red traces) rat. The gratings were sinusoidally modulated at a temporal frequency of 4 Hz (period 250 ms), and the principal component of the P-ERG response is on a temporal frequency twice the temporal frequency of the stimulus (second harmonic, two peaks and two troughs of the P-ERG response within one stimulus cycle). P-ERG recorded in response to a blank field is reported to show the noise level. It is evident that a response to a pattern of 0.5 cycles per degree (c/deg) is still present in the EE but not in the non-EE rat. *B)* Examples of retinal acuity estimate for one EE and one non-EE rat at P25. Acuity (arrow) estimated by steady-state P-ERG was calculated by extrapolating the linear regression through normalized data to noise level. *C)* P-ERG assessment of retinal acuity in non-EE (black) and EE (red) rats during postnatal development. Acuity of animals is plotted as groups of age. The bars indicate SEM. Shaded rectangle indicates the range of retinal acuity in non-EE adult (P60) rats. Two-Way ANOVA shows a significant effect of both age and environmental housing condition ($P<0.001$ in both cases) and a significant interaction between age and environmental housing condition ($P<0.001$). *Post hoc* Tukey test reveals a significant difference (*) from P25 to P34–35 between EE and non-EE groups ($P<0.05$). [non-EE rats, P19–P20: $n=5$, P22–P23: $n=5$, P25–P26: $n=5$, P28–P29: $n=5$, P34–P35 $n=5$, P44–P45: $n=5$; EE rats, P19–P20 $n=6$, P22–P23 $n=5$, P25–P26: $n=5$, P28–P29: $n=5$, P34–P35: $n=5$, P44–P45: $n=5$.] *D)* P-ERG acuity normalized to the acuity value at P44–P45 is plotted as a function of age for each experimental group, to show the leftward shift of the curve for EE animals, which illustrates the acceleration of visual acuity development produced by EE. *E)* Environmental enrichment influences development of cortical acuity. Visual evoked potentials (VEP) assessment of visual acuity in non-EE (dark gray) and EE (light red) rats during postnatal development. Cortical acuity of animals is plotted as groups of age. The bars indicate SEM. Shaded rectangle indicates the range of cortical acuity in non-EE adult (P60) rats. Two-way ANOVA shows a significant effect of both age and environmental housing condition ($P<0.001$ in both cases) and a significant interaction between age and environmental housing condition ($P<0.05$). *Post hoc* Tukey test reveals a significant difference (*) from P25 to P34–P35 between non-EE and EE groups ($P<0.05$) [non-EE rats, P19–P20: $n=4$, P22–P23: $n=4$, P25–P26: $n=5$, P28–P29: $n=5$, P34–P35: $n=5$, P44–P45: $n=5$; EE rats, P19–P20: $n=4$, P22–P23: $n=4$, P25–P26: $n=5$, P28–P29: $n=5$, P34–P35: $n=5$, P44–P45: $n=5$]. *F)* VEP acuity normalized to the acuity value at P44–P45 is plotted as a function of age for each experimental group to show the leftward shift of the curve for EE animals.

non-EE rats at P25-P26, as for P-ERG acuity development (Fig. 1, *E* and F).

Accelerated retinal acuity development in EE animals is induced during early phases of enrichment, and precocious eye opening is not responsible

EE rats open their eyes two days before non-EE rats (**Fig. 2*A***). We asked whether the precocious eye opening observed in EE rats gives a contribution to the accelerated retinal development (Fig. 2). To answer this question, we made two different experiments.

In the first one, we made a forced eye opening at P10 in non-EE rats (eye opening at P10, EO-P10) and recorded retinal acuity at P25-P26; we found that retinal acuity is not affected by this procedure (Fig. 2*C*; non-EE: 0.51±0.01 c/deg; EO-P10: 0.56±0.02 c/deg, difference not significant). Thus, a precocious visual experience is not sufficient to mimic the increase in acuity seen in EE animals at P25-P26.

In the second experiment, we kept animals enriched from birth in a dark room from P10 to P14 [EE-(DR_P10–14) rats] in order to abolish their advantage in visual experience due to the precocious eye opening;

