



UNIVERSITÀ  
DEGLI STUDI  
FIRENZE

Dipartimento di Neuroscienze, Psicologia, Area del Farmaco  
e Salute del Bambino

DOTTORATO TOSCANO DI NEUROSCIENZE  
CICLO XXX

Coordinatore Prof. Renato Corradetti

**Environmental enrichment, BDNF and experience-dependent epigenetic regulation of  
visual cortex plasticity in juvenile and adult rats**

Settore Scientifico Disciplinare "M / PSI-02"

**Dottorando**

Dott. Gabriele Sansevero

**Tutor**

Prof. Giovanni Cioni  
Dott. Alessandro Sale

Anni 2014/2017

## Table of contents

<b>1) Introduction.....</b>	<b>pag. 1</b>
<b>1.1) Developmental brain plasticity.....</b>	<b>pag. 1</b>
1.1.1) <i>Critical Periods or the right time to experience.....</i>	<i>pag 2</i>
1.1.2) <i>Disrupted visual experience during Critical Period.....</i>	<i>pag. 2</i>
1.1.3) <i>Physiological mechanisms of brain plasticity.....</i>	<i>pag. 5</i>
1.1.4) <i>Molecular substrates of ocular dominance plasticity.....</i>	<i>pag. 14</i>
1.1.5) <i>Experience-dependent plasticity in adult visual cortex.....</i>	<i>pag. 24</i>
<b>1.2) Impact of Environmental Enrichment on brain development and plasticity:         physiological and molecular aspects.....</b>	<b>pag. 26</b>
1.2.1) <i>Effects of environmental enrichment on brain anatomy.....</i>	<i>pag. 27</i>
1.2.2) <i>Environmental enrichment modifies electrophysiological responses.....</i>	<i>pag. 29</i>
1.2.3) <i>Environmental enrichment modulates gene expression.....</i>	<i>pag. 31</i>
1.2.4) <i>Effects of environmental enrichment on nervous system disorders.....</i>	<i>pag. 37</i>
<b>1.3) A new research paradigm: environmental enrichment and visual system         plasticity.....</b>	<b>pag. 40</b>
1.3.1) <i>Relationship between environment and developmental plasticity in the brain.....</i>	<i>pag. 40</i>
1.3.2) <i>Environmental enrichment and adult cortical plasticity.....</i>	<i>pag. 43</i>
<b>1.4) Aims of the Thesis.....</b>	<b>pag. 48</b>
<b>2) Material and methods.....</b>	<b>pag. 50</b>
<b>2.1) Part one: Experience affects CP plasticity in the visual cortex through an epigenetic         regulation of histone post-translational modifications.....</b>	<b>pag.50</b>
2.1.1) <i>Monocular deprivation.....</i>	<i>pag. 50</i>
2.1.2) <i>Rearing environments.....</i>	<i>pag.50</i>
2.1.3) <i>Single-unit recording.....</i>	<i>pag. 51</i>
2.1.4) <i>Injections of suberoylanilide hydroxamic acid.....</i>	<i>pag. 53</i>
2.1.5) <i>Visual cliff task.....</i>	<i>pag. 53</i>

2.1.6) Western blot.....	pag. 54
2.1.7) Chromatin immunoprecipitation.....	pag. 55
2.1.8) Real-time PCR.....	pag. 55
<b>2.2) Part two: Intranasal BDNF administration promotes visual function recovery in adult amblyopic subjects.....</b>	<b>pag. 56</b>
2.2.1) Animal treatment and surgical procedures.....	pag. 56
2.2.2) Intranasal administration protocol.....	pag. 57
2.2.3) <i>in vivo</i> electrophysiology.....	pag. 58
2.2.4) Behavioural assessment of visual acuity.....	pag. 59
2.2.5) Behavioural assessment of stereopsis in the visual cliff task.....	pag. 60
2.2.6) Western blot.....	pag. 61
2.2.7) Drug administration.....	pag. 62
<b>3) Results.....</b>	<b>pag. 63</b>
<b>3.1) Part one: Experience affects CP plasticity in the visual cortex through an epigenetic regulation of histone post-translational modifications.....</b>	<b>pag. 63</b>
3.1.1) OD plasticity in developing rats.....	pag. 63
3.1.2) EE enhances histone acetylation in the primary visual cortex.....	pag. 68
3.1.3) Histone acetylation at the BDNF gene promoter.....	pag. 70
3.1.4) A causal link between histone acetylation and CP plasticity.....	pag. 72
3.1.5) EE in adulthood induces H3 acetylation and reinstates plasticity in the rat visual cortex .....	pag. 73
<b>3.2) Part two: Intranasal BDNF administration promotes visual function recovery in adult amblyopic subjects.....</b>	<b>pag. 78</b>
3.2.1) Evaluation of BDNF pro-convulsive side effects.....	pag. 78
3.2.2) Intranasal BDNF infusion increases BDNF levels in V1.....	pag. 78
3.2.3) Visual function recovery in adult rats with reverse-occlusion.....	pag. 80
3.2.4) Visual function recovery in adult rats with unrestricted binocular sight.....	pag. 83
3.2.5) Blockade of BDNF signalling in V1 prevents recovery from amblyopia in BDNF-treated Rats.....	pag. 88

<b>4) Discussion.....</b>	<b>pag. 90</b>
<b>4.1) Part one: <i>Experience affects CP plasticity in the visual cortex through an epigenetic regulation of histone post-translational modifications</i>.....</b>	<b>pag. 90</b>
<b>4.2) 4.2) Part two: <i>Intranasal BDNF administration promotes visual function recovery in adult amblyopic subjects</i>.....</b>	<b>pag. 94</b>
<b>Appendix: <i>Scientific Production during the PhD</i> .....</b>	<b>pag. 98</b>
<b>References.....</b>	<b>pag. 100</b>

## **Introduction**

Environment has become a pivotal factor in biological studies since Charles Darwin proposed the theory of evolution in his masterpiece “On the origin of the species”[1]; at that time environment was interpreted as an agent able to influence populations and large numbers of individuals over the generations. In modern neuroscience, it is known that environment deeply interacts with single entities at any stage of their life: it exerts profound effects on the brain and, remarkably, it has been demonstrated that stimulating environmental conditions are able to affect brain plasticity with beneficial consequences throughout the entire lifespan. In this section I will provide an overview about the relationship between environment and plasticity, mostly focusing on the visual system which, over time, has become a milestone in this field of studies.

### **1.1) Developmental Brain Plasticity**

Brain plasticity can be defined as the capacity of neurons and neural circuitries to change functionally and structurally, in response to experience. This capability is fundamental for learning and memory processes, behavioural adaptability, brain development or repair. Experience, in form of external stimuli, is translated in patterns of electrical activation by neurons; through these patterns plasticity is able to reshape neuronal functionality at both molecular and physiological level: it can modulate the expression of ions channels and membrane receptors, modifying the efficacy of already existing synapses, moreover it can determine the formation of new synaptic contacts or the extinction of existing ones. At larger scale plasticity can influence the dendritic or axonal arborisation or cause the release of neuromodulators that can influence cells’ surrounding environment.

### *1.1.1) Critical Periods or the right time to experience*

Genes and environment cooperate during early phases of development to sculpt brain connections and functions. In this period, developing circuits are extremely sensitive to external stimuli and experience is able to severely influence their mature functionality, particularly during well-defined temporal windows called “critical periods”(CP) [2],[3],[4][5][6]. How early experience transforms the immature nervous systems into the precise patterns of connectivity that are required to mediate adaptive behaviour is a question of great interest for Neuroscience. Experimental evidence has shown that different regions of the brain have critical periods that occur at different times and are activated and regulated by distinct mechanisms[7]. The prevailing way to observe the heightened plasticity in developing brain and to investigate the basic mechanisms has been to deprive a subject of experience in one sensory modality and to investigate the consequences of this procedure on sensory development.

### *1.1.2) Disrupted visual experience during Critical Period*

Among sensory systems, visual cortex has been the leading model to investigate experience-dependent plasticity since Torsten Wiesel and David Hubel demonstrated for the first time that occluding one eye in developing kittens, a procedure called monocular deprivation (MD), dramatically threatened the binocularity properties of cortical neurons [8], causing an impressive loss in the number of neurons driven by the deprived eye and an increase in the number of neurons responding preferentially to stimuli presented to the open eye. Even if a large number of neuronal cells in the visual cortex receive input from both eyes, they show different degrees of preference for one of them, a property defined ocular dominance, OD. During the CP loss of sensory experience in one eye results in a marked shift of OD in favour

of the non-deprived one. In parallel with this lack of ability to drive cortical neurons, the deprived eye shows a strongly reduced visual acuity and its contrast sensitivity is blunted: the deprived eye becomes amblyopic[8],[9],[10],[11],[12]. It is worth to notice that spatial resolution in the deprived retina remains completely unaffected, suggesting that the modifications at the basis of the amblyopia occur at cortical level[13],[14],[15],[16]. Hubel and Wiesel observed that, in kittens, the susceptibility to MD changes with age, beginning abruptly near the start of the fourth week of life, being most robust during a specific time window (between sixth and eighth weeks) and then declining. It is possible to reverse the effects of MD by removing the eye occlusion but only during the CP; after CP closure, these effects become irreversible [17],[18],[19],[20],[21],[2]. There is little or no recovery from amblyopia in the adult. Moreover MD starting in adulthood produces no detectable outcome [9],[12].

At the anatomical level, juvenile MD causes a reduction of territories in the cortical layer IV which are driven by the deprived eye and innervated by lateral geniculate nucleus (LGN) with a subsequent expansion of those driven by the open eye [22]. However, the reorganization of the geniculo-cortical projections may not be the first modification induced by MD as it was believed in the past years: anatomical and functional changes occur within one day from the start of MD in cortical layers II and III, but not in layer IV [23],[24]. These findings suggest that MD elicits the first modifications at level of cortical neurons in the intracortical horizontal connections and that these changes are reflected in subsequent reorganization of the geniculo-cortical afferents.

MD effects on the OD of cortical neurons have been also demonstrated in rodents: the physiological responsiveness of neurons in the binocular zone of V1 shift towards the open eye, and this plasticity is confined to a well-defined critical period [25],[26],[27]. As in

other species, the OD shift in rodents is found in all layers, but it is more pronounced in extra granular layers than in layer IV [27], suggesting that intracortical as well as geniculocortical synapses undergo plasticity following MD. In the developing visual cortex of the mouse, this functional plasticity is accompanied by anatomical changes, as in higher mammals [28]. Moreover, it has been shown by in vivo two-photon microscopy that spine motility in the binocular region of V1 contralateral to the deprived eye is 35% higher than motility in nondeprived animals [24]. This increased spine motility may reflect structural destabilization which could precede a robust pruning of spine protrusions, probably correlated to the rapid reduction in the deprived-eye drive [29].

Another widely used paradigm to study the role of experience and environment on the development of the visual cortex is dark rearing (DR). In this protocol, animals are reared from birth in complete darkness, no visual information is available, and only the spontaneous electrical activity is present along the visual pathways. Animals reared in DR show an abnormal functional and anatomical maturation of their visual cortex, that appears immature far beyond the end of the CP. Dark-reared animals display several physiological deficits including a rapid habituation of visual cortical neurons, i.e. the visual response tends to disappear with repetition of stimulus presentations [30],[26]. Receptive fields of visual cortical cells are larger than normal, and neurons have a reduced orientation selectivity [26], anatomically ocular dominance columns are immature [30],[31], the visual spatial resolution (visual acuity) of dark-reared animals is extremely low, as measured electrophysiologically and behaviourally [26],[32]. Other parameters of visual responses are changed: cell responsiveness is lower, latency of visual response is increased and spontaneous activity is increased in DR animals [33],[32]. A total lack of visual experience also affects the fine morphology of visual cortical neurons, which exhibit



alterations in dendritic arborisation and in the size, morphology and density of dendritic spines [34],[35]. Light exposure, even if just for few hours, restores a regular developmental process, allowing the recovery of both neuronal response properties [36],[37] and normal anatomical features [35].

### *1.1.3) Physiological mechanisms of brain plasticity*

Once it has been defined the crucial role of experience and the existence of a CP during the development of visual system, it is necessary to understand what is changing in the nervous system following alterations in visual experience. However, changes in environmental experience can affect the sensory systems at multiple levels (deprivation may also affect orientation and direction selectivity) and the synaptic modifications underlying a shift in OD may occur at multiple synapses. Furthermore, there may be multiple forms of synaptic plasticity [38]. In conclusion there are several caveats to consider in establishing that variations in cortical responsiveness are due to particular synaptic modifications.

