Brain structural and functional development: genetics and experience

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ABBREVIATIONS

BDNF	Brain-derived neurotrophic factor
GABA	γ-Aminobutyric acid
IGF-1	Insulin-like growth factor 1
NMDA	*N*-methyl-D-aspartate

Brain development is the result of the combined work of genes and environment. In this paper we first briefly discuss how, in terms of cellular and molecular plasticity mechanisms, the richness of early environment can control developmental trajectories and can induce long-term changes in neural circuits that underlie enduring changes in brain structure and function. We then see that experience most effectively moulds neural circuit development during specific time windows called critical periods. After the closure of these privileged windows for plasticity, it is very difficult to promote repair from 'errors' in brain development. As an example, congenital cataracts, refractive defects, or strabismus, if not precociously corrected during development, cause permanent deficit in visual acuity of the affected eye, a condition known as amblyopia. Little or no recovery from amblyopia is possible in the adult. However, recent results show that by using protocols of enriched environment it is possible to design interventions, which, by acting on specific plasticity factors, enhance adult cortical plasticity and allow recovery from amblyopia. This suggests that a better knowledge of how experience and environment engage endogenous plasticity factors could help to design interventions aimed at promoting recovery from neurodevelopmental defects, even after the end of critical periods.

EXPERIENCE-DEPENDENT PLASTICITY AND BRAIN DEVELOPMENT

Brain development results from the combined work of genes and environment, where genes have the role of guiding the initial steps of brain development (e.g. neural tube formation and subdivision in specific regions) and the initial formation of neural connections and neural circuits. To express correctly the developmental programs underlying brain development, gene expression has to be tightly controlled. An important control on the probability of gene expression during neurodevelopment is exerted by epigenetic mechanisms, such as DNA methylation and histone acetylation; indeed, key processes such as cell fate specification, neurogenesis, and myelination are highly regulated at the epigenetic level.[1]

In recent years we have learned that epigenetic mechanisms can be affected by interaction of the developing subject with the environment.[2] Indeed, experience has the crucial role of guiding the final maturation of neural circuits and behaviour.[3–6] Visual system development has been a paradigmatic model in studying experience-dependent development:[3,4,6–8] in the absence of visual experience, as may be the case for children born with dense bilateral cataract[7] or for experimental deprivation of vision from birth in mammals (dark rearing[8]), visual cortical circuits do not complete their maturation and the developmental increase in visual acuity does not occur; if visual experience is defective, as in subjects with monocular cataract, refractive defects, or strabismus, development of visual cortical circuits is altered, causing a strong deficit in visual acuity of the affected eye, together with a strong reduction of binocular vision.[4–7]

The capability of experience to shape neural circuit development relies on the property of developing neural circuits to be modified, structurally and functionally, by experience-evoked neural activity, a process called neural plasticity. During development, enhanced levels of neural plasticity are crucial for experience to drive the maturation of neural circuits and functions.[3–6]

Steps leading to long-term experience-dependent plasticity in neural circuits involve several cellular and molecular factors, which include activation of glutamate *N*-methyl-D-aspartate (NMDA) receptors, balance between excitation and inhibition, neurotrophin action, and intracellular signalling pathway activation, eventually leading to gene transcription and, by epigenetic modifications, to long-term changes in gene transcription probability.[3–6] If one of these factors is missing, owing to genetic defects or experimental manipulations, experience might fail in successfully guiding neural circuit development. For instance, even in the presence of a normal visual experience, visual acuity does not mature if the action of nerve growth factor is blocked.[9] Thus, visual experience needs molecular 'tools' to carry on its role of guiding neural circuit development.

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Examples of experience/activity-dependent development are ubiquitous in the brain. More recently than in sensory systems, the presence of activity-dependent remodelling of developing connections has been shown for the corticospinal system,[10] suggesting the presence of activity-dependent competition between ipsi- and contralateral corticospinal system axons. In the case of an early brain lesion, the presence of activity-dependent competition would add to the effect of the lesion[11] and one consequence could be a remodelling of corticospinal system inputs similar to that found after cortical silencing, namely withdrawal of crossing fibres from the lesioned cortex and maintenance of ipsilateral ones. This type of reorganization in infants is associated with a worse motor outcome.[11]

This allows making a final comment on the first part of this paper: higher levels of plasticity, as found in very young animals, are not intrinsically an advantage. Plasticity in itself is neither good nor bad: it is simply the potential for changes in connectivity, it obeys 'electrical' and molecular rules, but whether the outcome of this change is adaptive or maladaptive will depend on how this potential for change is harnessed.

