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Enriched environment and acceleration of visual system development

Alessandro Sale [a,*], Elena Putignano [a], Laura Cancedda [b], Silvia Landi [a], Francesca Cirulli [c], Nicoletta Berardi [d,e], Lamberto Maffei [a,e]

[a] *Laboratorio di Neurobiologia, Scuola Normale Superiore, via Moruzzi 1, I-56100, Pisa Italy*
[b] *Division of Neurobiology, Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720-3200, USA*
[c] *Section of Behavioural Neuroscience, Department of Cell Biology and Neurosciences, Istituto Superiore di Sanità, Viale Regina Elena 299, Rome I-00161, Italy*
[d] *Dipartimento di Psicologia, Università degli Studi di Firenze, Firenze I-50100, Italy*
[e] *Institute of Neuroscience of CNR, via Moruzzi 1, I-56100, Pisa Italy*

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Abstract

Rearing mice from birth in an enriched environment leads to a conspicuous acceleration of visual system development appreciable at behavioral, electrophysiological and molecular level. Little is known about the possible mechanisms of action through which enriched environment affects visual system development. It has been suggested that differences in maternal behavior between enriched and non-enriched conditions could contribute to the earliest effects of enriched environment on visual development and that neurotrophins, BDNF in particular, might be involved. Here, we examined Brain Derived Neurotrophic Factor (BDNF) levels in the visual cortex during development and showed that an increase occurs in the first week of life in enriched pups compared to standard reared pups; BDNF levels at birth were equal in the two groups. This suggests a postnatal rather than a prenatal effect of environment on BDNF. A detailed analysis of maternal care behavior showed that pups raised in a condition of social and physical enrichment experienced higher levels of licking behavior and physical contact compared to standard reared pups and that enhanced levels of licking were also provided to pups in an enriched environment where no adult females other than the mother were present. Thus, different levels of maternal care in different environmental conditions could act as indirect mediator for the earliest effects of enrichment on visual system development.

Some of the effects of different levels of maternal care on the offspring behavior are long lasting. We measured the visual acuity of differentially reared mice at the end of the period of visual acuity development (postnatal day 45) and at 12 months of age, using a behavioral discrimination task. We found better learning abilities and higher visual acuity in enriched compared to standard reared mice at both ages.

Keywords: Environmental enrichment; Visual system development; Visual acuity; BDNF; Maternal care; Licking behavior

1. Introduction

Environmental enrichment is a widely used paradigm for investigating the influence of sensory experience on brain and behavior. The enriched environment was first defined by Rosenzweig et al. (1978) as "a combination of complex inanimate and social stimulation". "Enriched" animals are reared in larger cages and in larger groups and a variety of toys, tunnels, nesting material, stairs are present and changed frequently. In addition, animals are typically given the opportunity for voluntary physical activity on running wheels. Rearing animals in an enriched environment has profound effects on the adult organism, leading to anatomical changes (observed, for instance, in the cortex, hippocampus and cerebellum) in dendritic arborization, spine density and synapses per neuron (Rosenzweig, 1966; Greenough and Volkmar, 1973; Renner and Rosenzweig, 1987; Rampon et al., 2000). These morphological changes are associated with improved learning and memory and

* Corresponding author. Tel.: +39-050-3153205; fax: +39-050-3153210.
E-mail address: sale@in.cnr.it (A. Sale).

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enhanced neural plasticity (reviewed in van Praag et al., 2000) and reorganization of cortical somatosensory maps (Polley et al., 2004).

Despite the large amount of data on adult animals, the possibility that an enhanced sensory-motor stimulation provided by an enriched environment early in life could also affect the development of the nervous system has been explored only very recently. More complex dendritic arbors were found in cortical pyramidal cells following enrichment from weaning (Kolb, 1995) but little else is known. Focusing on visual system development as a paradigmatic model of nervous system development, we showed that postweaning environmental enrichment prevents dark rearing effects on rat visual cortical development (Bartoletti et al., 2004), while environmental enrichment from birth in mice results in a conspicuous acceleration of visual system development at behavioral, electrophysiological and molecular level (Cancedda et al., 2004). Some of the main changes we found in enriched mice were observed at a very early age (postnatal days 7–15, (P7–P15)), when pups still spend all their time in the nest. The precociousness of these events makes unlikely that they derive from a direct influence of the richness of the environment on pups. Therefore, we hypothesized that different levels of maternal care in the two environmental conditions could act as an indirect mediator for the earliest effects of enrichment on visual system development (Cancedda et al., 2004). The hypothesis that different levels of maternal care in enriched condition could induce the precocious development of the visual system in enriched animals is also suggested by results showing that variations in maternal care can affect BDNF levels and neural development of the offspring (Liu et al., 1997). Neurotrophins, and in particular, BDNF, have a major role in the control of visual cortical plasticity during a critical period early in life (reviewed in Berardi et al., 2003).

To date, the relationship between enriched environment and maternal care in rodents has rarely been investigated: a similarity between the effects of environmental enrichment in the adult and early-life handling stimulation (short periods of maternal separation provided daily to pups) has been suggested (reviewed in Fernandez-Teruel et al., 2002), but a comparison of maternal behavior between enriched and standard dams is still missing. Here, we investigated maternal care influence on the acceleration of visual system development in environmentally enriched mice by analyzing maternal behavior in enriched and standard reared mice during the first 10 days of life. In addition, we measured BDNF expression in the visual cortex from P0 to P25 to understand whether maternal behavior influences this important determinant of visual cortical development.

Early work by Levine (1957) has shown that early stimulation of neonatal rodents affects their endocrine and behavioral responses later in life. In particular, numerous studies have clearly indicated that manipulations of the mother–infant relationship have long-term consequences on neuroendocrine and behavioral responses (reviewed in Cirulli et al., 2003a), and enhanced learning and memory have been reported in adult animals that received high levels of maternal care during development (Liu et al., 2000). One outstanding change induced by environmental enrichment from birth in mice is an improvement in the adult visual acuity, a result shown by Prusky et al. (2000a) and recently confirmed by us using behavioral as well as electrophysiological methods (Cancedda et al., 2004). To assess whether this augmented visual acuity induced by environmental enrichment also persists into old age and whether environmental enrichment has a protective role on the known visual loss associated with aging, we studied visual acuity in 12 months-old mice reared from birth in enriched or standard environment.