Wiesel and Hubel proposed a mechanism in which OD plasticity operates through a competitive interaction between inputs from the two eyes for the control of cortical neurons, depending on the activity state of the postsynaptic neurons. This hypothesis was supported by the fact that binocular lid suture is not effective to shift OD columns in mammals [17],[27],[39]. An experiment performed by Stryker's laboratory showed that patterned vision is not necessary for visual cortical plasticity, and that an imbalance in spontaneous retinal activity alone can produce a significant OD shift, thus supporting the competitive view [40]. In addition, a reversible blockade of the discharge activities of cortical neurons by intracortical infusion of tetrodotoxin (TTX) or muscimol completely prevents the OD shift that would normally be seen after MD, or causes a paradoxical shift in favour of the

deprived eye [41],[42],[43],[44]. However, the mechanisms underlying binocular competition have remained elusive. The classic competition-based model is related to heterosynaptic mechanisms, where open eye inputs reduce the synaptic efficacy of the deprived inputs[45],[46]. Previous studies have implicated activity-dependent uptake of neurotrophins, as the mediator of binocular competition [47], but subsequent experiments have shown that neurotrophins actually have cell-specific effects, such as the regulation of inhibitory circuitry, which may provide an alternative explanation of their importance for OD plasticity [48], [49]. Chronic electrophysiological recordings in mice at the peak of the CP indicate that binocular competition may actually be the consequence of separable processes with distinct time courses mediating depression of deprived-eye and potentiation of non deprived-eye responses [50],[51],[52],[53].

***The homosynaptic view.*** It is tempting to speculate that the loss or gain of visual responsiveness of neurons in V1 during the critical period is simply the result of homosynaptic long-term depression (LTD) or potentiation (LTP) of excitatory connections somewhere in the visual circuit [54]. However, the role of LTP and LTD in OD plasticity is hotly debated. The induction of LTP has been extensively demonstrated at multiple synapses of the visual cortex *ex vivo*, although the mechanism appears to vary across layers [55]. Additionally, in rats, NMDA receptor (NMDAR)-dependent LTP can be induced in layers II/III and IV *in vivo* following tetanic stimulation of LGN, and this LTP is sufficient to increase the magnitude of visually evoked responses [56], suggesting that homosynaptic LTP, possibly at thalamocortical synapses, can mimic the effects of open-eye potentiation after MD. Many manipulations known to disrupt homosynaptic LTP have been applied during OD plasticity. One example of this is the finding that OD plasticity is disrupted in mice with either disrupted  $\alpha$ CaMKII autophosphorylation or lacking the protein entirely, which suggests a role

for LTP [27]. Similarly, open-eye potentiation is absent in mice with a postnatal deletion of NR1 targeted to cortical layers II-IV [57]. Further suggestions come from the recently discovered phenomenon of stimulus-selective response potentiation: in juvenile mice, the magnitude of visually driven thalamo-cortical responses in layer IV increases following repeated presentation of an oriented stimulus and this potentiation is dependent on NMDAR activation. Moreover, it has been shown that GluR1 delivery to synapses, that is crucial for LTP, is required for visual experience-dependent plasticity [58].

Stronger evidence exists that LTD-like mechanisms influence depression of deprived-eye responses. The biochemical signature of LTD (in terms of AMPA receptor phosphorylation and cell-surface expression) has been used as a 'molecular fingerprint' to ask whether similar changes occur in visual cortex following a period of MD. To date, this has been examined in the rat visual cortex and the results support the hypothesis that MD induces this type of LTD in visual cortex [59],[60]. A second approach to address whether LTD is induced by MD is to ask whether naturally occurring synaptic depression in vivo occludes LTD ex vivo. This issue has been recently examined in rodents: LTD measured in slices is reduced (occluded) by 3 days of MD in vivo in both layer IV and II/III [61]. Furthermore, the reduction in deprived-eye responses after lid suture is likely due to hebbian processes, as monocular inactivation with TTX (which prevents decorrelated inputs) blocks this depression [50]. However, the question of the relative contribution of this synaptic modification to the functional consequences of MD is still controversial.

An approach to this question has been to correlate deficits in LTD and OD plasticity in genetically or pharmacologically modified mice. A mutation that disrupts LTD dependent on metabotropic glutamate receptor (mGluR) does not alter the normal OD shift in response to MD. GAD65 knockout mice, which lack normal OD plasticity, show no deficit in induction of

LTP or LTD in layer II/III of mouse binocular visual cortex [62], while similar studies at younger ages show an impairment of LTD [63]. A dissociation of LTD and OD plasticity has been suggested also by the study of several protein kinase A (PKA) regulatory subunit mutants. For example, the RI $\beta$  knockout mouse has a deficit in layer II/III LTD but exhibits a normal OD shift after 4 days of MD [64]. However, two additional studies deleting both of the two RII subunits of PKA do not clarify all the mechanisms. RII $\alpha$  knockout mice display normal LTD in layer II/III, whereas both LTP and OD plasticity are reduced [65]. By contrast, RII $\beta$  knockout mice exhibit normal LTP at the same synapse, but lack both LTD and OD plasticity [66]. Also calcineurin, the only known Ca<sup>2+</sup>/calmodulin-activated protein phosphatase in the brain, has been identified as a molecular constraint involved in OD plasticity, but a transient increase in calcineurin activity, that prevents the shift of responsiveness in the visual cortex following MD, does not impair LTD induction. Given that many different plasticity mechanisms exist in the visual cortex [55], it is likely that a large portion of these seemingly conflicting results may be attributable to laminar differences between the molecular pathways supporting LTD and LTP, but essentially the LTD/LTP mechanisms alone are unlikely to account for the OD plasticity.

Several alternative hypotheses have been advanced to enlighten all the aspects of OD plasticity. For example, balanced levels of excitation and inhibition have shown to be critical for enabling plasticity [4],[67].

***Excitatory-inhibitory balance.*** In all species tested so far, anatomical and physiological evidences indicate that synaptic inhibition matures later than excitatory transmission in the neocortex [68],[69],[70],[71]. By controlling excitation, GABAergic circuits are ideally posed to control the engagement of activity-dependent synaptic modification. Thus, the mismatch in the maturation of excitation and inhibition may define a window of opportunity for

activity-dependent plasticity to occur. Taking advantage of gene-targeting technology, this hypothesis has been directly tested by reducing GABA synthesis or by prolonging glutamatergic synaptic responses, both adjustments yielding a similar shift of balance in favour of excitation in vivo. Mice carrying a targeted disruption of the GAD65 gene show an identical OD distribution to wild-type mice; however, the response to a brief period of MD during the CP is strikingly different. These mice, indeed, show no shift in their responsiveness in favour of the open eye and the cortical neurons continue to respond better to the contralateral eye input. Enhancement of inhibition obtained by local delivering of diazepam produces a complete OD shift in the infused mutant visual cortex [62].

Equally, in NR2A knockout mice, OD distribution is similar to control animals. Unlike GAD65 knockout mice, brief MD is able to induce a slight shift in favour of the open eye but, interestingly, the overall magnitude of this plasticity is significantly weakened. Long-term MD (> 2 weeks) produces no further shift, confirming that saturation is reached within 4 days. Also in this case, diazepam infusion concomitant with MD fully rescues OD plasticity [72]. A direct physiological consequence of excitatory-inhibitory unbalance in GAD65 and NR2A KO mice is enhanced activation in response to visual stimulation, as assessed by the observation that visual cortical neurons display a tendency for prolonged discharge outlasting the visual stimulus [72]. Whereas a robust prolonged discharge appears throughout life in both mutants, it is only evident early in life in wild-type animals before the CP, when intrinsic inhibition is weak and OD plasticity is absent. With the natural appearance of OD plasticity during the CP in wild-type mice, the prolonged discharge drops off sharply [73]. Taken together, these results indicate that a delicate balance between excitation and inhibition intrinsic to visual cortical circuits is necessary to detect the imbalanced activity between competing inputs from the deprived and non-deprived eyes.

Consistent with this view, the onset of the CP can be accelerated in wild-type animals by premature enhancement of GABA-mediated transmission [73]. Moreover, feedforward inhibition can enhance the precise timing of postsynaptic firing [74]. Specific spike timing-dependent windows for synaptic plasticity have been elucidated in developing and neocortical structures [75]. Spike-timing forms of plasticity rely upon physiologically realistic, millisecond-scale changes in the temporal order of pre- and post-synaptic action potentials. Prolonged discharge in both NR2A and GAD65 knockout mice would impair plasticity by altering the pattern of neural activity encoding visual input. The normal development of inhibitory circuitry, as well as diazepam infusion in transgenic mice, improve temporal processing of sensory input, allowing OD shift in response to MD to take place [67].

It is worth to point out that during development the inhibitory tone surpasses two functional thresholds in the visual cortex: the first one enables OD plasticity and the second one causes the end of the CP. A recent study shows that pharmacological reduction of intracortical inhibition obtained through the infusion of either MPA (an inhibitor of GABA synthesis) or picrotoxin (a GABAA antagonist) directly into the visual cortex reactivates OD plasticity in response to MD in adult rats [76]. Moreover, also other manipulations resulting in reductions of cortical inhibition promote adult plasticity [77].

Among the vast diversity of GABAergic interneurons in the neocortex, two major subclasses of parvalbumin-containing cells target the axon initial segment and soma. Both are ideally placed to control either spike initiation (chandelier cells) or back-propagation (basket cells) required for synaptic plasticity in the dendritic arbour [78],[79]. Because distinct GABAA receptor subunits are enriched at these two parvalbumin-cell synapses, their individual contributions to visual cortical processing and plasticity have been identified by point mutations that selectively remove diazepam sensitivity: systematic use of the mouse

knock-in technique showed that only one of these subtypes, the  $\alpha 1$ -subunit-containing interneurons (i.e. basket cells), drives cortical plasticity [80].

Two scenarios centred on the parvalbumin-positive basket cells have been proposed [4]. One is an 'instructive' model, in which powerful, fast somatic inhibition edits one-by-one the action potentials that can pass into the dendritic arbour by back-propagation through the cell body. Inefficient gating by weak inhibition at the soma would prevent a competitive outcome by allowing excess back-propagation and spurious coincident activity with infrequent inputs from the deprived retina. Consistently with this model, large-basket cells extend a wide, horizontal axonal arbour that can span ocular dominance columns in cat visual cortex and, receiving input from one eye, inhibits targets of the other eye [81]. An alternative 'permissive' model is based on the observation that basket cells are organized in electrically-coupled networks, endowed with the ability to detect synchrony [82], [83]. Whereas simultaneous inputs (for example, from the same eye) rapidly co-excite cells through gap junctions, even a 2 ms input jitter (for example, between opposite eyes) is sufficient to dampen the coupling by reciprocal GABAA synapses, which are also enriched in  $\alpha 1$  subunits [84]. As a result, basket cells are maximally active on a columnar scale, time-locked to release growth or plasticity factors when strong synchronous activity arrives in the neocortex.

***Homeostatic synaptic plasticity.*** Homeostatic synaptic plasticity mechanisms are emerging as important complements to Hebbian forms of plasticity in the activity-dependent refinement of synaptic connectivity [85]–[87]. Homeostatic plasticity acts to stabilize the activity of a neuron or neuronal circuit against perturbations that alter excitability, providing a robust mechanism for generating stability in network function in the face of experience-related changes in synaptic input. Plasticity phenomena that conform to this definition of

homeostatic plasticity include the activity-dependent regulation on intrinsic neuronal firing properties [88], [89], pre- and post-synaptic forms of excitatory synaptic plasticity, such as synaptic scaling, that adjust all of a neuron's excitatory synapses up or down in the right direction to stabilize firing [85], [86], the balancing of excitation and inhibition within neuronal networks [90], compensatory changes in synapse number [91], [92], and meta-plastic mechanisms that adjust the relative threshold of LTP and LTD induction [93], [94].

The best studied mechanism of homeostatic regulation is synaptic scaling of excitatory synapses, which was first described in dissociated rat cortical cultures, where blockade of activity with TTX increases and blocking GABA-mediated inhibition decreases the amplitude of miniature excitatory postsynaptic currents (mEPSCs) [95]. Interestingly, the rules for synaptic scaling depend on the synapse type: inhibitory synapses onto pyramidal neurons are scaled in the opposite direction from excitatory synapses, suggesting that the firing rate is regulated through reciprocal changes in excitation and inhibition [96].

Homeostatic adjustments in synaptic strength include post-synaptic and pre-synaptic modifications in synaptic function [92], [95] and require that neurons sense and translate changes in activity into compensatory changes in synaptic strength; despite the nature of the activity signal that controls synaptic scaling in inhibitory neuron is still debated, a very recent study demonstrated the involvement of the immediate early gene *Homer1a* in the scaling-down of pyramidal neurons during sleep [97]. Neurons could sense changes in their own firing rate through intracellular calcium levels and the modification of one or more intracellular signalling pathways (e.g. the calcium/calmodulin-dependent protein kinase family, the immediate early gene *Arc*, the polo-like kinase 2 and the cyclin-dependent kinase 5) globally scales synaptic weights up or down [98], [99]. Recently, several molecules important for trans-synaptic signalling and cell adhesion have been implicated in synaptic



scaling [100]. Finally, synaptic scaling could require widespread changes in network activity, perhaps through activity-dependent release of a soluble factor by many neurons or glial cells simultaneously, such as BDNF and TNF $\alpha$  [101], [52].

Homeostatic plasticity appears to stabilize circuit function in vivo in a number of organisms and brain areas [99]. Synaptic scaling has been most thoroughly studied in vivo in the visual system, using standard visual deprivation paradigms to mimic the activity blockade in culture. There is now increasing evidence that synaptic scaling in excitation and inhibition plays important roles during various CP of visual system development [99]. In particular, it has been suggested that the potentiation of non deprived-eye responses following MD might arise through homeostatic mechanisms that boost the excitability of cortical neurons in response to a drop of sensory input. A recent study using in vivo calcium imaging to monitor eye-specific activation of individual neurons within binocular layer II/III of visual cortex reported that binocularly driven neurons maintain their overall level of responsiveness to the two eyes, so that the decrease in the responsiveness to the deprived-eye stimulation is compensated by an increase in responsiveness to nondeprived-eye stimulation [51]. Interestingly, in monocular visual cortex, the population of neurons driven only by the deprived eye has homeostatic-mediated stronger responses after deprivation, as do all neurons after binocular deprivation [51].