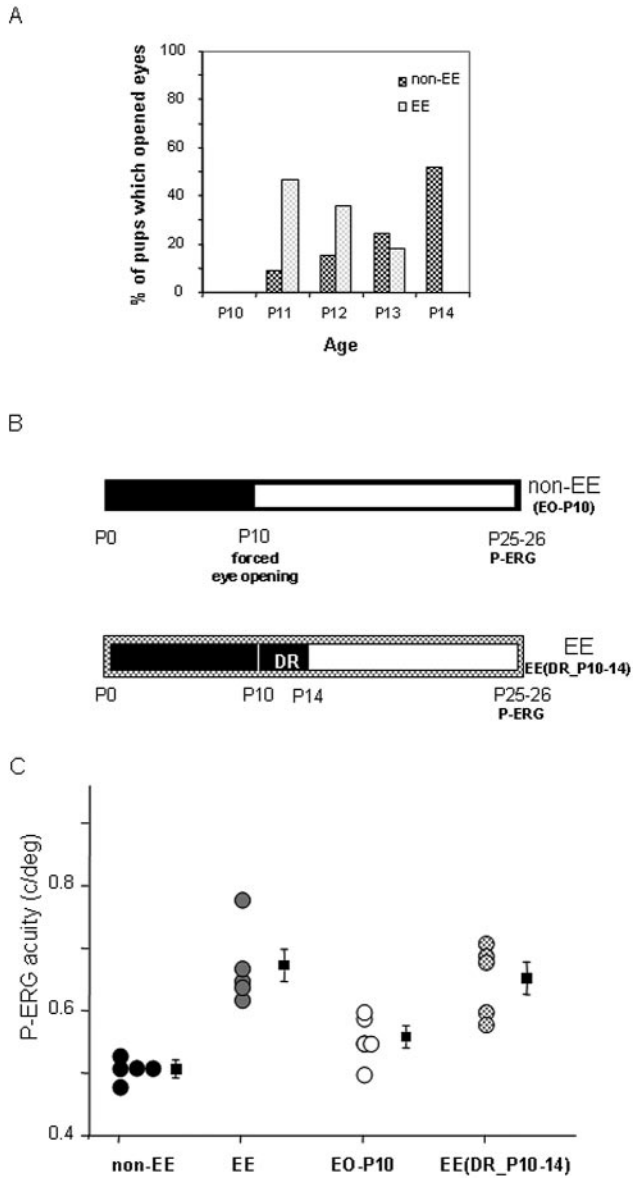

Figure 2. Precocious eye opening is not responsible for the effects of EE. *A)* Precocious eye opening is observed in EE rats, as shown by the percentage of EE and non-EE pups that opened their eyes at the age indicated on the abscissa. There is a clear difference between the two groups (Mann–Whitney rank sum test; $P<0.001$), with the median age at eye opening being P12 for EE and P14 for non-EE rats. *B)* Schematic protocol of the two experiments performed to evaluate the role of precocious eye opening in the accelerated retinal acuity development found in EE rats. *C)* Retinal acuity at P25–P26 for EE (same animals as in Fig. 1*C*, *D*), non-EE (same animals as in Fig. 1*C*, *D*), non-EE rats with a forced eye-opening at P10 (EO-P10) and EE animals dark reared from P10 to P14 [EE(DR_P10–14)] in order to abolish the visual experience advantage due to the precocious eye opening in EE. There is a significant difference between non-EE ($n=5$) and EE rats ($n=5$), while EE(DR_P10–14) ($n=5$) do not differ from EE rats and EO-P10 do not differ from non-EE rats [one-way ANOVA, $P<0.001$; post hoc Tukey's test, $P<0.05$].

we have found that at P25-P26, the acuity of this experimental group (0.65±0.03 c/deg) does not differ from that of EE group (Fig. 2*C*). Thus, precocious eye opening is not necessary for the accelerated maturation of retinal acuity produced by an enriched environment.

Our EE animals are enriched from birth until adulthood. To understand whether the effects of EE on retinal development stemmed from events caused by EE at an early or at a late postnatal age, we reared animals in EE until P10 and then transferred them to a standard environment at P10 (EEuntilP10; **Fig. 3*A***). P-ERG recordings at P25-P26 revealed that at this age EEuntilP10 rats had the same retinal acuity of EE animals enriched up to P25-P26 (see Fig. 3*B*; 0.64±0.03 c/deg; one-way ANOVA, $P=0.017$), showing that EE until P10 induces the same retinal functional maturation seen in animals exposed to EE until P25–26.

This suggests that factors influenced by EE and sufficient to trigger rat retinal acuity development are affected during the first 10 days of life.

BDNF role in retinal maturation of EE animals

BDNF is an important factor in RGC development, regulating both their morphological and functional maturation (14). We have investigated whether EE affected BDNF protein expression in the developing

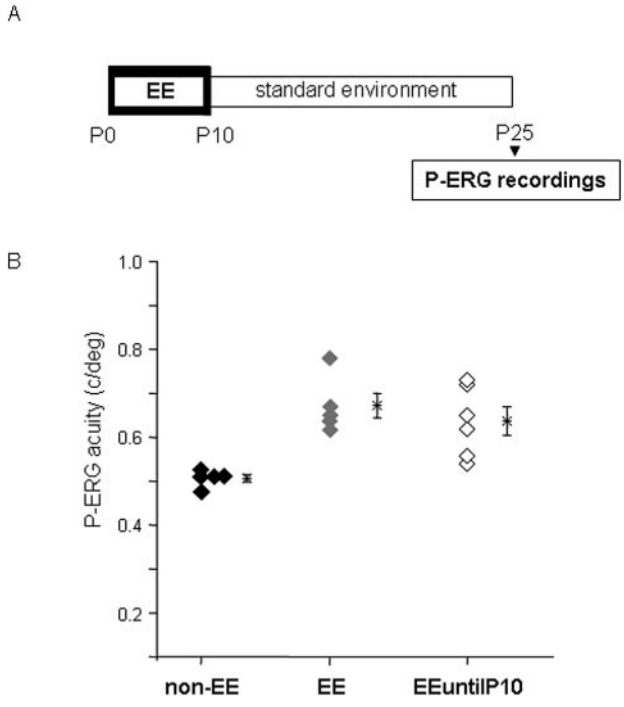

Figure 3. Early phase of EE is sufficient to induce accelerated retinal development. *A)* Experimental protocol. *B)* Retinal acuity values assessed at P25–P26 for animals enriched until P10 (EEuntilP10, $n=6$) and for rats enriched until P25 (EE rats, $n=5$). Visual acuity of EEuntilP10 rats is the same as for EE rats. Retinal acuity of P25–P26 non-EE rats is reported for comparison (same data as in Fig. 1). Retinal acuity in EEuntilP10 rats does not differ from that in EE rats (one-way ANOVA, $P<0.05$); both EE and EEuntilP10 rats differ from non-EE rats ($n=5$, one-way ANOVA, $P=0.002$), suggesting that ten days of enrichment are sufficient to induce EE effects on retinal functional development.