2. Materials and methods

2.1. Subjects and mating protocol

The animals (C57BL/6J mice) were housed in a room with a temperature of 21 °C, 12/12 light/dark cycle, and food and water available ad libitum. To avoid possible confounding effects deriving from the experience of previous motherhood only primiparous females were employed. Female mice were assigned either to enriched or to standard cages and put with males (one male for every mating cage) in their respective cage, from which males were removed after 10 days. At least 7 days before delivery, pregnant females were transferred to a new cage equivalent to that in which mating occurred and where behavioral observations were performed. With this procedure, both enriched and standard females received equivalent levels of stress deriving from cage transfer during pregnancy. Litters were not culled or sexed at birth so that handling of the pups has been minimized, avoiding any interference of cross-fostering with the enrichment procedure (see also Maccari et al., 1995). Sex ratio (proportion of males per litter) was recorded at weaning.

2.2. Rearing environments

Enriched environment, mating cage: consisted of a large cage (44 × 62 × 28 cm) with a wire mesh lid containing several food hoppers, a running wheel and differently shaped objects (tunnels, shelters, stairs) that were repositioned once a day and completely substituted with others once a week. Every

cage housed at least five dams and one male.

Enriched environment, behavioral observation cage, mother with filler females (EC): consisted of a cage totally equivalent to the enriched environment mating cage, but with only one dam (with her pups) and two additional "filler" females, tagged with natural white paint on the tail.

Enriched environment, behavioral observation cage, without filler females (ECwf): consisted of a cage equivalent to the enriched environment mating cage, but with only 1 dam with her pups.

Standard environment, mating cage: consisted of a standard laboratory cage ($26 \times 42 \times 18$ cm) housing four adults (three females and one male).

Standard environment, behavioral observation cage (SC): consisted of a cage equivalent to the standard environment mating cage, but with only one dam with her pups.

The behavioral observation cages were not changed for bedding material during the 10 days period of maternal care analysis. In enriched observation cages, objects were partially changed or repositioned once every 3 days.

2.3. BDNF immunoassay

Occipital cortices were dissected (between 8.30 and 9.30 a.m.) from 204 mice raised in EC and 174 SC mice and were frozen. Both cortices from several mice were pooled together to represent one sample (mean: 3100 μg of total proteins/sample). Proteins were extracted with lysis buffer (Igepal CA 630 1%, 10% Glycerol, 20 mM Tris–HCl pH 8, 137 mM NaCl, 0.5 mM Na_3VO_4, 1 μg/ml leupeptin, 10 μg/ml aprotinin, 1 mM PMSF) and quantified with Bio-Rad protein assay kit. One hundred microliter of BDNF standard and 100 μl of samples were run in duplicate (six dilutions) following the protocol from Emax-ImmunoAssay-kit, Promega. Standard samples were run for each plate and a standard curve was subsequently computed ($R^2 = 0.999 \pm 0.001$; $n = 6$). Optical absorbance was read at 450 nm with a microplate reader. Samples were considered BDNF positive when their signal was within the standard curve range. To determine the absolute amount of neurotrophin, a titration curve for each sample was generated and the midpoint of this curve was compared with the standard curve.

2.4. Maternal care observations

Six SC, 4 EC and 4 ECwf cages have been observed. Behavioral observations began when pups were 1 day of age and continued for 10 consecutive days. Maternal behavior was scored during six daily observation sessions of 75 min each for the first 10 days postpartum. The observation sessions occurred at 6.45 a.m., 9 a.m., 12 a.m., 3 p.m., 6 p.m. and 9 p.m.; the first and last sessions were during dark phase of the daily cycle, and were performed under dim red light illumination. For each session, the behavior of each female was scored every 3 min (150 observations per day), recording whether the target behavior was present or not.

Maternal behaviors scored (not mutually exclusive) were:

Pups alone: no adult females present in the nest.

Mother off pups: the dam is standing outside the nest area, not caring about the pups.

Mother in contact: the dam is in contact with a part of her body other than the tail to the body of at least one pup, not assuming the arched-back nursing (ABN) position. More passive nursing postures (dam's body lying down on her side with more than one pup attached to the nipples) or mother's self-grooming while nursing or in contact with the pups were included in this section.

ABN: the dam is crouching over pups. This position comports larger nourishment for the pups.

Maternal licking: the dam is licking any part of pup's body.

Non-maternal licking: at least one filler female is licking any part of pup's body.

Data are reported as the percentage of observations in which pups received the target behavior (number of observations in which the target behavior was recorded divided the total number of observations × 100, see Liu et al. (2000)). Data were analyzed with two-way repeated measures ANOVA.

2.5. Acute test of maternal retrieving

A test of retrieving behavior, which is defined as "carrying or pushing a pup to the nest by taking a part of the pup's body into the mouth", was performed between 10.30 and 11.30 a.m., when pups were P4. The dam and the offspring were removed from their home cage and placed in two separate clean cages for 7 min, during which litters were weighed. At the end of this period, four pups were put back in their home cage at 10 cm around one side of the nest, and the dam was reintroduced, directly on one pup. Three latencies were scored: the latency to retrieve the first pup (lat 1), the latency to retrieve the last pup (lat last) and the "on nest" latency, defined as the latency for dam to remain still in the nest (after the last pup retrieval) for at least 1 min (see also Cirulli et al., 2003b). In the EC, the test was immediately repeated for one filler female. Data were analyzed by the Kruskal–Wallis one-way analysis of variance on ranks.

Litters were weighed two additional times, at P11 (the day after the end of maternal behavior observa-

tions), and at P21 (weaning age). Two ways ANOVA of pup weights for different environmental conditions and postnatal ages showed that there was a significant effect of age ($P < 0.001$), but no difference was found among the three environmental conditions at the four ages analyzed during the first 21 days of life (P4, P6, P11, P21; two ways ANOVA, $P = 0.356$). No significant difference was found in litter sex ratio among the three groups (one-way ANOVA, $P = 0.289$; sex ratio means ± standard errors: 1.0 ± 0.2 for SC; 1.3 ± 0.2 for EC; 1.6 ± 0.3 for ECwf).