In support of the notion that synaptic scaling underlies gain of responsiveness to the non-deprived eye, blocking TNF $\alpha$  signalling in visual cortex either pharmacologically or genetically has no effect on the loss of responsiveness to the deprived eye but prevented the gain of responsiveness to the non-deprived eye [52]. It is important to notice that the mode of homeostatic plasticity within layer II/III of the visual cortex during the CP depends strongly on the method of visual deprivation: lowering visual drive through TTX or dark rearing

induces synaptic scaling, whereas eyelid suture causes an increase in the intrinsic excitability of monocular cortex pyramidal neurons [102]. This suggests that also the homeostatic response observed after MD is likely due to homeostatic intrinsic plasticity rather than synaptic scaling, but further studies will be necessary to elucidate this point.

In conclusion, these studies highlight the notion that experience-dependent plasticity is unlikely to be explained by a single form of synaptic plasticity, but rather arises through a complex interplay between multiple forms of change in synaptic strength, including modifications in inhibitory circuitry, homosynaptic depression and potentiation, and global changes in circuit gain.

#### *1.1.4) Molecular substrates of ocular dominance plasticity*

A complete understanding of CP plasticity requires linking the change in circuit function with the molecular mechanisms that make circuit changes possible. The molecular mechanisms that control the developmental plasticity of visual cortical connections are not fully understood. This paragraph reviews results establishing some factors as determinant for visual cortex plasticity.

***Glutamatergic receptors*** The properties of NMDA receptors (NMDARs) suggest that these molecules might play a central role in visual cortex plasticity, acting as ‘coincident detectors’ for Hebbian plasticity. The involvement of NMDARs in OD plasticity has been repeatedly proposed by pharmacological experiments [103], [104], but such manipulations have potent suppressive effects upon normal synaptic transmission. Recently, the use of different NMDAR antagonists or antisense oligonucleotides to reduce expression of NR1 subunit of the NMDA receptor has overcome this problem, showing that it is possible to block the effects of MD without affecting visual responses [105]. The direct dependence of OD

plasticity on NR1 subunits has been further demonstrated using conditional NR1-knockout mice [57].

An interesting property of NMDARs is that their subunit expression, determining the calcium influx, is developmentally and activity regulated. In particular, subunit composition varies in the visual cortex, from low to high NR2A/NR2B ratio, with a time course paralleling that of functional visual cortical development and the critical period [105]. It has been shown that in dark-reared animals the NR2A/NR2B ratio is lower than in light-reared animals [106]. However, recent results have demonstrated that NR2B over-expressing animals don't show an increased susceptibility to plasticity [107] and in mice with the deletion of NR2A subunit the sensitivity to MD is weakened, even if restricted to the normal critical period [72]. Interestingly, a very recent study highlights a co-regulation of OD plasticity and NMDAR subunit expression in GAD65 knockout mice. In the visual cortex of these animals there are reduced NR2A levels and slower NMDA currents. In addition, application of benzodiazepines, which rescues OD plasticity, also increases NR2A levels, suggesting that changes in inhibition would engage mechanisms that converge to regulate NMDA receptors, thereby enabling plasticity [108].

Further results establish a role for AMPA receptors (AMPA receptors) in the deprived-eye response depression following MD, reporting that a brief MD during the critical period alters AMPAR phosphorylation and reduces the expression of AMPARs on the surface of visual cortical neurons [109]. Finally, there is also direct evidence that metabotropic glutamate receptors (mGluRs) are involved in visual cortex plasticity, with distinct roles depending on the receptor subtype and cortical layer [55], [110]. Recently, using molecular genetic approach, it has been shown an important role for mGluRs in the regulation of OD plasticity

during development, since a 50% reduction in mGluR5 expression prevents OD plasticity induced by 3 days of MD [111].

**Neurotrophins.** There is a conspicuous number of observations suggesting that neurotrophins control visual cortical plasticity during the CP. Early studies in the rat demonstrated that intraventricular as well as intracortical infusion of NGF prevents OD shift following MD [112]–[114]. Moreover, infusion of antibodies that specifically activate the NGF receptor *trkA* equally blocks OD plasticity [115]. With the exception of NT-3, exogenous supply of all neurotrophins affects the outcome of MD. However, the effects of neurotrophins on OD plasticity are sometimes accompanied by alteration of other properties of visual cortical neurons, such as their pattern of discharge and orientation selectivity [114].

Other studies, which followed the opposite course of antagonizing the action of endogenous neurotrophins, have also shown that neurotrophins are important for normal visual cortical development and plasticity [116], [117]. In addition, neurotrophin production and release is developmentally regulated and depend on electrical activity, in particular on visual activity [118], [119]. In turn, neurotrophins can modulate electrical activity and synaptic transmission at both presynaptic and postsynaptic levels [48], [120]. They can have both fast actions, for instance by increasing transmitter release [121] or by directly depolarizing neurons [122], and slow actions, by modulating gene expression [120]. This reciprocal regulation between neurotrophins and neural activity might provide a means by which active neuronal connections are selectively strengthened. Indeed, neurotrophins seem to require the presence of electrical activity to exert their actions [123], [124].

The classic hypothesis ('neurotrophic hypothesis') states that competition for limited amounts of neurotrophins is the effector of activity-dependent plasticity in the cortex, and the conventional explanation for OD plasticity is that the deprived eye does not activate

cortical cells to the same amount of the open eye, thereby failing to stimulate them to release sufficient neurotrophins to sustain the deprived-eye pathway [123], [125].

The possibility of an anterograde action of neurotrophins as opposed to target-derived action has also emerged from literature [126], [127]. This significantly changes the frame of thought: in addition to thinking that cortex-derived factors guide stabilization of thalamic afferents on cortical neurons, we may have to consider that thalamic fibres themselves release factors which promote and guide the formation and maintenance of their synapses onto cortical neurons and that corticothalamic afferents may contribute to the development of the pattern of thalamocortical connectivity. However, some recent experiments show that a possible mechanism of action of neurotrophins on OD plasticity is an orchestrated modulation of synaptic efficacy, rather than a direct effect on thalamocortical afferents alone. In visual cortex synaptosomes, both NGF and BDNF potentiate glutamate and acetylcholine release, while only BDNF does so for GABA release. Like BDNF, NT4 potentiates GABA and glutamate release but is much less effective in enhancing acetylcholine release [128]. Putting this information together with data on the expression of trk receptors in the visual cortex and with data on retrograde transport of cortically injected NGF [129], it can be concluded that NGF is likely to act directly on cholinergic afferents from the basal forebrain and on a population of glutamatergic cortical neurons; BDNF targets are principally cortical glutamatergic pyramidal cells and inhibitory interneurons, whereas NT4 acts on glutamatergic thalamic afferents and probably pyramidal neurons and inhibitory interneurons [48].

In line with this, it has been shown that infusion of exogenous NGF in the cat has little or no effect on MD outcome and this seems related to a different cholinergic arborisation in the visual cortex of the two species. Similarly, BDNF infused in the cat visual

cortex paradoxically results in the expansion of connections subserving the deprived eye, as previously observed with the intracortical infusion of the GABA receptor agonist muscimol [130], [131].

The relationship between neurotrophins and the development of inhibitory processes has been investigated in detail, using an elegant transgenic mouse with postnatal overexpression of BDNF in the forebrain. In these animals, BDNF overexpression accelerates the maturation of intracortical GABA-mediated inhibition and this is paralleled by a precocious development of visual acuity with respect to wild type animals and an accelerated time course of the CP, which opens and closes one week earlier respect to control animals [32], [132], [133]. It should be noted that recent studies of a mutant mouse heterozygous for the null allele of BDNF demonstrate that a 50% reduction in the BDNF levels has no effect upon OD plasticity [134]. Similarly, Stryker and colleagues, using a conditional transgenic mouse, show that TrkB inactivation does not affect the induction of OD plasticity following MD [135]. However, given the redundancy of neurotrophin action on the modulation of synaptic transmission, these data do not exclude that neurotrophic factors play a fundamental part in the plasticity of visual cortex: the compensating action of other neurotrophins could account for the absence of alterations in visual cortex plasticity in these mutant mice. Moreover BDNF has been demonstrated to enhance plasticity in the adult visual cortex [136]–[138], acting at both the structural and functional level [139].

***Neuromodulatory systems.*** Several studies have tried to uncover the contribution of neuromodulators to cortical plasticity [140]. As with many other molecules involved in cortical plasticity, in rats, the distribution of different receptors and fibres is developmentally regulated and dependent on cortical input [141], [142]. The involvement of these transmitters in visual cortex plasticity was first investigated by Kasamatsu and Pettigrew who

showed that depletion of noradrenaline in kitten visual cortex disrupts OD plasticity [143]–[146] and infusion of noradrenaline in kitten visual cortex enhances plasticity [145], [147], [148]. Further experiments demonstrated that intracortical infusion of noradrenaline combined with MD reduces the proportion of binocular neurons in adult cat visual cortex, restoring neuronal plasticity to the normally aplastic visual cortex of adult animals [149]. In addition, OD changes are inducible in adult cat visual cortex by electrical stimulation of the locus coeruleus [149] or peripheral administration of an exogenous precursor of noradrenaline [150]. Experiments using osmotic minipumps to infuse  $\beta$ -adrenergic antagonists in kitten visual cortex indicated that activation of  $\beta$ 1-receptors seems to be mostly involved in regulation of OD plasticity [151]. Noradrenaline may support cortical plasticity through a NMDA receptor-gated mechanism. In vitro slice experiments have provided evidence that noradrenaline facilitates synaptic plasticity by enhancing NMDA receptor-mediated response component [152]. Noradrenaline could increase the probability of activation of NMDA receptors by its action on membrane  $K^+$  conductance [153] and second messengers, such as cAMP [154].

Also the involvement of acetylcholine in OD plasticity has been examined in kittens, through the lesion of basal forebrain. This alone results not sufficient to prevent OD shift in the visual cortex following MD, but combining this kind of lesion with depletion of cortical noradrenergic innervation reduces the physiological response to MD [155]. Further experiments using pharmacological compounds to chronically block cholinergic receptors in visual cortex showed that blockade of muscarinic but not nicotinic receptors disrupts OD shifts in visual cortex of monocularly deprived kittens. In particular, chronic blockade of muscarinic M1 but not M2 receptor subtypes prevents OD shift [156], [157]. Similarly to that

observed for noradrenaline, acetylcholine effects could be attributed to a facilitation of NMDA receptor activation [158].

The role of serotonin in OD plasticity has been investigated chronically infusing a specific neurotoxin into the visual cortex of kittens undergoing MD: the results showed that serotonin depletion prevents the susceptibility to experience-dependent modifications. Equally, the combined infusion of two broad serotonergic receptor antagonists reduces OD plasticity [159]. In addition, it has been demonstrated that the serotonin 5-HT<sub>2c</sub> receptor subtype plays a key role in activity-dependent synaptic modifications in visual cortex [160]. To explain the facilitatory action of serotonin in OD plasticity it has been proposed also in this case a mechanism associated with NMDA receptors [161], [162]. It is worth to point out, however, that administration of the selective serotonin reuptake inhibitor fluoxetine has been recently shown to restore OD plasticity in adult animals. The effects induced by fluoxetine are associated with a marked reduction of GABAergic inhibition, thus suggesting that serotonin could affect visual cortical plasticity also modulating intracortical inhibition [77].

***Intracellular signalling of cortical plasticity.*** Experiments using transgenic mice and/or pharmacological manipulations have identified three signalling kinases that can modulate synaptic strength and are critical for inducing OD plasticity: extracellular signal-regulated kinase 1,2 (ERK-1,2), cAMP-dependent protein kinase (PKA), and calcium/calmodulin-dependent protein kinase II alpha (CaMKII $\alpha$ ) [163]–[165]. These kinases may rapidly promote OD plasticity by directly phosphorylating plasticity-regulating molecules at the synapse (such as glutamate or GABA receptors) or cytoplasmic substrates crucial for synaptic transmission, neuronal excitability and morphological stabilization (e.g. synapsin I, potassium



channels, MAP2), or they may signal to the nucleus to mediate changes in gene transcription [166].

The intracellular mechanisms mediated by kinase signalling can lead to the activation of cAMP-responsive element-binding protein (CREB), which in turn controls CRE-mediated gene expression of proteins essential for establishment and maintenance of plastic changes [167], [168]. Indeed, it has been recently demonstrated that CRE-mediated transcription is upregulated by MD during the critical period in the visual cortex contralateral to the deprived eye and that CREB is necessary for OD plasticity [169], [170]. As with many other molecules that mediate changes in plasticity, CREB levels also decreases with age [169]. Recently, the regulation of chromatin structure as emerged as one of mechanisms regulating visual cortex plasticity, since it has been demonstrated the involvement of histone phosphoacetylation in OD plasticity. In juvenile mice, visual stimulation that activates CREB-mediated gene transcription also induces ERK-dependent MSK and histone H3 phosphorylation and H3-H4 acetylation, an epigenetic mechanism of gene transcription activation. In adult animals, ERK and MSK are still inducible; however, visual stimulation induces weak CREB-mediated gene expression and H3-H4 posttranslational modifications. Finally, stimulation of histone acetylation in adult animals by means of trichostatin is able to promote OD plasticity [171]. The gene expression modifications deriving from the induction of histone acetylation could explain the way by which long-term changes of brain circuitry take place.