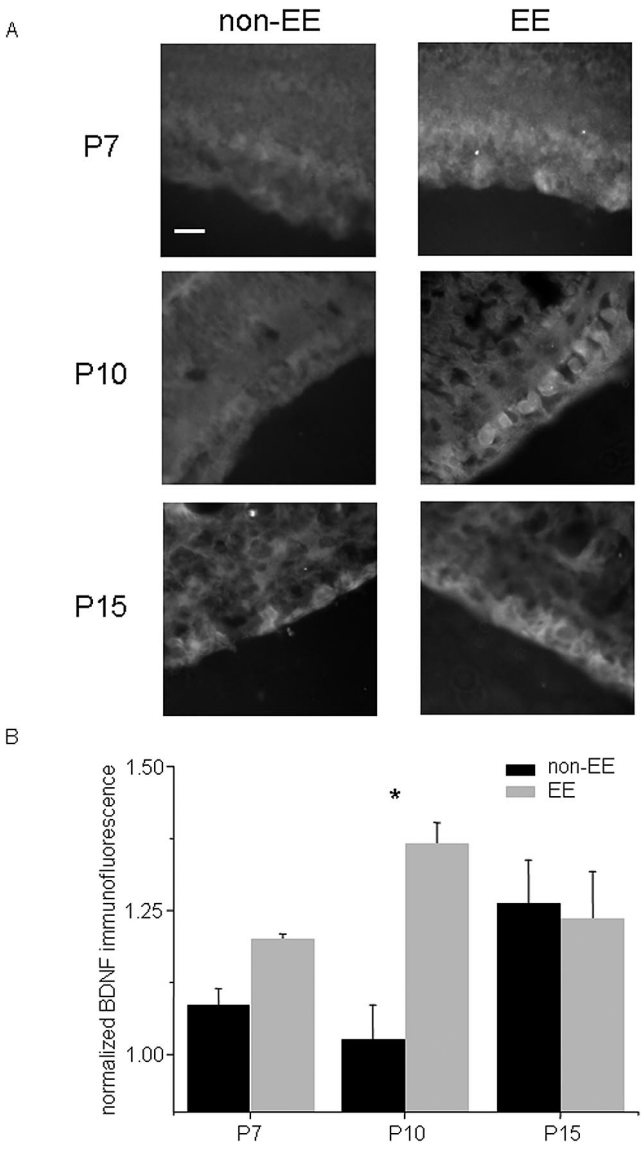

Figure 4. BDNF is precociously expressed in the RGCs of EE rats. *A)* Micrographs of EE and non-EE retinal sections immunostained for BDNF at different ages. BDNF immunolabeled cells are detectable at the level of RGC layer starting from P7 in EE but not in non-EE retinas. Scale bar is 20 μm. *B)* Quantitative analysis of BDNF immunofluorescence intensity normalized to background level in the RGC layer of non-EE (black) and EE rats (gray); the two groups differ significantly at P10 ($P<0.001$), while at P15 BDNF immunofluorescence is equal in the two groups ($P=0.73$) and at P7 the difference does not reach the significance level ($P=0.08$) (two-way ANOVA age per housing, post hoc Tukey's test).

retina, focusing on the ganglion cell layer. In normal rats, BDNF protein level is very low before P14 (26, **Fig. 4***A*); in EE animals, BDNF immunoreactivity is detectable in the RGC layer already at P7, and at P10, it is significantly higher than in non-EE rats (two-way ANOVA, housing per age, post hoc Tukey's test, Fig. 4*B*). Thus, EE causes a precocious BDNF development in the RGC layer.

To investigate whether the precocious expression of BDNF in EE animals is necessary for the accelerated maturation of retinal acuity caused by EE, we reduced BDNF levels in EE animals during the time window of its enhanced expression by intraocularly injecting BDNF antisense oligonucleotides having the same sequence as that previously employed to reduce BDNF protein levels in the developing rat retina (27, 23).

We first tested whether BDNF antisense oligos reduce BDNF expression in the retina of EE rats by immunohistochemistry. EE rats were intraocularly injected with BDNF antisense (EE-AS, $n=4$) or sense oligos (EE-S, $n=3$) at P6 and P9, and 48 h after the last injection the eyes were removed and processed for BDNF immunohistochemistry. The retinas from 3 untreated P11 EE rats were used as controls. We found, as previously shown with the same oligo sequence by (27) and (23) that BDNF antisense oligos decrease BDNF expression with respect to controls (**Fig. 5***A*), whereas the sense oligos do not (one-way ANOVA, $P=0.001$).

We then treated a group of EE animals by means of intraocular injections of BDNF antisense oligonucleotides (EE-AS, $n=7$) and another group by means of BDNF sense oligonucleotides as controls (EE-S, $n=5$) at P6 and P9 and recorded P-ERG at P25–26. We found (Fig. 5*B*) that retinal acuity in EE-S rats did not differ from that of EE rats (0.63 ± 0.01 c/deg), whereas acuity in EE-AS animals was significantly lower than in EE rats and did not differ from that of non-EE rats of the same age (0.49 ± 0.01 c/deg; one-way ANOVA, $P=0.001$). It is important to underline that we were able to make an internal comparison in the development of the retinal acuity in six of the EE-AS rats, in which one eye was treated with BDNF antisense oligos and the other was left untreated. In these animals (Fig. 5*C*), we have recorded visual acuity for both eyes, the antisense treated and the untreated; in all cases visual acuity in the treated eye was lower than in the untreated eye (EE-AS rat 1: 0.53 *vs.* 0.64 c/deg; EE-AS rat 2: 0.5 c/deg *vs.* 0.73 c/deg; EE-AS rat 3: 0.48 *vs.* 0.65 c/deg; EE-AS rat 4: 0.54 *vs.* 0.64 c/deg; EE-AS rat 5: 0.52 *vs.* 0.67 c/deg; EE-AS rat 6: 0.44 *vs.* 0.63 c/deg), and there was a statistically significant difference between the two groups of retinal acuity values (paired *t* test, $P=0.001$).