ENRICHED EARLY EXPERIENCE AND BRAIN DEVELOPMENT

Maternal influence can be considered one of the most important early sources of experience for the developing subject, regulating physical growth and promoting neural maturation of brain structures involved in cognitive functions.[12] One of the best-characterized effects of maternal influence is the long-term effect exerted on the hypothalamus–hypophysis–adrenal system of the offspring: once adult, the offspring of mothers providing high levels of maternal care show reduced fearfulness and stress levels compared with those displayed by offspring born from and cared for by less attentive mothers.[12] This effect is linked to epigenetics: Meaney and co-workers[12] have shown that differences in the hypothalamus–hypophysis–adrenal response to stress displayed by the offspring of highly or less caring mothers depend on different amounts of DNA methylation of the promoter of the glucocorticoid receptor gene in the hippocampus. This explains the typical phenotype of reduced stress response exhibited by subjects that have experienced intensive maternal care levels during infancy, because the efficiency of the feedback control on the production of stress hormones is enhanced by the higher number of hippocampal glucocorticoid receptors.

Another approach used to study the effects of the environment on brain and behaviour has been the enriched-environment approach. Originally defined as 'a combination of complex inanimate and social stimulation',[13] enriched environment consists of wide and attractive cages where animals are reared in large social groups, with running wheels to allow voluntary physical exercise, and the presence of a variety of stimulating objects, regularly changed and substituted with others to stimulate explorative behaviour, curiosity, and attentional processes. The definition of enriched environment is based on the comparison with alternative rearing conditions, such as the standard condition, in which animals are reared in small social groups and in very simple cages where only nesting material, food, and water are present.[6] Crucial elements in the enriched environment setting are novelty, cognitive activity, motor activity, social stimulation, satisfaction, and reward.[6]

What this paper adds

- Neural plasticity is high during early developmental stages called critical periods.
- Recovery from early neural dysfunctions is limited after the closure of the critical periods.
- Enriching the environment enhances brain plasticity, promoting recovery from neurodevelopmental defects.

The first studies using enriched environment documented an increase in cortical thickness, an increase in spine density and in dendritic tree complexity, as well as effects on neuromodulators such as acetylcholine and noradrenaline, and an improvement in learning and memory performance; more recently, a strong enriched-environment effect on synaptic plasticity has been shown.[6] It is important to emphasize that the increase in dendritic spine density found in enriched animals is a sign of increase in functional synaptic connectivity, which correlates with enhanced behavioural performance with respect to age-matched controls and, as we shall see in the next paragraph, with promotion of brain development. It is therefore very different from the situation of excess dendritic spines bearing immature, philopodium-like aspects, owing to a failure of pruning and of synaptic consolidation, found in some developmental disorders (e.g. fragile × syndrome) and correlating with behavioural and cognitive abnormality; indeed, enriched environment can strongly ameliorate the morphological immaturity of dendritic spines in an animal model of fragile X syndrome.[14]

We have recently exploited the protocol of enriched environment to investigate the impact of early environment on brain development, using the developing visual system as a model.

Our first findings showed that visual acuity development is robustly accelerated in mice and rats born in enriched environment[6] and that this could be attributed to enriched environment acting on factors involved in V1 development and developmental plasticity. In particular, enriched environment very precociously increases brain-derived neurotrophic factor (BDNF) expression in V1,[6] and it was already known that a precocious BDNF increase, obtained by a transgenic approach in mice, accelerates visual acuity development.[15] Enriched environment also accelerates the maturation of intracortical GABAergic (γ-aminobutyric acid-related) inhibitory circuitry in V1, a crucial factor for visual acuity maturation. GABAergic intracortical inhibition is indeed involved in the maturation of visual cortical neuron receptive fields,

which underlies visual acuity development.[6,15] Intracortical inhibition development is delayed by dark rearing, which delays both V1 receptive field maturation and visual acuity development; on the contrary, GABAergic inhibition development is accelerated in mice with precocious BDNF increase, in good correlation with accelerated development of visual acuity.[15]