Additional classes of molecules are also likely to be important for calcium-dependent cellular processes that may mediate brain plasticity. For example, a link between calcium signalling and cytoskeletal dynamics comes from a recent microarray screen, which has found that the calcium sensor cardiac troponin C (part of a complex that mediates calcium-

dependent actin-myosin interaction) is elevated in visual cortex during the critical period, and is regulated by visual activity [172]. Additionally, calcineurin, a calcium/calmodulin-activated phosphatase, has proven to be an effective negative regulator of OD plasticity: indeed, calcineurin overexpression reversibly prevents an OD shift during the CP in mouse [173], suggesting that the balance between protein kinases and phosphatases is critical for visual cortex plasticity.

**Extracellular environment.** Downstream effectors that implement the program initiated by the signalling mechanisms described in the preceding section are largely unknown. However, it is becoming clear that the extracellular environment, and in particular the extracellular matrix (ECM), plays an important part in controlling spine dynamics and visual cortical plasticity. Recent studies have shown a key role in OD plasticity for the major components of brain ECM, the chondroitin-sulfate proteoglycans (CSPGs). During development CSPGs condense at high concentration in lattice-like structures, called perineuronal nets (PNNs), which completely ensheath visual cortical neurons, in particular parvalbumin-positive neurons. The time course of PNN condensation in the visual cortex tightly matches the visual cortex CP for the effects of MD [174]. In addition, the development of CSPGs is regulated by visual activity, since the process of PNN condensation is prolonged by dark rearing [175], [176]. Enzymatic degradation of CSPGs in the adult visual cortex reactivates OD plasticity in monocularly deprived adult animals, suggesting that adult ECM exerts a powerful inhibitory control on OD plasticity [174].

The outcome of the study of ECM influence on OD plasticity led to analyse the role of endogenous extracellular proteases in the visual cortical plasticity during the CP. It has been shown that pharmacological inhibition of tPA hampers visual cortical plasticity [177], [178], and MD is ineffective in mice with deletion of the tPA gene both at the functional and

structural level. Plasticity can be rescued in tPA knockout mice by the exogenous administration of tPA during the period of MD. Moreover, the link between tPA and experience-dependent plasticity is strengthened by the observation that in wild type animals MD elicits a fast and transient increase of tPA activity during the CP but not in the adult [29].

The released tPA increases extracellular proteolysis directly or by the activation of plasmin. These proteases have a wide spectrum of targets and the available information is not sufficient to dissect which of these targets must be cleaved for plasticity to proceed. However, converging data point to an important role for tPA in 'freeing up' the extracellular matrix to promote the structural reorganization of connections during deprivation [29].

Another candidate for plasticity regulation has been revealed by a recent study highlighting the critical role of myelin, particularly via its interaction with the Nogo receptor (NgR). The authors first characterized the density and laminar distribution of NgR and its ligands in mouse visual cortex: while total levels of myelin as well as of NgR increase only slightly during the CP, layer IV shows the greatest increase in myelin. The main result is that the absence of either Nogo or NgR prevents the closure of the CP and preserves plasticity: indeed, these transgenic mice exhibit an undiminished OD plasticity, even when MD is imposed in four month-old animals. Interestingly, the Nogo/NgR-dependent regulation of visual cortical plasticity does not seem to involve a change in GABAergic inhibition or tPA activity, as parvalbumin and tPA immunoreactivity are normal in NgR knockout mice. Therefore, Nogo/NgR must act either independently or further downstream in the signalling cascade, presumably converging to regulate cortical anatomical rearrangements [179], [180].

In addition to the results described up to here, in the past few years several studies have investigated the molecular mechanisms of visual cortex plasticity using genetic screens,

and have opened the door for examination of new families of molecules in plasticity (e.g. proteins related to IGF-I pathway or immune/inflammation system signals). Expression of most of these molecules is developmentally regulated and differentially altered by sensory experience [172]. These studies further highlight that OD plasticity invokes a complex, interrelated set of mechanisms, involving a large number of molecules of different classes. An important goal for the field of cortical plasticity is to understand how the many molecular mechanisms guiding plasticity are recruited, how they interact and converge to permit and instruct plasticity, and over which time scale they act.

#### *1.1.5) Experience-dependent plasticity in adult visual cortex*

It is widely accepted that experience-dependent plasticity is a prominent feature of the developing visual cortex. However, adult cortical circuits can be still modified by a variety of manipulations, such as perceptual learning and visual deprivation [181]. Perceptual learning refers to a robust gain in performance on basic perceptual tasks that are induced by sensory experience and are dependent on practice [182]. Studies in both humans and animals have shown that in adults with normal vision practice can improve performance in various aspects of visual perception, including stimulus spatial frequency, orientation, luminance contrast, motion-speed and motion-direction discrimination [183], [184]. The characteristics of such learning processes suggests that they involve early stages along sensory pathways, in some instances primary sensory cortices.

Despite this recent progress in localizing the visual areas involved in perceptual learning, elucidation of the underlying mechanisms at the cellular level remains a challenge [181]. Interestingly, a number of studies over the last years suggest that perceptual learning may provide an effective way for treating amblyopia [184]–[186].

While the cortical modifications mediating perceptual learning appear to be induced by increased exposure to certain visual stimuli, significant changes can also be caused by deprivation of inputs in part or all of the visual field. Although it is induced by abnormal visual experience, the capacity of the adult cortex for such reorganization is functionally advantageous, since it allows the neuronal machinery rendered inactive by peripheral injury to be reused for processing other inputs. This could in turn facilitate functional recovery of perception. One form of visual deprivation is caused by lesioning a portion of the retina and thus causing a scotoma in the visual field. Retinal lesions initially silence the visual cortical region retinotopically mapped to the scotoma, but most cells recorded in the cortical projection zone of the retinal lesion exhibit 'ectopic' excitatory visual receptive fields which are displaced in the immediate vicinity of the lesion already after a few hours from the placement of lesions. The presence of ectopic receptive fields, combined with the presence of normal cortical representation of the retinal region surrounding the lesion, indicate a clear expansion of the cortical representation of the part of the retina near the lesion [187]–[189]. To determine the loci along the visual pathway at which the reorganization takes place, the course of topographic alterations in the primary visual cortex and dorsal lateral geniculate nucleus (LGN) have been compared. At a time when the cortical reorganization is complete, the silent area of LGN persists, indicating that changes in cortical topography are due to alterations of long-range intrinsic horizontal connections [190]–[192].

Another form of deprivation-related plasticity is OD plasticity. Even if this issue remains controversial, recent studies showed that in adult mice a long period of MD can cause a shift in cortical OD. The 2-3 days of MD effective in juveniles must be extended to at least 5 days in order to induce OD shift in adult mouse V1 [57], [193]. However, adult plasticity involves different functional changes in cortical circuits: while developmental OD

plasticity is due to a rapid reduction of responses to the deprived inputs followed by a later enhancement of non-deprived inputs [50], adult OD shifts in mice are primarily accounted for by increased responses to the non-deprived eye [57], [193]. Recent studies showed that prior experience can facilitate adult OD plasticity: indeed, inducing a saturating OD shift by brief eye closure in juvenile or adult mouse visual cortex enables faster and more persistent OD changes in response to a second MD several weeks later [194]. Similarly, housing adult animals in the dark for a brief period allows strong plasticity in the visual cortex [195], [196].

## **1.2) Impact of Environmental Enrichment on brain development and plasticity: physiological and molecular aspects**

Within the old 'nature versus nurture' debate a relevant progress in understanding the influence of environmental experience on the development, refinement and maintenance of appropriate nervous system connections was obtained by introducing environmental enrichment (EE) as an experimental protocol. EE is an alteration of the standard laboratory conditions that modifies the quality and intensity of environmental stimulation, reaching an optimization of the rearing environment. A comprehensive definition of EE was provided for the first time by Rosenzweig et al. [197] as 'a combination of complex inanimate and social stimulation'. Enriched animals are reared in large groups (6-10 individuals can be considered the most common used condition) and housed in wide stimulating environments where a variety of objects differently shaped (e.g. running wheels, platforms, boxes, toys, tunnels, shelters, stairs and nesting material) are present and changed frequently (specifically, the objects are completely replaced at least once a week). The goal of EE is to improve the animals' quality of life by providing them with high levels of multi-sensory stimulation, increasing physical activity and social interactions, stimulating natural behaviours and

cognitive abilities, since the novelty due to frequent objects' replacement attracts the explorative curiosity of most laboratory animals. The significance of EE is based on the comparison of the enriched conditions with the standard environment condition (SC), that consists in housing 2-5 individuals in laboratory standard cages where no particular objects are present except for food, water and litter, and with the impoverished environment condition (IC) that consists in housing the animals singly in cages identical to those used for SC or even smaller [198].

Although EE research has been mostly done in rodents, similar effects occur in several species of mammals (gerbils, ground squirrels, rabbits, cats and primates), and also in some avian species [199]–[204].

#### *1.2.1) Effects of environmental enrichment on brain anatomy*

In the initial studies, it has been observed that 30 days of exposition to an enriched living condition result in different brain weights between littermates housed in EE and SC or IC conditions, not imputable to differences in body weight [205], [206]. These changes have been noticed in the entire dorsal cortex, but the largest difference was found with respect to the occipital cortex (9.4%). This result prompted several groups to investigate in detail the anatomical outcome of living in EE, taking the visual cortex as preferred model. It has been widely reported that the cerebral cortex in EE animals is significantly thicker compared to littermates housed in impoverished and standard environments [207]–[211]. Subsequent studies have pointed out that exposure to EE leads, in different cortical layers, to an increment in size of neurons' cell soma and nucleus [212], dendritic branching and length [213]–[215], number of dendritic spines [216], synaptic size and density [217]–[220], postsynaptic thickening [209], gliogenesis [210] and angiogenesis [221]. Further experiments

revealed that significant cerebral effects of enriched versus impoverished (or standard) experience could be induced at any part of the life span and with relatively short periods of exposure [207], [222].

The anatomical changes are not limited to cortical regions: indeed, similar effects to that reported for cerebral cortex have been found for pyramidal cells of CA1 and CA3 and for dentate granule neurons of hippocampus [221], [223]–[225], for the striatum [226], the amygdala [227] and the cerebellum [228], [229]. Recent studies have shown that exposure to EE increases hippocampal neurogenesis and integration of the newly born cells into functional circuits. A short (3h) daily exposure to a complex environment for 14 days in adults is sufficient to induce a long-term increase in the rate of neurogenesis [230]–[233]. The definitive proof of increased neurogenesis in the hippocampus of EE animals has been provided by Kempermann et al. [230], [234] in mice and by Nilsson et al. [231] in rats using the proliferation marker bromodeoxyuridine (BrdU). EE induces an ≈70% increase in the number of new-born dentate gyrus cells, but this is not associated with any detectable changes in differentiation of the progenitor cells towards a neuronal or glial fate. Indeed, most (80-85%) of these cells expresses a neuronal phenotype, a proportion similar to that observed in naïve rats. This suggests that EE effects are expressed independently of the cell lineage, resulting in a net increase in both neuronal and glial cells in the dentate gyrus [231]. EE does not seem to affect proliferation of progenitor cells, rather it appears to increase the number of surviving newly formed granule cells in the dentate gyrus [230], [231]. Increased cell survival reflects differences in apoptotic rates: indeed, apoptotic assessment using the TUNEL method revealed a decreased proportion of neurons undergoing cell death [235].

In the rodent brain, thousands of new neurons are generated every day, with new-born cells contributing to tissue homeostasis and brain functions that underlie certain forms



of learning and memory [236]. New-born granule cells in the dentate gyrus might contribute to the improved performance in the spatial learning tests observed in EE animals [237], [238], [236]. To address this issue, it has been examined whether the increase in the number of surviving adult-generated cells following EE contributed to improved memory function by reducing neurogenesis throughout the EE period by means of antimetabolic agents. The antimetabolic treatment during EE completely prevented both the increase in neurogenesis and EE-induced long-term memory improvement, thus establishing that new-born cells in the dentate gyrus contribute to the expression of the mnemonic effects of EE [239]. However, this result is controversial [240] and EE results in many different types of structural and functional changes that could facilitate memory, including increased dendritic branching and spine and synapse number in the cortex and the hippocampus [241], [242].

#### *1.2.2) Environmental enrichment modifies brain electrophysiological responses*

Few studies have addressed the possible relationship between enriched living and electrophysiologically measurable modifications of synaptic transmission. In hippocampal slices taken from enriched housed rats excitatory postsynaptic potential (EPSP) slopes and amplitudes are greater with respect to age-matched controls [243]–[246]. Similarly, it has been demonstrated that EE selectively increases glutamatergic responses in the cerebral cortex of the rat: excitatory postsynaptic currents (EPSCs) display a large amplitude increase, accompanied by a rise-time decrease and reduced pair pulse ratio in layer II/III of the auditory cortex [247].