2.6. Eye-opening observations

From postnatal day 11, pups ($n = 26$ pups for EC, $n = 16$ pups for ECwf and $n = 16$ pups for SC group) were inspected for eye-opening twice a day at about 8 a.m. and 7 p.m. Eye-opening was defined as the initial break in the membrane sealing the lids of both eyes.

2.7. Behavioral assessment of visual acuity in 12 months-old mice reared in EC or SC from birth

Starting at P350, two groups of mice reared from birth in EC ($n = 5$) or SC ($n = 4$) were trained and tested in the visual water task (Prusky et al., 2000b) to assess their visual acuity. Visual water task trains animals to first distinguish a low (0.05 cycles/deg) spatial frequency vertical grating from gray, and then tests the limit of this ability at higher spatial frequencies. The apparatus consists of a trapezoidal-shaped pool with two panels placed side by side at one end. A midline divider is extended from the wide end of the pool into the middle, creating a maze with a stem and two arms. The length of the divider sets the choice point and effective spatial frequency. An escape platform is placed below the grating. Animals are released from the center at the end of the pool opposite the panels. The position of the grating and the platform is alternated in a pseudorandom sequence over training trials, while the mice are shaped to swim towards the grating in one of the arms of the maze. A trial is recorded as incorrect if an animal enters the arm without the platform. Animals are removed from the pool when they find the platform. Once 80% accuracy is achieved, the limit of the discrimination is estimated by increasing the spatial frequency of the grating until performance falls below 70% accuracy. The highest spatial frequency at which 70% accuracy is achieved is recorded as the visual acuity.

3. Results

We have recently demonstrated that rearing mice from birth in an enriched environment leads to a profound acceleration of important properties of visual system development (Cancedda et al., 2004). We found that raising mice in this condition causes a precocious maturation of visual acuity, measured both behaviorally and electrophysiologically. The mature visual acuity of EC and SC mice was also significantly different (Fig. 1(A)).

A profound visual system development acceleration was also found assessing the time course of visual cortex long-term potentiation (LTP) (Fig. 1(B)). The LTP induced by theta burst stimulation from the white matter (WM-LTP) is a well established in vitro model of visual cortical development, since the susceptibility to potentiation of layer III cortical neurons after stimulation of the white matter is present only during a critical period in the early life of rodents, being absent in adult animals. This critical period is delayed by dark rearing, which also delays visual cortical development, and accelerated by BDNF overexpression, which accelerates visual cortical development (Kirkwood et al., 1995; Huang et al., 1999). The developmental decline of WM-LTP magnitude in EC animals occurred precociously, with a significant difference between SC and EC groups at P20 (Fig. 1(B)).

These functional events were preceded and accompanied by changes in molecular pathways important for visual cortical development. In particular, EC pups possessed higher GAD65/67 levels at P7 and at P15 (Fig. 2(A)), and the time course of CRE/CREB induced gene expression in the visual cortex was accel-

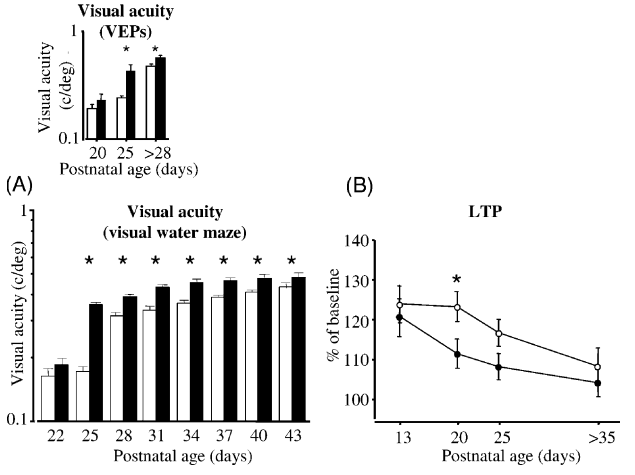

Fig. 1. Acceleration of visual system development by environmental enrichment. (A) Behavioral assessment of visual acuity in SC (white) and EC (black) groups at different ages, by visual water task. (a, *inset*) Electrophysiological assessment of visual acuity in SC (white) and EC (black) mice during postnatal development, by Visual Evoked Potentials (VEPs). Visual acuity of animals is plotted as groups of age; (B) Accelerated developmental decline of WM-LTP in EC mice (black) compared to SC mice (white), measured 30 min after TBS. The star indicates statistically significant differences between the two groups. The bars indicate SEM.

erated in EC mice (Fig. 2(B)). The role of CRE/CREB system in mediating the effects of enriched environment on visual system development was confirmed by an experiment in which this molecular pathway was pharmacologically enhanced through injections of rolipram, a specific inhibitor of the high-affinity phosphodiesterase type IV (Tohda et al., 1996; Kato et al., 1998; Nakagawa et al., 2002). This treatment mimicked the acceleration of visual acuity development elicited by environmental enrichment, with augmented visual acuity values in standard reared rolipram-treated mice at P25 (Fig. 2(C)).

3.1. Developmental BDNF levels in EC and SC visual cortex

We measured levels of BDNF protein in enriched and standard reared mice at different postnatal ages, from birth until P25, using a conventional ELISA protocol.

In both groups, BDNF protein levels exhibited a developmental increase which reached a plateau at P20 (Fig. 3). The effect of enrichment was very precocious: enriched pups possessed higher levels (~55%) of BDNF at P7. We detected no difference in the visual cortex between enriched and standard reared pups at P0 (Fig. 3). These results suggest a postnatal rather than a prenatal influence of environment, indicating that postnatal stimulation is responsible for the effects of enriched environment on BDNF. One main source of postnatal stimulation for the newborn is maternal care.

3.2. Maternal behavior observations

It has been shown that the offspring of mothers that exhibit high levels of pup licking have an increased expression of BDNF in the hippocampus at P8 (Liu et al., 2000), i.e. about the same age at which we found augmented BDNF levels in the visual cortex of enriched pups (Fig. 3). We therefore hypothesized that different (possibly augmented) levels of maternal care were provided to pups in enriched environment, and we performed detailed analysis of maternal behavior in standard and enriched conditions. We started studying maternal care using the same enrichment protocol employed in the earlier study, the classic enrichment protocol also including a complex social interaction due to the presence of numerous adult mice in the same cage (EC). The observations performed in this condition showed effects of environmental enrichment on maternal care levels received by pups and raised the question whether and to which extent maternal behavior could be influenced by a more simplified environmental enrichment protocol (physical vs. physical and social), in which no adult females other than the dam were present (ECwf).