Later on, a crucial mediator of enriched-environment effects was found to be insulin-like growth factor 1 (IGF-1); indeed, enriched environment increases brain IGF-1, exogenous supply of IGF-1 mimics enriched-environment effects, accelerating visual acuity development, and blocking IGF-1 action in enriched animals blocks enriched-environment effects on visual acuity development.[6] IGF-1 seems to be upstream of BDNF and inhibitory GABAergic circuit development,[6] and mediates not only enriched-environment effects on visual development but also the effects of an enriched early experience provided by massage, which recapitulates key features of maternal care.[16] We found that enriching early experience by massage accelerates brain, and in particular visual, development both in rat pups and in human babies,[16] in correlation with an increase in IGF-1; blocking IGF-1 in rat pups blocks massage effects.[16]

The importance of IGF-1 as a 'master mediator' of enriched-environment control on experience-dependent development has been reinforced by Wang et al.[17] They found that a mismatch between two visual developmental processes, ocular dominance development, and binocular matching of orientation selectivity development, can be caused by overexpression of BDNF. BDNF acts on ocular dominance development and cortical plasticity decline but is unable to drive binocular matching of orientation selectivity, with negative results for the quality of binocular vision; enriched-environment exposure and IGF-1 are able to correct this mismatch, ensuring an harmonic development of all properties of visual cortical neurons.

Since the richness of the environment seems to promote the expression of those same molecules that experience exploits to drive visual development, we asked whether it could be possible to use enriched environment to promote the expression of these factors when visual experience is lacking, such as in dark rearing, to promote visual development. We found that this is indeed the case: animals that are dark reared but living in enriched environment show normal visual acuity maturation.[6]

USING THE ENVIRONMENT TO PROMOTE PLASTICITY IN THE ADULT BRAIN

Critical periods are windows of heightened neural plasticity during development. After closure of critical periods, neural plasticity becomes very low and experience loses much of its capacity to modify neural circuits; this has been shown for several developmental processes, including visual and auditory development and language acquisition.[3,5,6,18,19] Different species show different critical periods for the same function, in good accordance with the different time course of development and lifespan in these species. On the other hand, different functions show different critical periods in the same species, in good accordance with the different time course of development for different brain areas.

Why does neural plasticity decline with closure of critical periods? Mechanistically, this is due to the progressive increase in the level of several factors exerting a brake on brain plasticity, such as intracortical GABAergic inhibition, extracellular matrix components like chondroitin sulphate proteoglycans (corticospinal system proteoglycans), cholinergic system-linked lynx protein, and a progressive decrease in plasticity factors, most notably epigenetic factors, such as histone acethylation.[3,18,19] The decline in plasticity can be explained in terms of the necessity to provide stability of neural functions once plasticity has fulfilled its main task, namely allowing experience to guide neural circuit maturation during critical periods.[3,19]

One of the most important consequences of the existence of critical periods is that if experience has been insufficient or inappropriate, with the consequent abnormal development of neural circuits and functions, even if the appropriate experience were to be provided after critical period end, the low levels of brain plasticity would prevent neural circuits changing and resuming typical development.[8,20]

Currently, a much discussed question in brain repair research is whether it is possible to correct the results of an incorrect development after critical period end. To study this possibility, we have exploited the model of critical period for recovery from amblyopia.[6] Congenital cataracts, refractive defects, or strabismus, if not precociously corrected during development, cause permanent amblyopia: little or no recovery from amblyopia is possible in the adult.[3,6,18,19] If the low levels of plasticity after critical period end are responsible for the limited recovery normally found in adults, enhancing plasticity should help to promote recovery. To boost plasticity, it could be useful to act on those factors that control critical period closure and limit adult plasticity.[3,6,18,19] Indeed, pharmacologically or genetically modulating these factors, bringing them back towards juvenile levels, enhances adult visual cortical plasticity and allows recovery from amblyopia. This has been shown for levels of intracortical inhibition, presence of chondroitin sulphate proteoglycans, which limit structural plasticity, myelin, histone acetylation, and lynx protein (see Sale et al.[6] and Bavelier et al.[19] for reviews).