Exploration of enriched environments elicits pattern of electrical activity in hippocampal neurons of area CA1 that are similar to patterns of electrical stimulation used to induce LTP in hippocampal slices. In two studies, long-term potentiation (LTP) and long-

term depression (LTD), two different paradigmatic models of synaptic plasticity, have been compared in hippocampal slices from enriched and control rats. These data indicate that significant enhancement of LTP and LTD occur in hippocampal area CA1 following 5-8 weeks of EE [248], [249]. Moreover, it is interesting to notice that enhancements in LTP and LTD, seen after a 5-week exposure to EE, are not reversed after 3-5 week exposure to standard housing [249]. Two phenomena may contribute to this enhancement. One is a facilitation of the induction of synaptic plasticity. The finding that paired-pulse facilitation is decreased in enriched rats compared with control animals suggests that exposure to EE enhances transmitter release and, thus, decreases the demand for presynaptic activation to reach the postsynaptic thresholds for inducing LTP and LTD. Consistently, LTP induction requires a smaller number of high-frequency stimuli in enriched animals and it is very likely that enhanced LTD is also due, at least in part, to a facilitation of its induction. EE may also actually increase the dynamic range of synaptic modification: indeed, repeated LTP and LTD induction produces larger synaptic changes in enriched than in control rats. These data reveal that exposure to different environmental experiences can produce long-lasting effects on the susceptibility to synaptic plasticity, involving pre- and postsynaptic processes [249].

Less is known about the changes induced by EE in other brain regions. However, in a recent work it has been demonstrated that EE significantly increases LTP and largely diminishes LTD in the anterior cingulate cortex (ACC). Sensory experience changes synaptic plasticity in the ACC via postsynaptic mechanisms, by altering the dynamic regulation of NMDA receptor subunits: indeed, the component of NR2B-containing NMDA receptors is enhanced in EE-exposed animals [250].

Surprisingly, little attention has been paid to changes in the response properties of cortical neurons after EE. Rich and stimulating environments improve the sensory

information processing of cortical neurons. The latency of evoked potentials recorded in the visual cortex of rats was shown to be significantly shorter after their rearing in a complex environment [251]. A similar result was reported for evoked potentials recorded in the vibrissae representational zone [252]. Hereafter, Coq and Xerri [253] reported that an enriched environment promoting tactile stimulation through palpation and manipulation of objects induces a selective expansion of the forepaw map areas serving the glabrous skin. Moreover, the expanded cutaneous zones display a finer-grained topographic organization characterized by smaller receptive fields. In the somatosensory 'barrel' cortex EE reduces the functional representation of the facial whiskers and extracellular recordings demonstrated suppressed evoked neuronal responses and smaller receptive fields in the cortical layer II/III of enriched rats [254]. Similarly, animals raised in enriched conditions have higher responsiveness to light stimuli, contrast sensitivity, as well as spatial and temporal frequency detection, and sharper orientation tuning in primary visual cortex with respect to impoverished animals [255]. Neurophysiologic responses are sensitive to EE also in the auditory cortex: EE substantially increases response strength, selectivity and directional sensitivity, but decreases threshold and latency of auditory responses [256]–[259].

### *1.2.3) Environmental enrichment modulates gene expression*

Efforts dedicated to understanding potential molecular mechanisms underlying the previously described changes of EE on brain and behaviour started several years ago, prompted by the promising goal to reveal molecules that can be manipulated to reproduce the beneficial effects of the enriched experience. It has been early found that an enriched experience causes increased rates of protein synthesis and increased amounts of proteins in the cortex [208]. Subsequent studies showed that EE lead to increased amounts and

expression of RNA in rat brain [260], [261]. New possibilities to further characterize brain molecular changes elicited by EE came from the development of gene chip analysis techniques, real-time PCR, and the recent next generation sequencing, allowing the simultaneous screening and comparison of differential gene activation in dependence on different environmental conditions. Although they produce only 'snapshots' of a highly dynamic process, such studies are instructive and suggest that a large number of genes change their expression levels in response to EE.

The two studies so far analysing the effects of enrichment on gene expression in the mouse [262] and rat cerebral cortex and hippocampus [263] reported changes occurring even after only 3h of enriched environment exposure, but persisting until two weeks from the start of enriched housing procedure. The differential expression of genes after 3 and 6 h of exploration in EE reveals the early molecular events resulting from environmental stimulation. Almost half (46%) of the environmentally responsive genes codes for proteins involved in macromolecule synthesis and processing, including enzymes involved in DNA, RNA and protein processing, and transcription factors and translational regulatory enzymes, standing at the beginning of molecular changes with variable target pathways and essential for various structural and functional endpoint changes in the nervous system.

A distinct group of genes found to be differentially expressed after brief EE encodes proteolytic proteins and chaperones involved in signalling and apoptosis (e.g. caspase-6 and the Bcl-2 associated protein Bax), indicating a molecular correlate of the antiapoptotic effect of enrichment training.

EE animals also show alterations in the expression of structural proteins involved in the establishment of new synapses and reorganization or strengthening of existing synapses; in particular, it has been shown that the expression of proteins belonging to cell-adhesion

molecule (CAM) family (e.g. integrins) and to associated Rho family, involved in the induction of events in surface adhesion, synapse formation and neuronal plasticity, is 3-fold increased during the early phase of enrichment. A cluster of genes encoding proteins implicated in synaptic vesicle trafficking and modulation of neurotransmitter release, including synaptobrevin and clathrin-AP2, is up-regulated after 3 and 6 h of enriched training. Changes in the expression of these genes clearly suggest that presynaptic processes are being modified by enriched experiences. Finally, it has been described that exposure to EE dynamically regulates the expression of a number of genes whose products are associated with neuronal excitability (e.g. the 78-kDa glucose-regulated protein and neurokinin A) [262]. Moreover, Pinaud et al. [264] demonstrated that animals exposed daily, for 1 h, to EE exhibit a marked up-regulation in the cerebral cortex, hippocampus and striatum of the immediate early gene Arc mRNA, an activity-dependent neuronal marker involved in multiple forms of neuronal plasticity.

By comparing the gene expression profiles following a short experience in EE with those from animals exposed to EE for two weeks, it results that some group of genes are equally regulated [263]: i) transcription factors, such as different zinc finger transcription factors (e.g. JunB, EIF-4E, Krox20, NGF1-B); ii) synapse-related molecules (e.g. synapsin, synaptogyrin, clathrin, Rho proteins); iii) proteolytic proteins and molecules mediating apoptosis (e.g. proteins belonging to Bcl family, ubiquitin-specific protease, ClpP protease, aspartyl aminopeptidase and prolidase). However, most of the genes regulated by a longer housing in EE are different from those whose expression levels change at the early stages of the enriched experience.

A number of genes associated with the regulation of neurotransmission and neuronal spiking activity are also affected by EE. The expression levels of different members of

neurotransmitter (e.g. glutamate, GABA, dopamine and noradrenaline) receptors and of ion channels and transporters (e.g. Na,K-ATPase and Na<sup>-</sup>, K-channels) are dynamically modified following EE [263]. Rampon et al. observed that the expression level of postsynaptic density 95 (PSD-95), important not only for anchoring the NMDA receptor at the postsynaptic membrane but also for coupling this receptor to pathways controlling synaptic plasticity, increases after 2 days and 14 days of enrichment. EE is also associated with changes in the expression of molecules downstream of the NMDA receptor, including up-regulation of calmodulin (that modulates clustering of neurotransmitter postsynaptic receptors) and down-regulation of neurogranin (that regulates calmodulin availability). In the same manner, long-term EE modulates the expression of a group of kinase/phosphatase network molecules (e.g. CaM kinase, PKC, calcineurin, protein phosphatase), playing a pivotal role in the remodelling of neuronal circuits [263]. Prolonged EE also alters the mRNA levels of many genes associated with structural changes occurring during neuronal growth and synaptogenesis (e.g. the cytoskeletal proteins dynactin and cortactin, N-cadherin, dynamin-like protein 1, myosin heavy chain) [225], [262], [263].

The study by Keyvani et al. [263] identified a differential regulation following EE also for the expression of growth factors/receptors (e.g. FGF-, IGF-receptors, BDNF, VEGF). Moreover, there are other molecules, whose expression is regulated by enriched experience, that might play an indirect role in the context of brain plasticity, i.e. metabolic enzymes (implicated in energy metabolism, oxidative stress and mitochondrial activity) and molecules involved in immune response (e.g. complement protein C1q, MHC class and T-receptor molecules). Noticeably, similar functional groups of genes were found to be regulated in different brain areas, particularly in the hippocampus and striatum. However, different expression patterns were found in distinct brain areas at the individual gene level and there

were only a few genes regulated in parallel in different brain regions: the higher responsiveness of the hippocampus to EE could be due to a more pronounced susceptibility of this structure for plasticity changes [263], [265], [266].

Neurotransmitter systems characterized by diffuse projections to the entire brain are particularly sensitive to environmental stimuli. First studies by Rosenzweig et al. [206], [267] reported an increase in acetylcholinesterase activity, indicating an effect on the cholinergic system. Subsequent studies confirmed and extended this initial observation to other neurotransmitter systems. It has been shown that EE augments mRNA expression levels of serotonin 1A receptor and serotonin concentration in the cerebral cortex and hippocampus [268]–[270]. Enriched experience increases also noradrenaline concentration and potentiates  $\beta$ -adrenoceptor signalling pathway in the cerebral cortex, cerebellum and brainstem [271], [272].

Alterations of neurotransmitter aminoacids systems in mice subjected to differential housing have initially been revealed by Cordoba et al., [273]: in particular, a significant increase for aspartate was found in the spinal cord, whereas glutamate significantly decreased in colliculi and cerebral cortex; similarly, glycine increased in the cerebral cortex and decreased in colliculi and pons-medulla, while GABA increased in the spinal cord, pons-medulla and cerebellum and decreased in the thalamus and hypothalamus. Subsequent studies failed in uncovering a clear influence of EE on GABAergic transmission, but showed that enrichment significantly affects excitatory glutamatergic system. In this context, Rampon et al. [274] investigated the influence of EE on knockout mice in which NMDA receptor was selectively deleted in the CA1 subregion of the hippocampus: they found that the learning deficits exhibited by these mice in three hippocampus dependent behavioural tasks were rescued after two months of EE, thus establishing that CA1 NMDA receptor

activity seems not essential for experience-induced behavioural and synaptic plasticity. An explanation proposed by Tsien group is that the compensation might be due to an enhancement in connectivity outside the functionally deleted hippocampus, for instance in the neocortex. This possibility is strengthened by another study, aimed at investigating learning and memory function in transgenic mice in which the NMDA receptor function is enhanced in the forebrain via overexpression of the NR2B subunit. These mice show overall improvement in their performances in learning and memory tasks; however, EE do not further increase their already augmented abilities. The occlusion of the effects induced by environmental stimulation suggests the existence of overlapping mechanisms between EE and genetic enhancement of the NMDA receptor functions [275].

As a step toward the detailed dissection of the molecular mechanism underlying EE, Tang et al. presented biochemical evidence that GluR1, NR2A and NR2B proteins in the forebrain begin to increase after 2 weeks of EE, indicating that NMDA and AMPA receptors functions might be directly modified by environmental experience. An alteration in the expression of AMPA and NMDA subunit receptor following EE has been observed also in the hippocampus [276], [277].

The group of molecules with potent functions that most likely respond to external stimuli are neurotrophic factors (or neurotrophins), a class of secreted proteins promoting neuronal development, survival and plasticity which comprises NGF, BDNF, NT-3 and NT-4. EE plays a powerful role in the modulation of synthesis and secretion of neurotrophic factors throughout the brain, resulting in higher levels of mRNA for NT-3, NGF and BDNF in the visual cortex and hippocampus [278] and of a candidate-plasticity gene, the nerve growth factor induced-A (NGFI-A or Zif/268), throughout the brain [279], moreover, EE results in



increased protein levels of NGF, BDNF and NT-3 in several brain regions, including cerebral cortex, hippocampus, cerebellum and basal forebrain [280], [281].

Neurotrophins act on neurons by binding to two distinct classes of membrane receptors: one class consists of a single receptor, p75, that binds NGF and other neurotrophins with relatively low affinity; the other class consists of multiple receptor tyrosine kinases, including trkA, trkB, trkC and their isoforms. Immunohistochemical analysis of brain tissue from the medial septal area reveals higher staining intensity and fibre density with both the low-affinity and the high-affinity NGF receptors in EE animals [282]. It has been also demonstrated that EE dynamically affects the protein levels of full-length and truncated TrkB in the different regions of the visual system [283]. In addition, EE increases hippocampal phosphorylation of the transcription factor cyclic-AMP response element-binding protein (CREB; [284]), which is known to regulate BDNF expression.

A study by Fischer et al. [285] suggests that EE-induced effects might be mediated, at least in part, by chromatin remodelling. They demonstrated for the first time that EE increases the acetylation of histone 3 and 4 (H3, H4) in the hippocampus and, to a lesser extent, in the cortex of wild-type mice. Histone post-translational modifications regulate chromatin susceptibility to transcription: high levels of histone acetylation on a specific DNA segment is generally correlated with increased transcription rates. This strongly suggests that epigenetic control of gene transcription through histone acetylation could be the final gate opened by EE to promote plasticity [3], [198], [286].