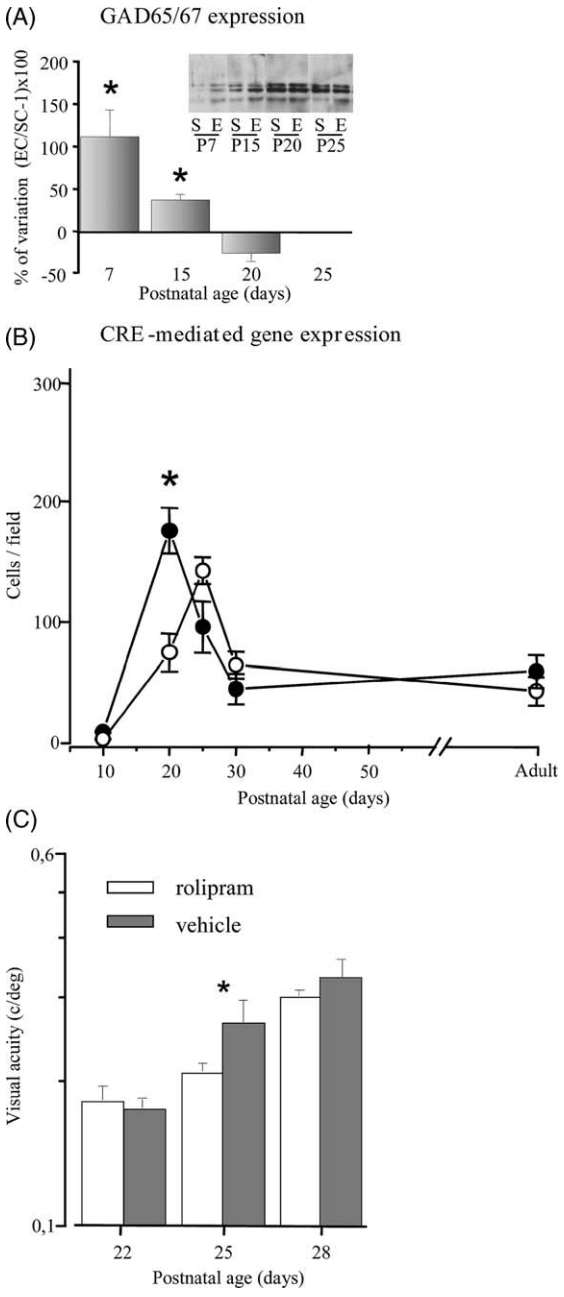

Fig. 2. Acceleration of visual system development by environmental enrichment. (A) Higher levels of GAD 65/67 expression in EC pups: percentage of variation of GAD 65/67 expression in EC and SC occipital cortices computed as $((EC/SC) - 1) \times 100$ at different postnatal ages. (a, *inset*) Representative gel of western blot for GAD 65/67 expression at different ages in occipital cortices of SC (S) and EC (E) mice; (B) Acceleration of CRE-mediated gene expression in the binocular visual cortex of EC mice: quantification of the density of X-gal positive cells for the SC (white) and EC (black) animals at the indicated postnatal ages; (C) Pharmacological activation of cAMP/CREB system by rolipram mimics the enriched environment effects on mice visual acuity development: behavioral assessment of visual acuity in animals treated with the inhibitor of the phosphodiesterase type IV rolipram (dark gray) and vehicle (white) at different ages. Visual acuity of animals is plotted as groups of age. The star indicates statistically significant differences between the two groups. The bars indicate SEM.

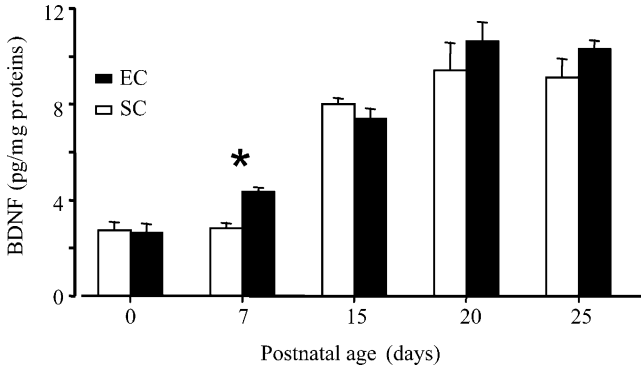

Fig. 3. Higher levels of BDNF protein in EC pups: developmental increase of total protein levels of endogenous BDNF in occipital cortices of EC (black) and SC (white) mice. The star indicates that the BDNF levels in EC and SC groups are different (*t*-test $P < 0.001$). Bars indicate SEM.

3.3. *Pups alone in the nest and mother off pups*

Maternal care in rodents occurs in bouts, alternated with periods of maternal absence during which pups remain alone in the nest. We therefore examined the important parameter of time spent alone by pups in the nest in absence of any adult females. This time was dramatically lower in EC than in SC and in ECwf. Indeed, while it was low during the first days post-birth and successively augmented in SC and in ECwf, the pups in the EC were virtually never alone in the nest, since, when the mother was absent, she was always replaced by one or both adult fillers and vice versa (Fig. 4(A)). The time spent alone by pups was significantly lower in SC than in ECwf (Fig. 4(A)). To better characterize the maternal contribution to these results, we compared the frequency of *mother off pups* behavior among the three environmental conditions (Fig. 4(B)): SC dams spent significantly less time out of the nest than dams in the two enriched conditions, but no difference was found between EC and ECwf.

3.4. *Arched-back nursing*

The frequency of ABN was high during the first days *postpartum* and declined during the subsequent days (Fig. 5(A)) in the same way in the three environmental conditions; no difference was found in the time spent by dams in ABN. However, since SC dams spent significantly less time out of the nest than dams in the two ECs (see above), this implies that SC dams tent to assume other positions than ABN more frequently. This was confirmed by the analysis of *mother in contact* behavior, which showed statistically higher frequency for SC dams than for EC and for ECwf, while non-difference was found between the two ECs (Fig. 5(B)).