Thus, the reduction of retinal BDNF blocks the effects of EE on the RGC functional maturation analyzed by means of P-ERG.

DISCUSSION

In this study, we have investigated the effects of EE on the development of retinal physiological responses. Our results show that retinal functional development is sensitive to the experience provided by the environment.

The acceleration in visual acuity determined by enrichment is not dependent on vision since EE is effective in causing it before P10 (that is before eye opening) and forced eye opening at P10 is not. This is in line with previous findings that show that it is possible to modulate the outcome of visual deprivations by varying

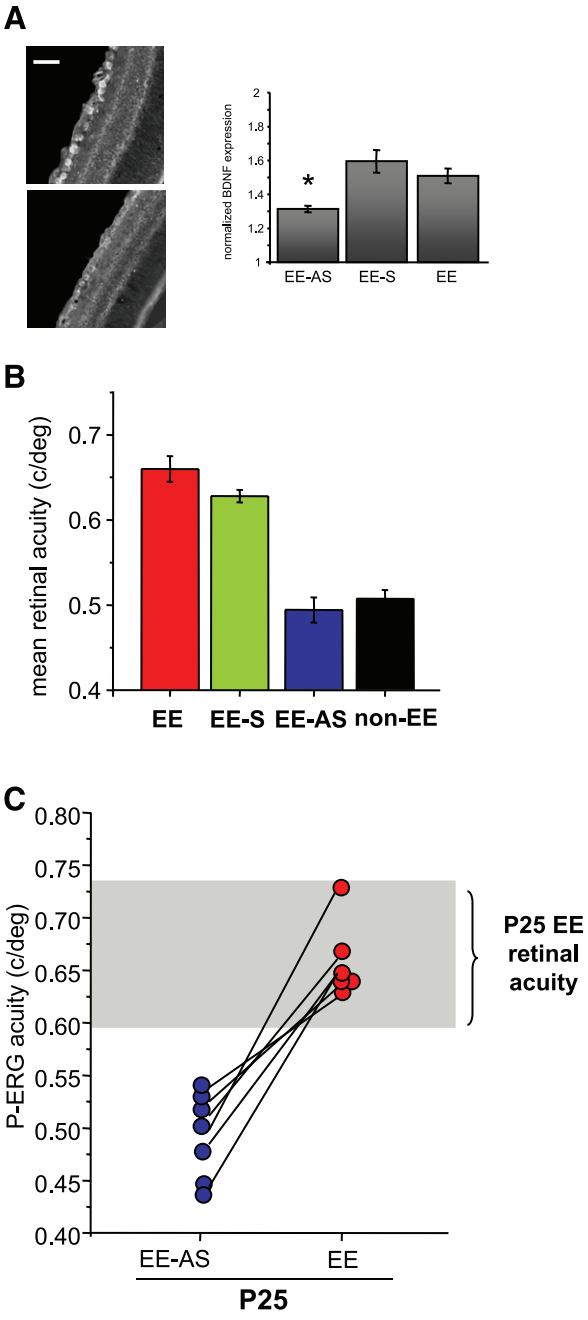

Figure 5. BDNF is necessary for EE effects on retinal functional development. Injections of BDNF antisense oligonucleotides block the accelerated maturation of retinal acuity seen in EE animals. *A*) left: Examples of BDNF staining in the retina of a sense-treated eye (top) and of an antisense-treated eye (bottom) in EE P11 rats. Injections made at P6 and P9. Scale bar is 50 μm. *A*) right: Quantitative analysis of BDNF immunofluorescence normalized to background level in RGC layer of EE P11 rats treated with antisense (EE-AS, $n=4$), sense (EE-S, $n=3$), and left untreated (EE, $n=3$). BDNF immunofluorescence in EE-AS rats is significantly lower than in EE-S and EE rats; the latter two groups do not differ (one-way ANOVA, $P<0.003$, *post hoc* Tukey's test). *B)* Mean retinal acuity in EE, EE treated intraocularly with BDNF sense oligo (EE-S), EE treated with antisense oligo (EE-AS), and non-EE rats. Animals for EE and non-EE groups as in Fig. 1*C, D*. Retinal acuity of EE-AS rats differs from that of EE animals but not from that of non-EE animals, while retinal acuity in EE-S rats differs from that of non-EE and EE-AS rats but not from that of EE rats (one-way ANOVA, $P<0.001$, *post hoc* Tukey's test, $P<0.05$). *C)* In six EE animals treated with BDNF antisense oligos in one eye and left untreated in the other eye, retinal acuity was determined at P25 for both eyes; for each animal, the retinal acuity of the treated and of the untreated eye are reported in figure, joined by a dotted line. The acuity of the BDNF antisense-treated eye is significantly lower than that for the fellow eye (paired *t* test, $P<0.001$).