#### *1.2.5) Effects of Environmental Enrichment on nervous system disorders*

Since EE is a rather mild and non-invasive treatment, the results obtained in animal models can be of great interest and applicability also for humans in many different fields, from

psychology to medical clinic. The available results indicate that exposure to stimulating environmental conditions results in beneficial psychological and behavioural outcomes. Ramey and Ramey have shown that, starting at an early age, the use of a comprehensive enriched environment can increase IQ by a mean of 15 points in children from disadvantaged homes [287]. Moreover, it has been demonstrated that subjects who participated in an enrichment program (nutritional, educational, and physical enrichment) at the age of 3-5 years have lower scores for schizotypal personality and antisocial behaviour at the age of 17 years and for criminal behaviour at the age of 23 years, compared with control subjects [288].

It is also important to underline that, thanks to its ability to modulate brain plasticity at different levels [289], EE is also a potentially useful tool in studies aimed at ameliorating functional phenotypes in neurodevelopmental disorders. Neurodevelopmental intellectual disorders are a complex group of diseases, characterized by cognitive disabilities, that affect 2-3% of human population [290]. Paradigmatic cases of such diseases are Rett syndrome and Down syndrome. Interestingly, it has been proposed that both syndromes may have a common key etiologic mechanism consisting of a general dysregulation of the cerebral balance between excitatory and inhibitory drive, leading to impaired synaptic plasticity in several brain structures [290]. This view is mainly based on studies performed in transgenic murine models of these pathologies. Indeed, detailed electrophysiological analysis in mice carrying conditional deletion of MeCP2 or neuron-specific expression of mutated protein forms shows cortical and hippocampal reductions in cell activity [291], [292] and reduced LTP expression in various brain regions [293], an effect generally attributed to over-inhibition. Recently, it has been elegantly demonstrated that MeCP2 knockout mice display a progressive shift in cortical excitation/inhibition balance favoring inhibition, through early

upregulation in the expression of parvalbumin GABAergic neurons synapsing onto cortical pyramidal neurons [294]. In Ts65Dn mice, the prime model of Down syndrome [295], a large number of studies have shown that the cognitive impairment is mainly related to overinhibition in temporal lobe circuitries [296]–[299], with a central role of excessive inhibition being confirmed by the demonstration that administration of GABA-A and GABA-B receptor antagonists reverses the main deficits in this model [300], [301]. In agreement with these data, analyses of human post-mortem brain samples have shown increased density of GABA receptors in people with Rett syndrome [302] and an impaired balance between excitatory and inhibitory systems in Down syndrome tissues [303].

The beneficial impact of EE may be optimised by early applications of EE paradigms starting during the critical periods for functional maturation. Pre-weaning EE stimulates BDNF expression in the brain, leads to a partial rescue of motor and cognitive abilities, and reverses cortical LTP deficits in mouse models of Rett syndrome, increasing the number of cortical excitatory synapses without any changes in the density of inhibitory synapses [304]. Moreover, early EE in Ts65Dn mice produces recovery of spatial memory and hippocampal synaptic function, with a concomitant increase in BDNF amount and reduction of the inhibitory transmission but without upregulation of the excitatory tone [305].

Besides neurodevelopmental disorders, exposure to EE has also remarkably beneficial effects in animal models of various other nervous system disorders, including neurodegenerative diseases [285], [306], [307] and different types of brain injury [308]–[310].

### **1.3) A new research paradigm: Environmental Enrichment and visual system plasticity**

Some years ago, the lab led by Lamberto Maffei and Alessandro Sale at the Neuroscience Institute of CNR in Pisa started a series of studies focusing on visual system development and plasticity in environmentally enriched rodents. In this new approach, the rigorous and highly quantitative methodology typical of visual system research has been combined with the theoretical framework of the EE paradigm; this resulted in quite a powerful new tool in which the visual system has served as a model to study the effects of EE, and, at the same time, EE has emerged as a tool to probe visual-circuit plasticity and to unravel the underlying molecular factors.

#### *1.3.1) Relationship between environment and developmental plasticity in the brain*

The most striking effect on visual system development elicited by an EE paradigm starting at birth is a marked acceleration in the maturation of visual acuity (VA), a very sensitive and predictive index of visual system maturation. This has been initially assessed in the mouse, both electrophysiologically by visual-evoked potential (VEP) recordings and behaviourally by a discrimination task (visual water box task) [311], and then replicated in the rat [312]. The acceleration effect is strong, as in enriched animals VA development is 7 days beforehand with respect to control animals: in the timescale of human visual development, it would be as a child reached his final VA at around three years of age (i.e. approximately two years before the age at which children's acuity development normally ends). This precocious VA development induced by EE is accompanied by a precocious developmental decline of the possibility to induce LTP of layer II-III field potentials after theta-burst stimulation of the white matter in the visual cortex, a well-established in vitro model of developmental plasticity [311].

The study of molecular mechanisms underlying the effects of EE revealed that one crucial factor is the neurotrophin BDNF: mice reared from birth in EE have increased levels of the BDNF protein in their visual cortex at P7 [311], [313]. The acceleration of visual cortical development in EE animals closely resembles that previously reported in transgenic mice overexpressing BDNF in the forebrain [132]. A widely accepted model is that precocious higher BDNF levels triggers the development of the inhibitory GABAergic system, which, by affecting receptive field development and synaptic plasticity, could determine both the faster maturation of VA and the accelerated decline of synaptic plasticity. In line with this hypothesis, an increased expression of the GABA biosynthetic enzymes GAD65 and GAD67 has been found in EE pups at both P7 and P15 [311], [313]. Another molecular factor crucially involved in the EE effects on visual system development turned out to be IGF-I. IGF-I is increased postnatally in the visual cortex of enriched rats, and post-weaning administration of IGF-I in this structure mimics EE effects on VA acceleration. Furthermore, blocking endogenous IGF-I action in the visual cortex of developing EE subjects completely prevents EE effects on VA maturation [314]. One of the targets of BDNF and IGF-I signalling is the activation of CREB. Cancedda et al. [311] demonstrated that EE from birth accelerates the time course of CRE/CREB-induced gene expression and that treatment of non-EE mice with rolipram, a specific inhibitor of the high-affinity phosphodiesterase type IV that activate cAMP system, resulting in an increased phosphorylation of the transcription factor CREB, partially mimics EE effects on CREB pathway and on visual acuity development. Even if the work by Cancedda and colleagues focused on the visual system, it is very likely that the EE effect is not specific to the visual cortex, as suggested by the influence on CRE-mediated gene expression observed also in the other cortical areas [315], [316].

The surprising finding that EE affects BDNF and GABAergic inhibition before eye opening indicates that some of the EE effects on visual system development could be totally independent of vision. This issue has been addressed by Bartoletti et al. [317] in a study in which EE and dark rearing (DR) have been combined together. Lack of visual experience from birth prevents the VA development and prolongs the duration of the critical period in standard housed animals [26]. These effects can be completely counteracted by providing DR animals with the opportunity to experience EE while in the dark: DR-EE rats show a normal closure of the critical period for OD plasticity and a normal VA development. Also in this case the effect of EE is very similar to that found in BDNF overexpressing mice, in which a rescue of DR effects on visual system development is evident [32], and the influence of EE on GABAergic inhibitory circuits has been confirmed [317].

A more recent finding is the demonstration that also retina development is affected by experience provided by EE both at the electrophysiological and molecular level. Landi and colleagues monitored the development of retinal responses in enriched and non-enriched rats using pattern electroretinogram, a sensitive measure of retinal ganglion cells (RGCs) function. Retinal acuity development is sensitive to EE on the same time scale as cortical acuity [312]. Furthermore, enriched mice displayed a pronounced acceleration in the process of RGC dendrite segregation into ON and OFF sublaminae [318]. BDNF turned out to be a key molecule in both processes, as demonstrated by the higher BDNF levels in the RGC layer of enriched animals and by the lack of EE effects in the retina of enriched pups in which BDNF was blocked by means of antisense oligonucleotides [312], [318]. A clear influence of EE on retinal development has also been reported during prenatal life. Data by Sale et al. demonstrated that exposing pregnant females to EE (maternal enrichment) determines a marked acceleration of retinal anatomical development in the embryos, accelerating the

migration of neural progenitors and anticipating the time-course of naturally occurring cell death. Interestingly, the effects found in the foetus are mediated by IGF-I. Anatomical modifications, indeed, are accompanied by a marked increase in IGF-I expression in the retinas of enriched pups and in the milk of mothers. Furthermore, the neutralization of IGF-I in enriched mothers by means of administration of antiIGF-I antiserum prevents the action of maternal enrichment on retinal development, and chronic IGF-I injection to standard pregnant females mimics the effects of EE in the foetuses [138]. Strikingly, the effects of early enrichment were mimicked in human preterm infants through massage treatment that resulted to accelerate the development of the visual function through IGF-I signalling [319].

Interestingly, maternal care levels have been directly linked with epigenetic changes, [320], [321]; these findings together with the ones already reported by Fisher and colleagues [285] strongly suggest that chromatin remodelling might represent a direct link between EE and its long lasting effects on the visual cortex. Moreover, in the last years, many studies demonstrated an epigenetic control of experience-dependent gene transcription in developmental-dependent plasticity [171], [322] and enlightened the role of new molecules involved in the regulation OD plasticity, such as MicroRNAs (miRNAs)[323].

### *1.3.2) Environmental enrichment and adult cortical plasticity*

Promoting plasticity in the adult nervous system could pave the way for novel therapeutic strategies for the treatment of brain injuries and neurological disorders in adulthood, when recovery and functional rehabilitation are very hard to achieve. In the visual system, a widely diffused pathology (2-4% of incidence in the general population) for which no suitable treatment is still available in the adult, is the above mentioned amblyopia [186].

Strikingly, it has been demonstrated that adult amblyopic rats exposed to EE for 3 weeks immediately after being subjected to reverse suture (i.e., reopening of the long-term deprived eye and closure of the eyelids in the fellow eye) undergo a full recovery of their visual functions, both in terms of visual acuity and binocular vision [138], [324]. Beneficial effects on visual discrimination abilities were detectable at both the electrophysiological and behavioural level, and outlasted the end of the treatment for at least 10 days. Recovery of plasticity in enriched animals was accompanied by increased expression of BDNF, decreased density of CSPG perineuronal nets, and a three-fold reduction in GABA release detected in the visual cortex contralateral to the previously amblyopic eye through in vivo brain microdialysis, without any significant change in the release of glutamate. The reduction in the intracortical inhibition-excitation balance elicited by EE was causally linked with the functional rescue of visual functions, as definitely demonstrated by the complete lack of visual acuity and ocular dominance recovery in enriched rats intracortically infused in their visual cortex with the benzodiazepine Diazepam [138]. This has been one of the first demonstrations that reducing GABAergic inhibition promotes adult visual cortical plasticity, in agreement with Takao Hensch's work on the fundamental role of inhibition as a crucial regulator for the critical period in V1 [4], [7].

It is interesting to point out that while, during development, an increase in BDNF levels elicited by EE promotes the maturation of the GABAergic system in the visual cortex, in adult animals enhanced BDNF is associated with reduced GABA release in the EE setting. Distinct processes have been proposed to link these molecular factors together in different periods of the animal life [325]. Specifically, while it is well established that an early increase in BDNF is a prime trigger for the maturation of inhibitory circuitry in the immature brain [49], on the contrary, the increased expression of BDNF in adult animals may be one of the



consequences of a reduced inhibition/excitation balance, which can increase the expression of various genes involved in neural plasticity.

More recently, it has been assessed the possibility to rescue visual acuity in long-term deprived adult rats exposed to EE immediately after silencing of retino-thalamic projections of the non-amblyopic eye due to optic nerve dissection [326]. This represents a case of particularly relevant clinical interest, since a significant number of amblyopic patients lose their better eye due to accidents or ocular illnesses, thus becoming severely visually impaired [327], [328]. No spontaneous recovery of visual abilities was detected in animals reared under standard environmental conditions, but a full rescue of visual acuity was achieved in monocular rats exposed to EE, accompanied by lower numbers of GAD67-positive cells and increased BDNF expression in the visual cortex [326].

Given its totally non-invasive nature, EE appears as a promising strategy to counteract visual impairments in human amblyopia. One step up toward the application of the EE paradigm to clinics is investigating the role of independent EE components (e.g., social, sensory, motor) in reproducing the beneficial effects elicited by the entire enriched experience, and then designing therapeutic approaches based on the most promising and effective variables. Recently, it has been evaluated the efficacy of motor activity, social stimulation, and enhanced visual stimuli in promoting amblyopia recovery in the rat model [329]. The results showed a full recovery of ocular dominance and visual acuity both in animals experiencing high levels of voluntary motor activity in a running wheel and in rats exposed to a protocol of visual enrichment consisting in a rotating visual drum. A strong involvement of visual experience in the recovery process is suggested by the results obtained with amblyopic animals maintained under classic EE conditions in a completely dark room, which did not display any sign of visual function recovery. This is not at odds with

previous results by the group of Quinlan and co-workers [196], showing that a period of dark exposure before the animals were reverse-sutured and exposed-back to normal light conditions promotes vision recovery in adult amblyopic rats; in the other paradigm, indeed, the animals were reverse-sutured and immediately transferred to darkness in an EE setting, therefore lacking a period of reverse-suture experience in the light [329].