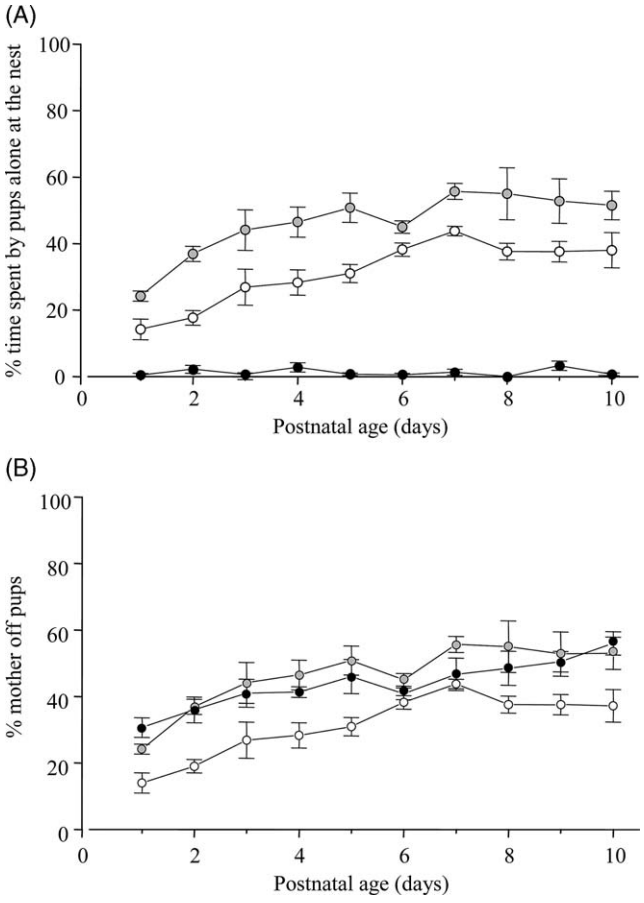

Fig. 4. (A) Frequency of "pups alone" recordings during the first 10 days postpartum in SC (white), EC (black) and ECwf (gray). Two-way repeated measures ANOVA revealed a significant effect of age and environmental housing condition ($P < 0.001$) and a significant interaction between age and environmental housing condition ($P < 0.001$). SNK post hoc analysis revealed that all groups differ statistically ($P < 0.05$); (B) Frequency of "mother off pups" recordings during the first 10 days postpartum in SC (white), EC (black) and ECwf (gray). Two-way repeated measures ANOVA revealed a significant effect of age and environmental housing condition ($P < 0.001$). SNK post hoc analysis revealed a statistical difference ($P < 0.05$) between SC and EC and between SC and ECwf, but not between EC and ECwf. Bars indicate SEM.

3.5. *Licking behavior*

One of the most critical maternal behaviors for normal growth of the newborn is ano-genital and oral licking, which has been associated with hormonal regulation of growth and with the development of neuroendocrine and behavioral responses to stress (Liu et al., 1997). We found that pups in the EC group experienced higher levels of licking than pups in the two other environmental conditions. Importantly, while these levels declined progressively in SC and in ECwf, they remained stably high among the 10 days of observations in EC (Fig. 6(A)). This was mostly due to the conspicuous contribution of licking provided by fil-

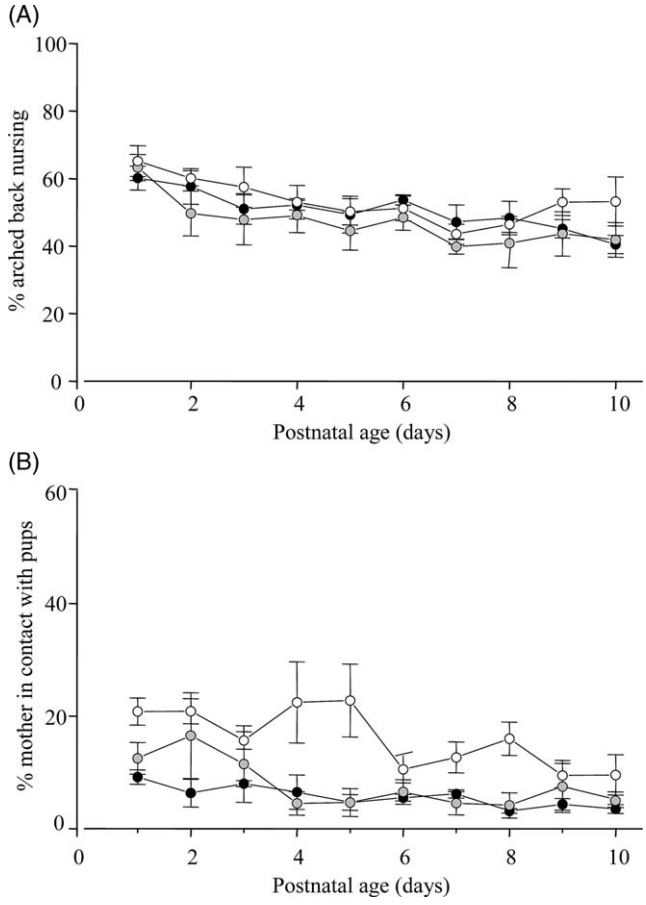

Fig. 5. (A) Frequency of "arched-back nursing" recordings during the first 10 days postpartum in SC (white), EC (black) and ECwf (gray). Two-way repeated measures ANOVA revealed a significant effect of age ($P < 0.001$) but not of environmental housing condition ($P = 0.418$); (B) Frequency of "mother in contact" recordings during the first 10 days postpartum in SC (white), EC (black) and ECwf (gray). Two-way repeated measures ANOVA revealed a significant effect of age ($P < 0.05$) and environmental housing condition ($P < 0.001$) and a significant interaction between age and environmental housing condition ($P < 0.05$). SNK post hoc analysis revealed a statistical difference ($P < 0.05$) between SC and EC and between SC and ECwf, but not between EC and ECwf. Bars indicate SEM.