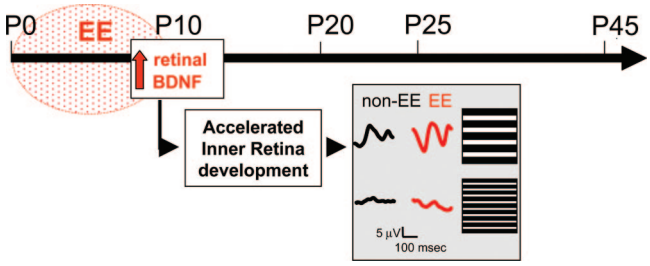

Figure 6. Proposed model of our experimental results. EE (dotted oval) increases retinal BDNF at an early postnatal age (around P10); the increase in BDNF triggers an accelerated inner retina development, reflected by the accelerated retinal acuity development assessed by pattern ERG. Waveform in the inset represents the P-ERG in response to gratings of low (upper row) and high (bottom row) spatial frequency in a P25 enriched (EE) and nonenriched (non-EE) rat.

the environmental conditions (8) or the availability of BDNF to cortical neurons (33). These data suggest that developmental factors that are not under visual experience control may contribute to visual system development.

There are several molecules important for nervous system development and plasticity that are modified by EE in adult animals (11, 34). The results that 10 days of enrichment are sufficient to induce an accelerated retinal functional maturation equal to that observed after a period of enrichment prolonged until P45 suggest that EE acts on molecular factors, the expression of which is influenced precociously, when pups are still immobile and dependent on the mother.

In the work of Cancedda and co-authors (7), we have found that BDNF protein level is enhanced precociously (P7) in the visual cortex of enriched mice. Our results on BDNF development in the RGC layer show that the expression of this neurotrophin is precociously affected by EE also in the retina, suggesting that BDNF could be one of the molecular factors that EE acts upon to prime P-ERG acuity development.

Even if the increase of BDNF has been always associated with physical exercise (11), the study of Liu (35) shows that high levels of maternal care enhance BDNF mRNA expression in rat hippocampus. We have recently shown that different levels of maternal care are found in EE (36). Thus, enhanced levels of maternal care induced by EE could justify our results for BDNF expression in the retina.

The results obtained by injecting BDNF antisense oligos in the eye of EE rats clearly show that BDNF is a key molecule in the retinal functional development

driven by EE since its reduction in the retina during the time window of its enhanced expression blocks the retinal functional development seen in EE animals.

Despite the fact that a clear role for BDNF has emerged in the developmental regulation of RGC dendritic and axonal arborization *in vivo* (37, 38) and in the preservation of retinal function in models of retinal degeneration (39), we have provided for the first time a direct demonstration of BDNF involvement in the functional development of retinal circuitry *in vivo*.

In the vertebrate retina, several studies have shown that TrkB is expressed primarily by amacrine cells and retinal ganglion cells (40) and in green-red sensitive cones (41); therefore, a large number of retinal cells is potentially responsive to BDNF. Indeed, it is known that initial expression of TrkB in rodent retina is in RGCs and in the developing IPL at P0 followed by the expression in the INL, OPL, and Muller cells by P6 and later (42).

BDNF could act directly on RGCs; in particular, BDNF might play a role in the activation of functional NMDA receptors (43) and in the developmental expression of specific ionic channels necessary for repetitive firing (44) in early retinal ganglion cell development. Many findings have demonstrated that BDNF shapes RGC dendritic morphology (45, 46, 47, 48). Interestingly, it has been shown that locally released BDNF can have an autocrine/paracrine action on RGCs, promoting the retraction of RGC dendritic arborization in *Xenopus* tadpoles (49). However, it is not clear whether there is a correspondence between the development of dendritic arborization and the maturation of receptive field size and consequently of retinal acuity. In turtle, it has been observed that experimental manipulations that modify spontaneous activity *in vivo* not only affect receptive field properties but also alter the dendritic arborizations of RGCs (50). However, an old work by Rusoff and Dubin (51) in kittens reported that there is no correlation between the dendritic field and the receptive field development in RGCs; indeed, receptive fields of RGCs in kittens are larger than in adults but RGC dendritic arborizations are smaller, suggesting that the progressive decrease in receptive field size is paralleled by an increase in RGC dendritic field with development. However, although the contribution of the shaping of RGC dendritic arborizations to the maturation of their receptive fields needs further elucidation, we do not exclude the possibility that a remodeling action of retinal BDNF on RGC dendrites may contribute to the P-ERG acuity maturation. Indeed, the increased levels of retinal BDNF may guide the maturation of RGC receptive fields by a selective pruning of RGC dendritic arborizations. This dendritic structural elaboration within areas where presynaptic terminals are located may reorganize the spatial distribution of synapses, contributing to the maturation of the center-surround ganglion cell organization.

Moreover, it is known that the spatial tuning of RGCs depends not only on processes in the outer retina but also on processes performed in the inner retina by amacrine cells and, in particular, by GABAergic amacrine cells (52, 53, 54). GABAergic amacrine cells are a heterogeneous population, colocalizing other neuroactive substances, such as peptides or classical neurotransmitters (55, 56, 57, 58). Dopaminergic amacrine cells, which also express GABA (59, 56) and form both dopaminergic and GABAergic synapses onto other amacrine cells (57), are responsive to BDNF. They express TrkB (40), and their development is accelerated by BDNF intraocular injections from P8 to P14 (13). Dopaminergic amacrine cells have been shown to contribute to the spatial organization of the receptive fields of RGCs (59, 60). In addition, it has been suggested that retinal dopamine level affects visual acuity development: children with phenylketonuria who experienced very high phenylalanine levels in the first postnatal days and who should therefore have particularly low levels of dopamine in the retina, have lower than normal visual acuity (61). It is conceivable that an accelerated development of amacrine dopaminergic cells promoted by the higher retinal BDNF levels in enriched animals could contribute to the accelerated retinal acuity development observed in EE rats.