In contrast to motor and visual enrichment, social stimulation alone did not favour restoration of normal visual acuity and ocular dominance. The EE components effective in triggering recovery from amblyopia were associated with decreased GABA release in synaptosome analysis, without any change in the release of glutamate, thus resulting in a decreased intracortical inhibition/excitation ratio [329].

These findings might encourage the implementation of new environmental strategies devoted to promote stimulation of the amblyopic eye in adult patients as a way to increase their chance to undergo significant visual functional improvements. Accordingly, a growing body of evidence in humans shows that experimental paradigms analogous to EE, such as playing video games or being trained in visual perceptual learning (PL) tasks, can be quite successful in eliciting amblyopia recovery in adult subjects [330]–[333]. In search for possible cellular and molecular mechanisms underlying PL effects, Sale and colleagues [334] set up a model of visual PL in rodents [334]. They first trained a group of animals to practice in distinguishing between two vertical gratings differing only for their spatial frequency; then, they made the spatial frequencies of the two stimuli progressively more similar to each other, until the animal performance fell to chance level. A daily discrimination threshold was measured, revealing a progressive improvement of discrimination performance with training. Control animals were required to only discriminate between an unchanging grating and a homogeneous grey panel (a purely associative task), matching the overall swim time

and number of training days with those of PL rats. Activation of V1 circuitries in trained animals was confirmed by the strong selectivity of the PL process for grating orientation. Within 1 h from the last discrimination trial, LTP from layer II–III of V1 slices appeared occluded in PL animals compared with controls, both when testing its inducibility in vertical connections (stimulating electrode placed in layer IV) and when stimulating at the level of horizontal connections (stimulating electrode placed in layer II/III). Moreover, a significant leftward shift toward increased amplitudes of field excitatory postsynaptic potentials was found in the input/output curves of trained animals compared with controls [334]. Thus these data fulfil two of the most commonly accepted criteria used to relate LTP with learning, i.e., occlusion and mimicry, indicating that visual discrimination improvements in PL rats can be explained in terms of long-term increments of synaptic efficacy in V1. This is consistent with the critical role for LTP in mediating learning in other brain areas such as the amygdala, the hippocampus, and the motor cortex [335]–[337].

An involvement of LTP-like processes in visual PL has been also suggested by Cooke and Bear [338], who showed that a form of robust experience-dependent plasticity in V1 called stimulus-specific response potentiation (SRP) and consisting in amplitude increases in layer 4 VEPs after repeated presentation of a sinusoidal grating stimulus, is blocked by previous thalamocortical LTP induced with a theta burst electrical stimulation of the dorsal lateral geniculate nucleus.

In agreement with evidence in human subjects, Sale et al. [334] found a marked recovery of visual functions in amblyopic rats practicing our visual PL task; on the contrary, control animals practicing a visual associative task which did not induce LTP-like changes in V1, did not show any recovery. Visual PL, but not visual associative learning, is accompanied by a robust decrease of the inhibition-excitation ratio [329]. They proposed that practice

with visual PL through the amblyopic eye promotes recovery of visual functions via an LTP-like potentiation of synaptic input from the amblyopic eye on visual cortical neurons; this potentiation is likely to be favoured by the lowered inhibition-excitation balance. Considering that the inhibition-excitation balance is also impaired during development in amblyopic human subjects and that excessive inhibition levels are involved in the degradation of their spatial vision abilities [339], [340], these results provide a strong rationale for future therapeutic attempts in humans based on a non-pharmacological manipulation of the GABAergic tone. In line with this, repetitive TMS (rTMS), a treatment increasing cortical excitability, transiently improves contrast sensitivity in adult amblyopic subjects [341].

#### **1.4) Aims of the Thesis**

Despite the efforts towards an understanding of the impact of EE on visual functions, several fundamental open questions remain concerning the mechanisms underlying the effects induced by exposure to stimulating environmental conditions on visual system development and plasticity.

On the one hand, focusing on visual system development, while it is known that an epigenetic remodelling of chromatin structure controls developmental plasticity in the visual cortex, three main questions have remained open: i) which is the physiological time course of histone modifications? ii) Is it possible, by manipulating the chromatin epigenetic state, to modulate plasticity levels during the CP? iii) How can we regulate histone acetylation in the adult brain in a non-invasive manner?

On the other hand, one fundamental aim in EE studies is to find out those molecular factors that are more suitable of being artificially manipulated to mimic or to strengthen the

impact of the environment on brain health and plasticity. Among the potential mechanisms underlying the beneficial effects of the most successful treatments proposed so far for enhancing visual cortex plasticity in adult subjects, BDNF emerges as one promising candidate [77], [136]–[138]. BDNF has been reported to promote neural plasticity at both the structural and functional level [139], thus being a very attractive target for the implementation of strategies aimed at drug delivery in amblyopic subjects, with the aim to favour synaptic potentiation and functional recovery of neural connections conveying sensory information from the amblyopic eye to the visual cortex. This promising approach, however, is thwarted by the impossibility for BDNF delivered via peripheral administration to efficiently cross the blood-brain barrier [342].

Focusing on these open issues, the present Thesis is structured in two main parts. In the first part, I investigated the link between histone acetylation and cortical plasticity in the rat visual system, both during the CP and in adult subjects. In the second part, I explored the impact of BDNF intranasal administration as a potentially safe and useful procedure and an effective method to induce visual function recovery in adult amblyopic rats.

## Materials and Methods

### *Animal treatment and surgical procedures*

The study was approved by the Italian Ministry of Public Health (protocol authorized by ministerial decree no. 282/2013-B). The animals were housed in a room with a temperature of 22°C and a 12 h light/dark cycle. In all experiments, experimenters were blinded to experimental conditions.

### **2.1) Part one: *Experience affects CP plasticity in the visual cortex through an epigenetic regulation of histone post-translational modifications***

#### *2.1.1) Monocular deprivation*

Rats were anesthetized with avertin (2,2,2-tribromoethanol solution, 200 mg/kg) at different postnatal days (P; i.e., P21, P28, P35, and  $\geq$  P75). The animals were allowed to recover from anaesthesia and were returned to their cages. Eyelid closure was inspected daily until complete cicatrization. Rats showing occasional lid reopening (observed with a surgical microscope) were not included in the experiments.

#### *2.1.2) Rearing environments*

EE consisted of a large cage (100 x 50 x 82 cm) with two or more floors linked by stairs, containing several food hoppers, running wheels, and differently shaped objects (platforms, boxes, toys, tunnels, shelters, and nesting material), which were repositioned once per day and completely substituted with others once per week. During the CP, every cage housed at least one female rat with her litter plus three additional filler females; in adulthood, the enriched caged housed six to eight rats of the same gender together. Housing under

standard conditions (SCs) consisted of a standard cage (40 x 30 x 20 cm) housing one female rat with her litter (during the CP) or two to three adult rats of the same gender. Litter and food were provided *ad libitum*, and conditions were the same in both environmental conditions. For the assessment of CP plasticity, rearing under SCs or with EE was started the day of mating and continued until the litters reached the ages at which the analyses were performed. For the assessment of visual cortex plasticity in the adult, differential rearing was started at P60.

### *2.1.3) Single-unit recordings*

After 1 week of MD, animals were anesthetized with intraperitoneal injection of urethane (1.4 g/kg; 20% solution in saline; Sigma-Aldrich) and placed in a stereotaxic frame. Body temperature was maintained at 37°C. A hole was drilled in the skull, corresponding to the binocular portion of the primary visual cortex (binocular area Oc1B), contralateral to the deprived eye. A micropipette filled with NaCl (3M) was inserted into the cortex, 4.8–5.2mm (depending on animal age) lateral with respect to the  $\lambda$  point. Both eyes were kept open by means of adjustable metal rings. For single-unit recordings, the positions of receptive fields were mapped using a hand-held stimulator. Only cells with receptive fields within 20° of the vertical meridian were included in the analysis. Spontaneous activity, peak response, and receptive field size were determined from peristimulus time histograms (PSTHs) recorded in response to computer-generated bars (size, 3°; drifting speed, 38.15°/s; contrast, 100%), averaged over 10–20 stimulus presentations. Electrical signals were amplified (25,000-fold), bandpass filtered (500 Hz–5 kHz), digitized (12 bit resolution), and averaged in synchrony with the stimulus. The analysis was performed off-line by an experimenter who was blind to the treatment conditions of the animals. OD classes were evaluated on the basis of the ratio

of contralateral to ipsilateral peak response. More specifically, neurons in ocular dominance class 1 were driven only by stimulation of the contralateral eye; neurons in ocular dominance classes 2 (ratio of contralateral to ipsilateral peak response,  $\geq 2$ ) and 3 (ratio of contralateral to ipsilateral peak response, between 1.2 and 2) were binocular and preferentially driven by the contralateral eye; neurons in ocular dominance class 4 were equally driven by the two eyes (ratio of contralateral to ipsilateral peak response, between 0.83 and 1.2); neurons in ocular dominance classes 5 (ratio of contralateral to ipsilateral peak response, between 0.5 and 0.83) and 6 (ratio of contralateral to ipsilateral peak response,  $\leq 0.5$ ) were binocular and preferentially driven by the ipsilateral eye; and neurons in ocular dominance class 7 were driven only by the ipsilateral eye. For each animal, the bias of the OD distribution toward the contralateral eye [contralateral bias index (CBI)] was calculated as follows:  $CBI = [(N(1) - N(7)) + 2/3 (N(2) - N(6)) + 1/3 (N(3) - N(5)) + NTOT] / 2NTOT$ , where  $N(i)$  is the number of cells in class  $i$ , and NTOT is the total number of recorded cells in a specific animal. Additionally, for each cell an OD score was calculated as follows:  $\{[\text{peak(ipsi)} - \text{baseline(ipsi)}] - [\text{peak(contra)} - \text{baseline(contra)}]\} / \{[\text{peak(ipsi)} - \text{baseline(ipsi)}] + [\text{peak(contra)} - \text{baseline(contra)}]\}$ , where peak is the maximal spike frequency evoked by visual stimulation, ipsi is the ipsilateral eye, baseline is the mean spiking frequency in the absence of stimulation, and contra is the contralateral eye. OD score cumulative distributions were computed for each group. Cell responsiveness was assessed according to standard criteria in terms of the amplitude of modulation of cell discharge in response to an optimal visual stimulus (peak response divided by spontaneous discharge). Receptive field size was determined from PSTHs, assuming as a visual response the signal above a value equal to the mean spontaneous discharge + 2 SDs [26].



#### *2.1.4) Injections of suberoylanilide hydroxamic acid*

Suberoylanilide hydroxamic acid (SAHA) was dissolved in 100% DMSO at a concentration of 50 mg/ml, and then was further diluted to 25 mg/ml in a solution of 50% DMSO and 50% saline. Injections (25 mg/kg) were given intraperitoneally from P12 to P18 and were alternated daily between left and right sides of the abdomen. All animals were injected during the same time interval each day (11:00 A.M. to 12:00 P.M.).

#### *2.1.5) Visual cliff task*

I used the procedure previously described by [324]. The apparatus consisted of a rectangular arena (100 x 40 cm<sup>2</sup>) constructed in poly(vinyl chloride) with black walls and bordered by black curtains to prevent the escape of the animal. The arena was divided into two 50x40 cm<sup>2</sup> Plexiglas plates. A moving platform, the depth of which could be varied by means of a mechanical scissor jack, was placed below each glass plate. A patterned floor consisting of 3 cm black-and-white checked photographic paper covered the surface of the platform. Incandescent lamps placed below the two patterned floors illuminate both surfaces to equate the brightness of the two sides. A video camera was hanging on the apparatus, and was connected to a computer by which the experimenter could observe and record the behaviour of the rat. Testing took place in a quiet room. The arena was divided into a shallow and a deep side. On the shallow side, the patterned floor was positioned immediately below the glass plate, while on the deep side the checked platform was moved to 29 cm below the glass plate. Each animal was placed on the shallow side, and the total time the rat spent exploring each side of the arena was automatically recorded by the Noldus EthoVision system. The trial ended after 5 min. The arena was cleaned between trials with an alcohol solution. A discrimination index was calculated as follows:  $(ts-td)/ttot$ , where

*ts* and *td* are, respectively, the time spent exploring the shallow side and the deep side of the arena, and *ttot* is the total time of the test procedure. Each animal was tested only once.