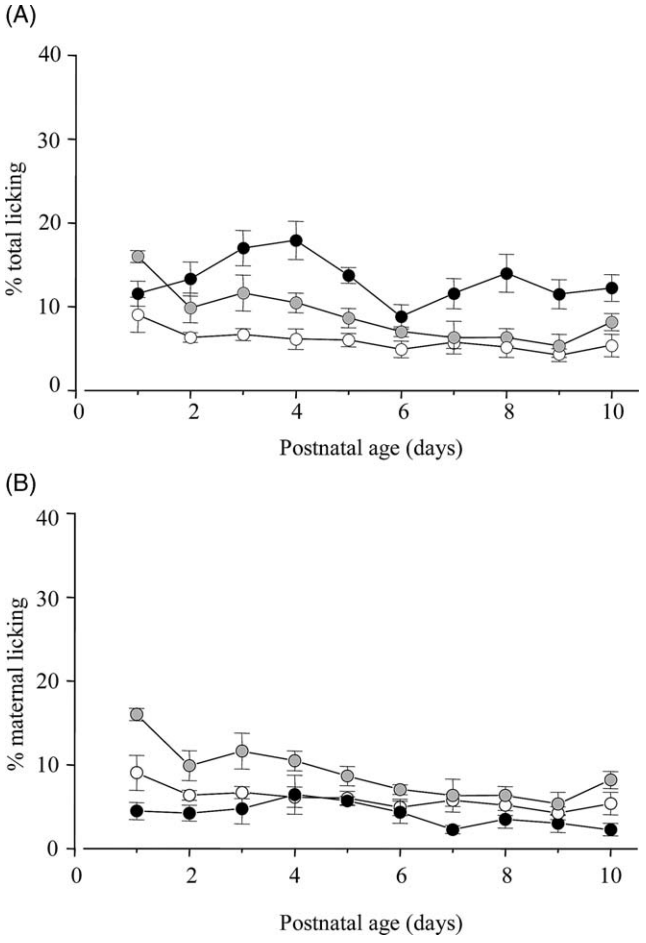

Fig. 6. (A) Frequency of "licking" recordings during the first 10 days postpartum in SC (white), EC (black) and ECwf (gray). Maternal and non-maternal licking have been summed. Two-way repeated measures ANOVA revealed a significant effect of age and environmental housing condition ($P < 0.001$) and a significant interaction between age and environmental housing condition ($P = 0.006$). SNK post hoc analysis revealed that all groups were statistically different ($P < 0.05$); (B) Frequency of "maternal licking" recordings during the first 10 days postpartum in SC (white), EC (black) and ECwf (gray). Two-way repeated measures ANOVA revealed a significant effect of age and environmental housing condition ($P < 0.001$). SNK post hoc analysis revealed a statistical difference ($P < 0.05$) between SC and ECwf and between ECwf and EC, but not between EC and SC. Bars indicate SEM.

ler females in addition to the proper maternal licking. A focalized analysis of licking behavior exhibited only by dams (excluding the contribution of filler females) showed that enriched mothers in the ECwf exhibited significantly more licking than enriched mothers in the EC condition and SC mothers (Fig. 6(B)).

3.6. Acute test of maternal retrieving

To better characterize maternal behavior, we performed test of maternal retrieving, a behavioral item most often used to assess maternal competence in a challenging situation (Cirulli et al., 2003a). No difference was found in the three retrieving latencies among dams in different environmental conditions or between latencies recorded in filler females and dams (Kruskal–Wallis one-way ANOVA on ranks: $P = 0.268$, $P = 0.369$ and $P = 0.258$, respectively, for lat 1, lat last and "on nest" latency).

3.7. Eye-opening

A significant effect of environmental condition on the timing of eye-opening was found (one-way ANOVA on ranks, $P < 0.001$). A multiple comparison procedure revealed that eye-opening was significantly accelerated by about 2 days in EC with respect to

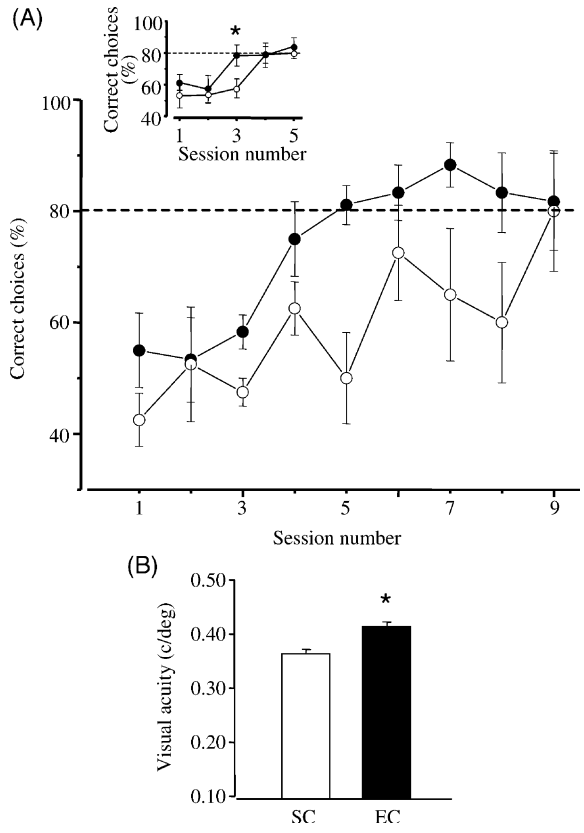

Fig. 7. (A) Enhanced learning abilities in middle-aged mice enriched from birth: percentage of correct responses during the training sessions of the visual water task in enriched (black) and standard (white) mice. Two-way repeated measures ANOVA revealed a significant effect of environmental housing ($P < 0.05$) and learning session ($P < 0.001$). (a, *inset*) For comparison, the performance in learning the same task previously recorded in differentially reared young mice is reported; (B) Enhanced visual acuity in enriched mice: visual acuity was higher in enriched mice compared to standard mice (*t*-test, $P < 0.05$). The bars indicate SEM.

ECwf and SC mice, while no difference was found between the latter two groups. The percentage of pups which opened their eyes at P12, P13 and P14, for EC, SC and ECwf, was: 77%, 6% and 0% at P12; 8%, 19% and 6% at P13; 15%, 75% and 94% at P14, respectively.