For instance, promoting histone acetylation or degrading chondroitin sulphate proteoglycans in adult V1, coupled with reverse suture (reopening the formerly deprived eye and closing the fellow eye), promotes a complete recovery of visual acuity in adult animals made amblyopic by monocular deprivation since critical period beginning.[21,22] In control animals, subjected to reverse suture but receiving a control treatment, visual acuity of the deprived eye remains low, with no sign of recovery from amblyopia.

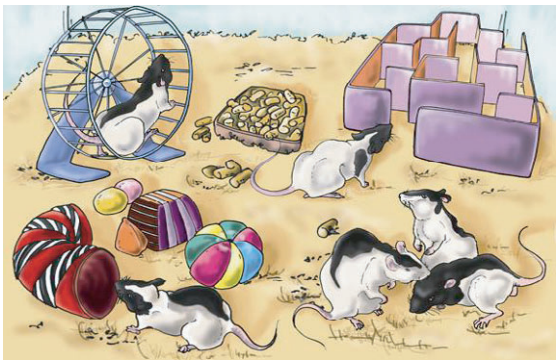
Because enriched environment acts on the same plasticity factors that have been targeted pharmacologically, including intracortical inhibition, chondroitin sulphate proteoglycans,

and epigenetic factors, we tested whether enriched environment could promote recovery from amblyopia in adult rats. We found that adult amblyopic rats subjected to reverse suture and exposed to enriched environment for 3 weeks undergo a full recovery of their visual functions, both in terms of visual acuity and binocular vision (Fig. 1).[6]

PERCEPTUAL LEARNING AND RECOVERY FROM AMBLYOPIA

Another protocol that might engage cortical plasticity and that might be considered akin to enriched environment is perceptual learning. According to a classic definition, perceptual learning is the ability to improve performance with practice in a perceptual task. We recently set up a protocol for visual perceptual learning in rodents. The task consisted of discriminating the spatial frequency of a test grating from that of a reference grating with a two-alternative forced-choice procedure in a Prusky water box.[23] We started with a pair of gratings of very different spatial frequency and, once the animal had learnt to swim reliably towards the monitor displaying the low-frequency reference grating, we progressively increased the spatial frequency of the test stimulus, thus reducing the difference between the spatial frequencies of the test and the reference gratings. The minimum spatial frequency difference discriminable was taken as the discrimination threshold. We showed that animal performance improved with practice, reaching a plateau after a few days of training.[23]

We found that visual perceptual learning engages synaptic plasticity in V1, increasing the synaptic strength of intracortical connections with respect to controls, a process that mimics long-term potentiation. Accordingly, the physiologically potentiated connections were resilient to further potentiation in response to artificial electrical stimulation,[20] showing that potentiation of V1 intracortical connections by perceptual learning occludes long-term potentiation. Interestingly, it is the incremental training, the practice in discriminating increasingly less different



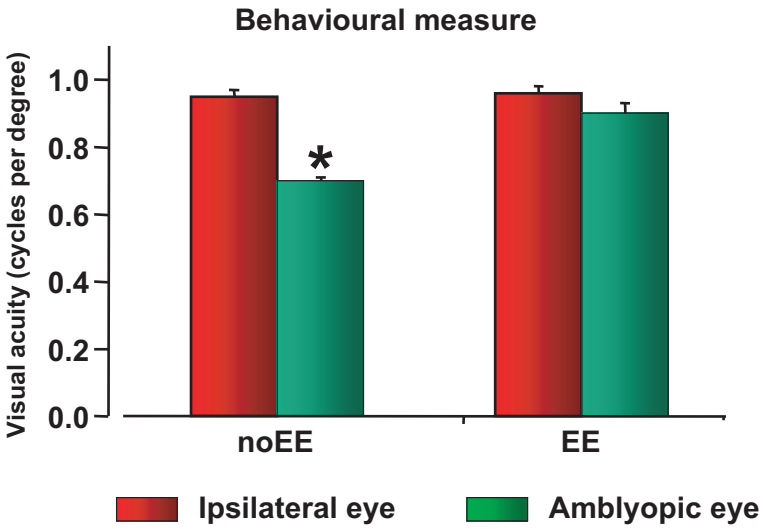


Figure 1: Environmental enrichment promotes recovery from amblyopia in adult rats. The graph reports results obtained with a behavioural assessment of visual acuity in the visual water box. Visual acuity was lower in the formerly deprived eye than in the other eye in non-enriched rats (paired *t*-test, $p<0.05$), whereas no difference was found between the visual acuity of the previously amblyopic eye and that of the fellow eye in enriched animals (paired *t*-test, $p=0.100$). *Statistical significance ($p<0.05$). Error bars, standard error of the mean. EE, environmental enrichment; noEE, no environmental enrichment.