Other subtypes of amacrine cells also respond to BDNF during development as documented by changes in the expression of neuropeptides colocalizing with GABA (14, 15). Thus, BDNF could contribute to the accelerated retinal acuity development found in EE animals also through an action of these amacrine cells. It has to be reminded, however, that different amacrine subpopulations might perform different functions in shaping the RGC-receptive fields, not all related to visual acuity. For instance, neuropeptide Y-expressing amacrine cells seem to contribute to low spatial frequency detection (62).

In conclusion, our results are the first evidence that an increased stimulation, such as that provided by EE, can affect the development of retinal visual responses accelerating the maturation of its functional properties by means of factors precociously activated in the retina and that the effects of EE require retinal BDNF action. FJ

We thank C. Orsini for helpful technical assistance. This work was supported by MIUR COFIN to L.M., Finanziamento Ricerce de Base (FIRB) grant Ricerce di Base Neuroscienze (RBNE) 01RZH4_002, Fondo Integratio Speciale Ricerce (FISR) project/Neurobiotechnology3/2.

REFERENCES

1. Baro, J.A., Lehmkuhle, S., and Kratz, K. E. (1990) Electroretinograms and visual evoked potentials in long-term monocularly deprived cats. *Invest. Ophthalmol. Vis. Sci.* **31,** 1405–1409
2. Fagiolini, M., Pizzorusso, T., Berardi, N., Domenici, L., and Maffei, L. (1994) Functional postnatal development of the rat primary visual cortex and the role of visual experience: dark rearing and monocular deprivation. *Vision Res.* **34,** 709–720
3. Fine, I., Wade, A. R., Brewer, A. A., May, M. G., Goodman, D. F., Boynton, G. M., Wandell, B. A., and MacLeod, D. I. (2003) Long-term deprivation affects visual perception and cortex. *Nat. Neurosci.* **6,** 915–916