3.8. *Visual acuity measurement in differentially reared 12 months-old mice*

Given that living in an enriched environment has a strong protective role against negative effects of aging on memory (Frick et al., 2003; Kempermann et al., 2002) and visual function can deteriorate significantly with age (Fiorentini et al., 1996) we wondered whether the enhancement in visual acuity induced by environmental enrichment in mice also persists into older age. We used a behavioral method (visual water box task) to measure visual acuity in 12 months-old mice reared from birth in enriched or standard condition. This method also allowed a comparison of learning abilities between the two groups. The first result we found was a marked impairment of SC mice in learning the associating task in the visual water maze. Fig. 7(A) reports the learning curves for the two groups (for comparison, in Fig. 7(a, inset), it is also reported the performance in learning the task previously recorded in young enriched and standard reared mice): while five sessions were required for enriched mice to reach the criterion of 80% of correct choices, standard mice reached this level only at the ninth session. Comparing the performance of middle-aged mice with that of young mice raised in correspondent environmental conditions shows a more pronounced impairment of cognitive abilities in standard reared mice than in enriched mice. Moreover, although the effect was small, the visual acuity of enriched mice was significantly higher than that of standard mice (Fig. 7(B); means: 0.42 ± 0.007 cycle/deg for enriched mice; 0.37 ± 0.008 cycle/deg for standard reared mice, *t*-test, $P < 0.05$).

4. Discussion

Neurotrophins have a major role in the control of visual cortical plasticity during a critical period early in life (reviewed in Berardi et al., 2003). In particular, BDNF exerts profound influence on the development of the visual system. BDNF overexpressing mice generated by Huang et al. (1999) exhibit a pronounced acceleration in both the development of visual acuity and the time course of ocular dominance and synaptic plasticity. We have recently demonstrated that changes very similar to these, which have been obtained through genetic engineering techniques, can also be induced by environmental enrichment, a naturalistic condition of increased environmental complexity (Cancedda et al., 2004). It is known that environmental enrichment increases neurotrophin expression in the hippocampus and visual cortex in the adult (Ickes et al., 2000; Pham et al., 2002). Here, we show that environmental enrichment enhances BDNF levels in the visual cortex during development: indeed, BDNF levels, not affected at the age of birth, are markedly augmented after the first life week in enriched pups compared to standard reared pups.

A possible source for these very precocious effects induced by environmental enrichment is maternal care influence, and we performed a detailed analysis of maternal behavior in different environmental conditions. We found that enriched pups experience higher levels of maternal care compared to standard reared

pups and we suggest that these variations might cause an acceleration in the visual system development. The first two weeks of life in rodents are characterized by a general absence of direct interaction between the environment and the newborn, which remains the whole time in the nest, totally dependent on the mother, that can be considered the most important source of sensory experience for the developing pup (Hofer, 1984; Liu et al., 2000). EC pups received a continuous physical contact due to the presence of adult females in the nest and also experienced the highest levels of licking, provided from both the dam and the filler females. A continuous tactile stimulation can strongly influence pup development, possibly providing a source for the earliest changes we observed in enriched mice, as the precocious eye-opening. It has been demonstrated that even 1 h of maternal separation in rat produces a decrease in the activity of ornithine decarboxylase (Wang et al., 1996), which is an important enzyme necessary for normal growth (Marton and Morris, 1987), and that this effect can completely be prevented through artificial tactile stimulation with a brush at a frequency resembling that of maternal licking (Pauk et al., 1986). Even more noteworthy, enhanced levels of licking were also recorded when the social contribution to maternal care was excluded, in the ECwf, although they were significantly lower than in EC. Therefore, maternal behavior seems to be highly plastic in response to environmental demands, with dams shifting from moderate licking levels when other females contribute to pup care, to intermediate levels in SC, to sustained licking in a complex environment where no social care is possible.

Increased dam licking has been reported in handled animals, a result that has been interpreted as evidence that the long-lasting neurobehavioral changes induced by handling can be at least partially mediated by altered maternal care (Liu et al., 1997; Pryce et al., 2001). Our results show that ECwf dams provide enhanced levels of licking to pups, while the time they spend out of the nest is significantly longer (i.e. enriched pups are more frequently observed to be alone in the nest); we propose for the first time that rising pups from birth in an enriched environment may result in a kind of handling effect (a mechanism of increased care following maternal separation), whereby the pups are separated from the mother not artificially, but as a result of her disposition to explore more frequently in a complex environment than in a standard cage. This observation that enrichment from birth and handling procedure can share a common mechanism promoting higher levels of maternal care is new.

Notwithstanding the fact that these differences in maternal care lie within a normal physiological range, they are likely to have an important role in the visual system acceleration observed in enriched pups. Indeed, variations in maternal care can affect BDNF levels and neural development of the offspring (Liu et al., 2000) and artificial manipulations and tactile stimulation in pups can influence eye-opening in rodents (Barnett and Burn, 1967; Smart et al., 1990). Furthermore, tactile stimulation influences the expression of hormones implicated in the control of pup development (Kuhn and Schanberg, 1998; Schanberg et al., 2003) and can affect the adult pattern of cortical cell dendritic fields (Kolb and Gibb, 1999). We suggest that in a precocious developmental phase, enhanced levels of maternal care in the enriched condition with respect to the standard condition can result in molecular changes in the visual cortex of pups, for instance, leading to the elevated BDNF expression at P7, which in turn could elicit the enhanced inhibitory levels observed at P7–P15. Since BDNF expression has a major role in the regulation of visual cortex development and plasticity (Huang et al., 1999), the effects of enriched environment on visual cortical development could be at least partially explained by a precocious BDNF expression due to increased maternal care levels. However, maternal behavior can control other factors potentially important for visual system development, such as growth factors crossing the placental barrier and present in maternal milk, that are involved in the regulation of the development. One is IGF-I, which is considered a strong modulator of fetal and neonatal somatic and organ growth (Olanrewaju et al., 1996). Since IGF-I receptors are present in the occipital cortex (Frolich et al., 1998), IGF-I could influence the expression of molecules relevant for visual cortical plasticity such as nerve growth factor (NGF) and BDNF. In the adult, exposure to enriched environment has protective effects against neurodegeneration that have been demonstrated to be mediated by IGF-I induced BDNF expression (Carro et al., 2000; Thoenen and Sendtner, 2002), but it is not known whether this is also true for the developing brain.