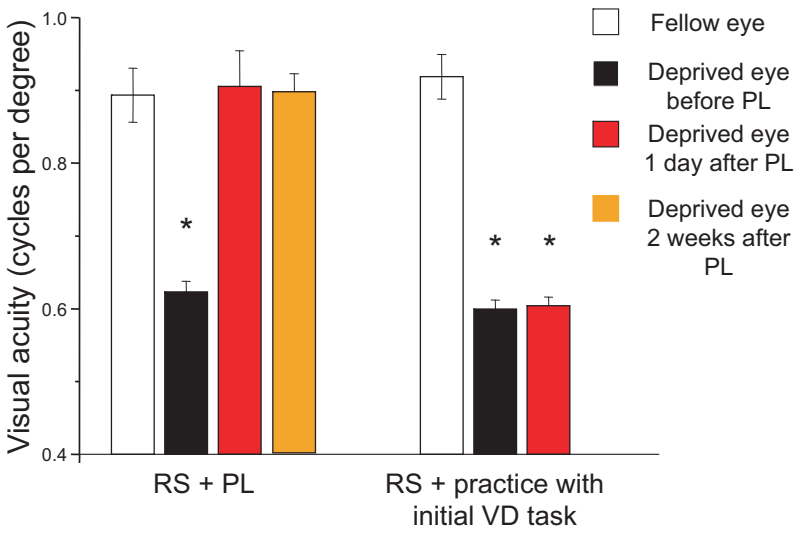


Figure 2: Perceptual learning promotes amblyopia recovery in adult rats. The graph reports results obtained with a behavioural assessment of visual acuity in the visual water box. Visual acuity was lower in the formerly deprived eye than in the fellow, non-deprived eye immediately after reverse suture; after perceptual learning practice, no difference is present between the visual acuity of the previously amblyopic eye and that of the fellow eye, as assessed both 1 day after the end of perceptual learning practice and 2 weeks later (one-way analysis of variance [ANOVA]); by contrast, just learning and practising the initial visual discrimination task, with no incremental training, was ineffective in promoting recovery of acuity (one-way ANOVA). *Statistical significance with respect to visual acuity of the fellow eye. Error bars, standard error of the mean. PL, perceptual learning; RS, reverse suture; VD, visual discrimination.

spatial frequencies, that leads to perceptual learning and to strengthening of V1 connections.[23]

Thus, visual perceptual learning enhances the strength of V1 synaptic connections and promotes their potentiation. It could therefore be possible that practising perceptual learning with the amblyopic eye might promote potentiation of its efficacy in driving cortical neurons, promoting recovery from amblyopia in adult animals. We found that this is indeed the case. Visual acuity was assessed first for the non-deprived eye, with the deprived eye still closed; then, after reverse suture, visual acuity of the deprived eye was measured immediately before and then after the end of the perceptual learning procedure. Only perceptual learning caused an increase in visual acuity for the deprived eye, which returned to normal levels. Just learning and practising the initial step of the visual discrimination task, which does not potentiate V1 intracortical connections, was ineffective in promoting recovery of acuity.[20] The improvement caused by perceptual learning was still evident 2 weeks after the end of perceptual learning, suggesting that the effects of perceptual learning outlast the training period (Fig. 2). Interestingly, intracortical inhibition in V1 was reduced in animals undergoing perceptual learning, suggesting that perceptual learning acts through a modulation of this key factor.[20]

Perceptual learning has been recently used in humans with amblyopia, with good and long-lasting effects on amblyopic eye performance (see Bavelier et al.[19]). Our results provide a possible mechanism of action that goes towards a better understanding of training effects and might help in designing better recovery strategies.

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