4. Tian, N., Copenhagen, D. R. (2001) Visual deprivation alters development of synaptic function in inner retina after eye opening. *Neuron* **32,** 439–449
5. Tian, N., and Copenhagen, D. R. (2003) Visual stimulation is required for refinement of ON and OFF pathways in postnatal retina. *Neuron* **39,** 85–96
6. Vistamehr, S., and Tian, N. (2004) Light deprivation suppresses the light response of inner retina in both young and adult mouse. *Vis. Neurosci.* **21,** 23–37
7. Cancedda, L., Putignano, E., Sale, A., Viegi, A., Berardi, N., and Maffei, L. (2004) Acceleration of visual system development by environmental enrichment. *J. Neurosci.* **24,** 4840–4848
8. Bartoletti, A., Medini, P., Berardi, N., and Maffei, L. (2004) Environmental enrichment prevents effects of dark-rearing in the rat visual cortex. *Nat. Neurosci.* **7,** 215–216
9. Maffei, L., and Fiorentini, A. (1981) Electroretinographic responses to alternating gratings before and after section of the optic nerve. *Science* **211,** 953–955
10. Ickes, B. R., Pham, T. M., Sanders, L. A., Albeck, D. S., Mohammed, A. H., and Granholm, A. C. (2000) Long-term environmental enrichment leads to regional increases in neurotrophin levels in rat brain. *Exp. Neurol.* **164,** 45–52
11. Cotman, C. W., and Berchtold, N. C. (2002) Exercise: a behavioral intervention to enhance brain health and plasticity. *Trends Neurosci.* **25,** 295–301
12. Pham, T., Winblad, B., Granholm, A.C., and Mohammed, A. H. (2002) Environmental influences on brain neurotrophins in rats. *Pharmacol. Biochem. Behav.* **73,** 167–75
13. Cellerino, A., Pinzon-Duarte, G., Carroll, P., and Kohler, K. (1998) Brain-derived neurotrophic factor modulates the development of the dopaminergic network in the rodent retina. *J. Neurosci.* **18,** 3351–3362
14. Cellerino, A., Arango-Gonzalez, B.A., and Kohler, K. (1999) Effects of brain-derived neurotrophic factor on the development of NADPH-diaphorase/nitric oxide synthase-positive amacrine cells in the rodent retina. *Eur. J. Neurosci.* **11,** 2824–2834
15. Cellerino, A., Arango-Gonzalez, B., Pinzon-Duarte, G., and Kohler, K. (2003) Brain-derived neurotrophic factor regulates expression of vasoactive intestinal polypeptide in retinal amacrine cells. *J. Comp. Neurol.* **467,** 97–104
16. Cohen-Cory, S., and Lom, B. (2004) Neurotrophic regulation of retinal ganglion cell synaptic connectivity: from axons and dendrites to synapses. *Int. J. Dev. Biol.* **48,** 947–56
17. Berardi, N., Domenici, L., Gravina, A., and Maffei, L. (1990) Pattern ERG in rats following section of the optic nerve. *Exp. Brain Res.* **79,** 539–546
18. Domenici, L., Gravina, A., Berardi, N., and Maffei, L. (1991) Different effects of intracranial and intraorbital section of the optic nerve on the functional responses of rat retinal ganglion cells. *Exp. Brain Res.* **86,** 579–584
19. Rossi, F.M., Pizzorusso, T., Porciatti, V., Marubio, L.M., Maffei, L., and Changeux, J. P. (2001) Requirement of the nicotinic acetylcholine receptor beta 2 subunit for the anatomical and functional development of the visual system. *Proc. Natl. Acad. Sci.* **98,** 6453–6458
20. Di Cristo, G., Berardi, N., Cancedda, L., Pizzorusso, T., Putignano, E., Ratto, G. M., and Maffei, L. (2001) Requirement of ERK activation for visual cortical plasticity. *Science* **292,** 2337–2340
21. Paxinos, G., and Watson, C. (1986) *The Rat Stereotaxic Brain.* Academic Press, Sidney, Australia.
22. Ma, Y. T., Hsieh, T., Forbes, M. E., Johnson, J. E., and Frost, D. O. (1998) BDNF injected into the superior colliculus reduces developmental retinal ganglion cell death. *J. Neurosci.* **18,** 2097–2107
23. Mandolesi, G., Menna, E., Harauzov, A., von Bartheld, C. S., Caleo, M., and Maffei, L. (2005) A role for retinal brain-derived neurotrophic factor in ocular dominance plasticity. *Curr. Biol.* **15,** 2119–2124
24. Pollock, G. S., Vernon, E., Forbes, M. E., Yan, Q., Ma, Y. T., Hsieh, T., Robichon, R., Frost, D. O., and Johnson, J. E. (2001) Effects of early visual experience and diurnal rhythms on BDNF mRNA and protein levels in the visual system, hippocampus, and cerebellum. *J. Neurosci.* **21,** 3923–3931
25. Vecino, E., Garcia-Grespo, D., Garcia, M., Martinez-Millan, L., Sharma, S.C., and Carrascal, E. (2002) Rat retinal ganglion cells co-express brain derived neurotrophic factor (BDNF) and its receptor TrkB. *Vision Res.* **42,** 151–157
26. Seki, M., Nawa, H., Fukuchi, T., Abe, H., and Takei, N. (2003) BDNF is upregulated by postnatal development and visual experience: quantitative and immunohistochemical analyses of BDNF in the rat retina. *Invest. Ophthalmol. Vis. Sci.* **44,** 3211–3218
27. Menna, E., Cenni, M.C., Naska, S., and Maffei, L. (2003) The anterogradely transported BDNF promotes retinal axon remodeling during eye-specific segregation within the LGN. *Mol. Cell Neurosci.* **24,** 972–983
28. Vorwerk, C. K., Naskar, R., Schuettauf, F., Quinto, K., Zurakowski, D., Gochenauer, G., Robinson, M. B., Mackler, S. A., and Dreyer, E. B. (2000) Depression of retinal glutamate transporter function leads to elevated intravitreal glutamate levels and ganglion cell death. *Invest. Ophthalmol. Vis. Sci.* **41,** 3615–3621
29. Garrett, K. L., Shen, W. Y., and Rakoczy, P. E. (2001) In vivo use of oligonucleotides to inhibit choroidal neovascularisation in the eye. *J. Gene Med.* **3,** 373–383
30. Thaler, S., Rejdak, R., Dietrich, K., Ladewig, T., Okuno, E., Kocki, T., Turski, W.A., Junemann, A., Zrenner, E., and Schuettauf, F. (2006) A selective method for transfection of retinal ganglion cells by retrograde transfer of antisense oligonucleotides against kynurenine aminotransferase II. *Mol. Vis.* **12,** 100–107
31. Alter, J., Lou, F., Rabinowitz, A., Yin, H., Rosenfeld, J., Wilton, S.D., Partridge, T.A., and Lu, Q. L. (2006) Systemic delivery of morpholino oligonucleotide restores dystrophin expression bodywide and improves dystrophic pathology. *Nat Med.* **12,** 175–177
32. Galli-Resta, L., Novelli, E., and Viegi, A. (2002) Dynamic microtubule-dependent interactions position homotypic neurones in regular monolayered arrays during retinal development. *Development* **129,** 3803–3814
33. Gianfranceschi, L., Siciliano, R., Walls, J., Morales, B., Kirkwood, A., Huang, Z.J., Tonegawa, S., and Maffei, L. (2003) Visual cortex is rescued from the effects of dark rearing by overexpression of BDNF. *Proc. Natl. Acad. Sci. U. S. A.* **100,** 486–491
34. Gomez-Pinilla, F., Ying, Z., Roy, R.R., Molteni, R., and Edgerton, V. R. (2002) Voluntary exercise induces a BDNF-mediated mechanism that promotes neuroplasticity. *J. Neurophysiol.* **88,** 2187–2195
35. Liu, D., Diorio, J., Day, J.C., Francis, D.D., and Meaney, M. J. (2000) Maternal care, hippocampal synaptogenesis and cognitive development in rats. *Nat. Neurosci.* **3,** 799–806
36. Sale, A., Putignano, E., Cancedda, L., Landi, S., Cirulli, F., Berardi, N., and Maffei, L. (2004) Enriched environment and acceleration of visual system development. *Neuropharmacology.* **47,** 649–660
37. Lom, B., and Cohen-Cory, S. (1999) Brain-derived neurotrophic factor differentially regulates retinal ganglion cell dendritc and axonal arborization in vivo. *J. Neurosci.* **19,** 9928–9938
38. Hu, B., Nikolakopoulou, A.M., and Cohen-Cory, S. (2005) BDNF stabilzes synapses and maintains the structural complexity of optic axons in vivo. *Development* **132,** 4285–4298
39. Okoje, G., Zimmer, J., Sung, J., Gehlbach, P., Deering, T., Nambu, H., Hackett, S., Melia M., Esumi, N., Zack, D.J., and Campochiaro, P. A. (2003) Increased expression of brain-derived neurotrophic factor preserves retinal function and slows cell death from rhodopsin mutation or oxidative damage. *J. Neurosci.* **23,** 4164–4172
40. Cellerino, A., and Kohler, K. (1997) Brain-derived neurotrophic factor/neurotrophin-4 receptor TrkB is localized on ganglion cells and dopaminergic amacrine cells in the vertebrate retina. *J. Comp. Neurol.* **386,** 149–160
41. Di Polo, A., Cheng, L., Bray, G. M., and Aguayo, A. J. (2000) Colocalization of TrkB and brain-derived neurotrophic factor proteins in green-red-sensitive cone outer segments. *Invest. Ophthalmol. Vis. Sci.* **41,** 4014–4021
42. Rohrer, B., Korenbrot, J. I., LaVail, M. M., Reichardt, L. F., and Xu, B. (1999) Role of neurotrophin receptor TrkB in the maturation of rod photoreceptors and establishment of synaptic transmission to the inner retina. *J. Neurosci.* **19,** 8919–8930
43. Ladewig, T., Fellner, S., Zrenner, E., Kohler, K., and Guenther, E. (2004) BDNF regulates NMDA receptor activity in developing retinal ganglion cells. *Neuroreport.* **15,** 2495–2499