One possible model put forward to explain the accelerated visual acuity development and visual cortical plasticity decline in BDNF overexpressing mice (Huang et al., 1999) was that higher BDNF levels would accelerate the development of the inhibitory GABAergic system, which, by affecting receptive field development and synaptic plasticity, could explain both the faster maturation of visual acuity and the precocious decline of cortical plasticity. We found that the expression of GABA biosynthetic enzymes, GAD65/67, was indeed increased in enriched pups at very early ages (P7–P15), suggesting that one mediator of BDNF action could be the intracortical inhibition. However, enrichment could act on visual cortical development also via other mechanisms, in particular through changes at morphological level. We have already mentioned in Introduction that environmental enrichment in the adult causes

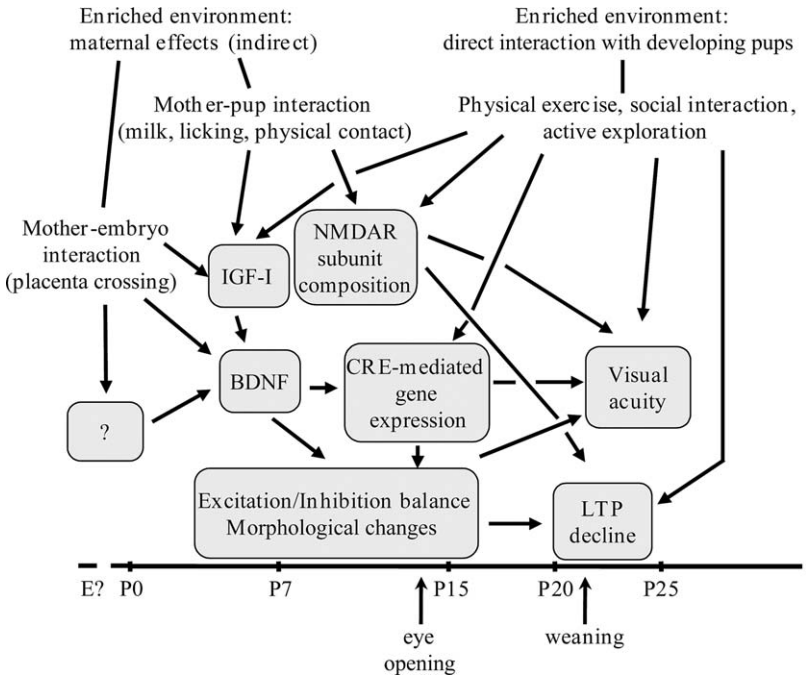

Fig. 8. Proposed model of environmental enrichment effects on visual system development (see text for details).

striking effects on dendritic arborization and spine density. Although nothing is known on the effects of enrichment on the morphological maturation of visual cortical neurons and of their thalamic afferents, it is well known that BDNF can affect the dendritic growth of pyramidal neurons in the visual cortex (McAllister et al., 1995; Wirth et al., 2003) in an activity dependent manner (McAllister et al., 1996) and that BDNF release from single cells can elicit local dendritic growth in nearby developing visual cortical neurons (Horch and Katz, 2002). BDNF has also been shown to affect spine formation in the hippocampus (Tyler and Pozzo-Miller, 2003) and facilitates translocation of initiation factor 4 in dendritic spines in cultured neurons, thus affecting local translation events at synapses (Smart et al., 2003). Actin-based plasticity in dendritic spines has been suggested to be a major factor in synaptic plasticity both during development and in the adult (Matus, 2000). Spine formation and motility are well known to decline during the critical period of the visual cortex and in the adult visual cortex spines are remarkably stable (Grutzendler et al., 2002); recently, the decline of spine motility has been shown to be delayed by lack of visual experience (Majewska and Sur, 2003). Therefore, an action on dendritic maturation and spine plasticity could be a possible cellular mechanism of enriched environment on visual cortical development and plasticity.

Other molecules potentially important for the effects of enrichment are NMDA receptors (NMDAR), which have a crucial role in ocular dominance plasticity and in development of receptive fields properties in the visual cortex (Bear et al., 1990; Roberts et al., 1998; Ramoa et al., 2001; Fagiolini et al., 2003). NMDAR subunit expression in the hippocampus is influenced by maternal care (Liu et al., 2000) and if this is also true for the visual cortex NMDAR could be involved in mediating the effects of increased maternal care on visual cortical development. Interestingly, NMDAR appear to be involved in regulating spine formation and stabilization (Kim and Lisman, 1999; Sala et al., 2001).

In conclusion, we propose a model in which two distinct temporal phases during pup development are differently controlled by the richness of the environment (Fig. 8). In the first phase, enhanced levels of maternal care may elicit some molecular changes in the brain of enriched pups that can trigger the precocious development of their visual system. Later, after the earlier eye-opening (~P12), when pups become to actively explore the surroundings, the complex sensory-motor stimulation provided by enriched environment may directly influence visual system development, for instance, inducing the precocious CRE-mediated gene expression in the visual cortex (Cancedda et al., 2004). A direct influence of enriched environment could have a major role in visual acuity acceleration and enhancement in EC, even if a contribution of differential maternal care cannot be excluded, as also previously suggested by Prusky et al. (2000a). The importance of this later phase of visual system development based on direct interaction between the developing subject and the

complexity of the environment is underlined by a recent work showing that postweaning enrichment lead to normal visual acuity maturation in dark reared rodents (Bartoletti et al., 2004).

Furthermore, the persistence of this effect of enrichment on the functional output of the visual system was investigated here by measuring visual acuity in differentially reared middle-aged mice. We found that the increased visual acuity elicited by environmental enrichment from birth was long lasting, also persisting during aging. Comparing visual acuity at 12 months of age to that recorded in young by Prusky et al. (2000a) shows comparable visual loss associated with aging for standard condition and enriched mice (respectively 15% and 25% of the values recorded in young), while the performance of enriched mice was much better than that of standard mice in learning the associating task in the visual water box. Given that both environmental enrichment (started even at old ages) and enhanced maternal care during development have been associated with long-lasting improved cognitive function (Liu et al., 2000; Frick and Fernandez, 2003; Frick et al., 2003), it is difficult to distinguish the different contribution provided by these treatments in ameliorating the learning abilities of EC mice studied in the present work. Future studies in which differential rearing is limited to only the first two weeks of life (i.e. until eye-opening, the period during which different maternal care levels were demonstrated) should provide further insights into the different contributions exerted by maternally mediated effects and by the direct interaction of pups with the environment on visual system development.

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