44. Rothe, T., Bahring, R., Carroll, P., and Grantyn, R. (1999) Repetitive firing deficits and reduced sodium current density in retinal ganglion cells developing in the absence of BDNF. *J. Neurobiol.* **40,** 407–419
45. Purves, D., Snider, W. D., Voyvodic, J. T. (1988) Trophic regulation of nerve cell morphology and innervation in the autonomic nervous system. *Nature* **336,** 123–128
46. McAllister, A. K., Lo, D. C., and Katz, L. C. (1995) Neurotrophins regulate dendritic growth in developing visual cortex. *Neuron.* **15,** 791–803
47. Schwartz, P. M., Borghesani, P. R., Levy, R. L., Pomeroy, S. L., and Segal, R. A. (1997) Abnormal cerebellar development and foliation in BDNF-/- mice reveals a role for neurotrophins in CNS patterning. *Neuron* **19,** 269–281
48. Xu, B., Gottschalk, W., Chow, A., Wilson, R. I., Schnell, E., Zang, K., Wang, D., Nicoll, R. A., Lu, B., and Reichardt, L. F. (2000) The role of brain-derived neurotrophic factor receptors in the mature hippocampus: modulation of long-term potentiation through a presynaptic mechanism involving TrkB. *J. Neurosci.* **20,** 6888–6897
49. Lom, B., Cogen, J., Sanchez, A. L., Vu, T., and Cohen-Cory, S. (2002) Local and target-derived brain-derived neurotrophic factor exert opposing effects on the dendritic arborization of retinal ganglion cells in vivo. *J. Neurosci.* **22,** 7639–7649
50. Sernagor, E., and Mehta, V. (2001) The role of early neural activity in the maturation of turtle retinal function. *J. Anat.* **199,** 375–383
51. Rusoff, A. C., and Dubin, M. W. (1978) Kitten ganglion cells: dendritic field size at 3 weeks of age and correlation with receptive field size. *Invest. Ophthalmol. Vis. Sci.* **17,** 819–821
52. Cook, P. B., and McReynolds, J. S. (1998) Modulation of sustained and transient lateral inhibitory mechanisms in the mudpuppy retina during light adaptation. *J. Neurophysiol.* **79,** 197–204
53. Flores-Herr, N., Protti, D.A., and Wassle, H. (2001) Synaptic currents generating the inhibitory surround of ganglion cells in the mammalian retina. *J. Neurosci.* **21,** 4852–4863
54. Taylor, W. R. (1999) TTX attenuates surround inhibition in rabbit retinal ganglion cells. *Vis. Neurosci.* **16,** 285–290
55. O'Malley, D.M., Sandell, J.H., and Masland, R. H. (1992) Co-release of acetylcholine and GABA by the starburst amacrine cells. *J. Neurosci.* **12,** 1394–1408
56. Haverkamp, S., and Wassle, H. (2000) Immunocytochemical analysis of the mouse retina. *J. Comp. Neurol.* **424,** 1–23
57. Contini, M., and Raviola, E. (2003) GABAergic synapses made by a retinal dopaminergic neuron. *Proc. Natl. Acad. Sci. U. S. A.* **100,** 1358–1363
58. Masland, R. H. (2005) The many roles of starburst amacrine cells. *Trends Neurosci.* **28,** 395–6
59. Witkovsky, P. (2004) Dopamine and retinal function. *Doc. Ophthalmol.* **108,** 17–40
60. Jensen, R. (1986) Effects of dopamine and its agonists and antagonists on the receptive field properties of ganglion cells in the rabbit retina. *Neuroscience* **17,** 837–855
61. Munakata, Y., Casey, B. J., and Diamond, A. (2004) Developmental cognitive neuroscience: progress and potential. *Trends Cogn. Sci.* **8,** 122–128
62. Sinclair, J. R., Jacobs, A. L., and Nirenberg, S. (2004) Selective ablation of a class of amacrine cells alters spatial processing in the retina. *J. Neurosci.* **24,** 1459–1467

Received for publication April 14, 2006.
Accepted for publication August 22, 2006.