#### 2.1.6) *Western blot*

To avoid circadian effects, all animals were killed during the same time interval each day (10:00 A.M. to 12:00 P.M.; light phase). After decapitation, visual cortices and hippocampi were removed rapidly and frozen on dry ice. Tissue was then homogenized in a hypotonic lysis buffer containing the following (in mM): Tris 10, pH 7.5; EDTA 1; sodium pyrophosphate 2.5;  $\beta$ -glycerophosphate 1; sodium orthovanadate 1; and phenylmethylsulfonylfluoride 1; with aprotinin 10  $\mu$ g/ml; leupeptin (Sigma-Aldrich) 10  $\mu$ g/ml; and IGEPAL CA-630 (Sigma-Aldrich) 1%. Histones were extracted from the nuclear fraction by the addition of five volumes of 0.2 M HCl and 10% glycerol, and the insoluble fraction was pelleted by centrifugation (18,000  $\times g$  for 30 min at 4°C). Histones in the acid supernatant were precipitated with 10 volumes of ice-cold acetone followed by centrifugation (18,000  $\times g$  for 30 min at 4°C). The histone pellet was then resuspended in 9 M urea. Protein concentration was determined by a Bio-Rad assay. Protein extracts of each sample were separated by electrophoresis and blotted; filters were blocked and incubated overnight at 4°C with primary antibodies (anti-acetyl-H3, 1:300 dilution, Millipore; anti-H3, 1:300 dilution, Millipore). Blots were then rinsed and incubated in infrared-labeled secondary antibodies (anti-mouse IRDye 680LT 1:20000 or anti-rabbit 800CW 1:20,000, Li-Cor Biosciences). Filters were scanned using an Odyssey IR scanner (Li-Cor), and densitometry analysis was performed with Image Studio Software version 3.1 (Li-Cor).

### 2.1.7) Chromatin immunoprecipitation

To avoid circadian effects, all animals were killed during the same time interval each day (10:00 A.M. to 12:00 P.M.; light phase). Visual cortices were removed, and the fresh tissue was immediately frozen and stored at -80 °C. Chromatin immunoprecipitation (ChIP) was performed according to the ChIP Kit (ab500, Abcam) guidelines. Briefly, tissue was cross-linked using 1% formaldehyde (10 min at room temperature), and the reaction was stopped by adding 0.125 M glycine. Samples were then sonicated, and DNA was fragmented (400–1500 bp range), using the Bioruptor (Diagenode) for 17 min (30 s “ON,” 30 s “OFF,” power high) at 4°C. Protein–DNA complexes were immunoprecipitated using protein A-coated beads, previously incubated with 1 µg of the specific antibody anti-ACh3 (lys 9 –14; catalog #06 –599, Millipore). Samples were incubated overnight at 4°C, and the beads were washed and treated with Proteinase K for 30 min at 55°C. Finally, DNA was purified according to the manufacturer protocol and subjected to quantitative PCR.

### 2.1.8) Real-time PCR

Levels of specific histone modifications at the P1 and P3 BDNF gene promoter were determined by measuring the amount of these promoters in chromatin immunoprecipitates by use of real-time PCR (Applied Biosystems Step One, Thermo Fisher Scientific). Input DNA (nonimmunoprecipitated DNA) and immunoprecipitated DNA were PCR amplified in triplicate using SsoAdvanced Universal SYBR Green Supermix (catalog #172–5271, Bio-Rad). The following primers were used to selectively amplify portions of BDNF P1, BDNF P3, and  $\beta$ -tubulin promoters: P1: forward, 5'-CCCCGCTGCGCTTTTCTGGT-3'; reverse 5'CAATTTGCACGCCGCTCCTTAC3'; P3: forward, 5'-GCGCGGAATTCTGATTCTGGTAAT-3'; reverse, 5'GAGAGGGCTCCACGCTGCCTTGACG-3';  $\beta$ -tubulin: forward, 5'-

TAGAACCTTCCTGCGGTCGT-3'; reverse, 5'-TTTTCTTCTGGGCTGGTCTC-3'. The relative quantities of immunoprecipitated DNA fragments were calculated by using the threshold cycle number (Ct) obtained during the exponential growth of the PCR products and normalized to the mean input Ct values of control animals [323]. Moreover, total RNA from rat visual cortex samples was purified by RNA nucleospin (Macherey-Nagel) as recommended by the manufacturer. cDNA was reverse transcribed using the Reverse Transcriptase Core Kit (Eurogentech). The following primers were used to amplify BDNF exons (IV and IX). Tubulin was used as an internal control for normalization: BDNF exon IV mRNA: forward, TGCGAGTATTACCTCCGCCAT; reverse, TCACGTGCTCAAAAGTGTCAG; and BDNF exon IX mRNA: forward, GAGAAGAGTGATGACCATCCT; reverse, TCACGTGCTCAAAAGTGTCAG. Quantitative real-time PCRs were performed using the SYBR PCR Mastermix (Applied Biosystems)

## **2.2) Part two: *Intranasal BDNF administration promotes visual function recovery in adult amblyopic subjects***

### *2.2.1) Animal Treatment and Surgical Procedures*

All experiments were performed in accordance with the approved guidelines and regulations of the Italian Animal Research Ethic Committee. All procedures were conducted in Long-Evans rats of both genders, and were approved by the Italian Ministry of Public Health. Rats were anesthetized with avertin (2,2,2 Tribromoethanol solution, 200 mg/kg) and mounted on a stereotaxic apparatus.

Monocular deprivation (MD) was performed through eyelid suturing at postnatal day (P) 21. Eyelid closure was inspected daily until complete cicatrization; subjects with even minimal spontaneous re-opening were excluded. Adult rats (P70) were either subjected to

reverse suture (RS) or to reopening of the deprived eye without RS. In RS rats, the long-term deprived eye was re-opened using thin scissors, while the other eye was sutured shut. Great care was taken, during the first week after both MD and RS, to prevent inflammation or infection in the previously deprived eye through topical application of antibiotic and cortisone. One day after RS or eyelid reopening, rats were divided in two groups, i.e. were either treated with intranasal BDNF or with intranasal saline. Thus, four groups were included in this study: BDNF-RS, SAL-RS, BDNF-bin, and SAL-bin.

### *2.2.2) Intranasal administration protocol*

From P70 rats received, every two days, intranasal infusions. Infusions were performed in awake animals. A solution of recombinant human BDNF (Harlan Laboratories, 1  $\mu$ M in saline) was administered intranasally with a Gilson pipette, 3  $\mu$ l at a time, alternating the nostrils, with a lapse of 2 minutes between each administration, for a total of 14 times. The administration was repeated 7 times, at 2 day intervals. During these procedures, the nostrils were always kept open. As control, rats were treated with saline. In order to assess possible undesired side-effects deriving from BDNF intranasal administration, I monitored animal survival and seizure susceptibility through behavioural observations. Behavioural observations began the second day of treatment and were protracted until the end of the entire protocol. Rat survival and the presence of seizures were qualitatively inspected during observation sessions of 120 min each, every 5 min, following a modified version of a previously published protocol.

### 2.2.3) *In vivo electrophysiology*

At the end of the intranasal treatment, the animals (n = 7 for both BDNF-RS and SAL-RS rats; n = 10 for BDNF-bin and n = 7 for SAL-bin rats) were anesthetized by i.p. injection with a mix of Zolazepam and Tiletamine (Zoletil-100, 40mg/kg, Virbac) and Xylazine (Xilor, 10mg/kg, Sigma) and placed in a stereotaxic frame. Additional doses of anesthetic were used to keep the anaesthesia level stable throughout the experiment. Body temperature was continuously monitored and maintained at ~37°C by a thermostated electric blanket during the experiment. A hole was drilled in the skull, corresponding to the binocular portion of the primary visual cortex (binocular area Oc1B) contralateral to the long-term-deprived eye. After exposure of the brain surface, the dura was removed, and a micropipette (2 MΩ) filled with NaCl (3 M) was inserted into the cortex 5 mm from (intersection between sagittal- and lambdoid-sutures). Both eyes were fixed and kept open by means of adjustable metal rings surrounding the external portion of the eye bulb.

Visual acuity was measured through both eyes using visual evoked potentials (VEPs). During recording through one eye, the other was covered by a black adhesive tape. To record VEPs, the electrode was advanced at a depth of 100 or 400 μm within the cortex. At these depths, VEPs had their maximal amplitude. Signals were band-pass-filtered (0.1-100 Hz), amplified, and fed to a computer for analysis. At least 50 events were averaged in synchrony with the stimulus contrast reversal. Transient VEPs in response to abrupt contrast reversal (0.5 Hz) were evaluated in the time domain by measuring the peak-to-baseline amplitude and peak latency of the major negative component. Visual stimuli were horizontal sinusoidal gratings of different spatial frequencies and contrast, generated by a VSG2/2 card running custom software and presented on a monitor (20 x 22 cm; luminance 15 cd m<sup>-2</sup>) positioned 20 cm from the rat's eyes. Visual acuity was obtained by extrapolation to zero

amplitude of the linear regression through the data points in a curve where VEP amplitude is plotted against log spatial frequency [138]. Ocular dominance(OD) was measured by calculating the contralateral to ipsilateral VEP ratio (C/I ratio), i.e. the ratio of VEP amplitudes recorded by stimulating the eye contralateral and ipsilateral, respectively, to the visual cortex where the recording is performed. During recording through one eye, the other was covered by a black adhesive tape.

#### *2.2.4) Behavioural assessment of visual acuity*

Six BDNF-bin and 6 SAL-bin rats were used for the behavioural assessment of visual acuity. I first measured visual acuity of the open eye (not deprived). Then, visual acuity of the amblyopic eye was measured (by temporary occlusion of the fellow eye) three times, i.e. after eyelid reopening (at P70), immediately at the end of the intranasal treatment and 4 weeks after the end of the intranasal treatment. To measure visual acuity, I used the visual water task [20],[343] which trains animals to first distinguish a low ( $0.1 \text{ cycles deg}^{-1}$ ) spatial frequency vertical grating from grey, and then tests the limit of this ability at higher spatial frequencies.

The apparatus consisted of a trapezoidal shaped pool with two panels placed side by side at one end. A midline divider was 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 was placed below the grating. Animals were released from the centre at the end of the pool opposite the panels. The position of the grating and the platform was alternated in a pseudorandom sequence over training trials while the rats were shaped to swim towards the grating in one of the maze

arms. A trial was recorded as incorrect if an animal entered the arm without the platform. Animals were removed from the pool when they found the platform.

Once 80% accuracy was achieved, the limit of the discrimination was estimated by increasing the spatial frequency of the grating. Visual acuity has been taken as the spatial frequency corresponding to 70% of correct choices on the sigmoidal function fitting the psychometric function. During each session, the experimenter was blind to the experimental group.

#### *2.2.5) Behavioural assessment of stereopsis in the visual cliff task*

I used  $n = 8$  BDNF-RS,  $n = 5$  SAL-RS rats,  $n = 7$  BDNF-bin and  $n = 6$  for SAL-bin rats. I followed the procedure previously described in [324]. The apparatus consisted of a rectangular arena (100 x 40 cm) constructed in poly(vinyl chloride) with black walls and bordered by black curtains to prevent the escape of the animal. The arena was divided into two 50 x 40 cm Plexiglas plates. A moving platform, the depth of which could be varied by means of a mechanical scissor jack, was placed below each glass plate. A patterned floor consisting of 3 cm black-and-white checked photographic paper covered the surface of the platform. Incandescent lamps placed below the two patterned floors illuminated both surfaces to equate the brightness of the two sides. A video camera was hanging on the apparatus, and was connected to a computer by which the experimenter could observe and record the behavior of the rat. Testing took place in a quiet room. The arena was divided into a shallow and a deep side. On the shallow side, the patterned floor was positioned immediately below the glass plate, while on the deep side the checked platform was moved to 29cm below the glass plate.



Each animal was placed on the shallow side, and the total time the rat spent exploring each side of the arena was automatically recorded by the Noldus EthoVision system. The trial ended after 5 min. The arena was cleaned between trials with an alcohol solution. A discrimination index was calculated as follows:  $(t_s - t_d) / t_{tot}$ , where  $t_s$  and  $t_d$  are, respectively, the time spent exploring the shallow side and the deep side of the arena, and  $t_{tot}$  is the total time of the test procedure. Each animal was tested only once.

#### 2.2.6) *Western blot*

In order to avoid circadian effects, all animals (BDNF-bin:  $n = 7$ ; SAL-bin:  $n = 7$ ) were sacrificed during the same time interval each day (10:00–12:00 h; light phase). After decapitation, brains were rapidly removed and the visual cortex was dissected and frozen on dry ice. Proteins were extracted with lysis buffer (20 mM Tris-HCl pH 7.45, 150 mM NaCl, 10 mM EDTA, 0.1 mM  $\text{Na}_3\text{VO}_4$ , 1mM PMSF, 1  $\mu\text{g}/\text{ml}$  leupeptin, 1  $\mu\text{g}/\text{ml}$  aprotinin, 1% Triton X-100 and 10% glycerol), and the total concentration of the samples was assessed with a protein assay kit (Bio-Rad) using a bovine serum albumin-based standard curve. Protein extracts (50  $\mu\text{g}$ ) were separated by electrophoresis and blotted; filters were blocked and incubated overnight at 4°C with anti-BDNF primary antibodies (1:500, Santa Cruz). Filters were also probed with anti- $\beta$ -tubulin antibody (1:15,000 dilution, Abcam) as an internal standard for protein quantification. Blots were then rinsed in Tween buffered saline (TPBS), incubated in infrared labelled secondary antibodies IRDye 700 CW or 800 CW (1:20,000 dilution, Li-Cor Biosciences), washed in TPBS and briefly rinsed in PBS. Filters were scanned using an Odyssey® IR scanner and densitometry analysis was performed with Odyssey® imaging software 3.1. Antibody signal was calculated as integrated intensity of the region defined around the band of interest. Protein amount was evaluated measuring the signal of

the band of interest and dividing it by the signal of  $\beta$ -tubulin band on the same filter. Samples were run in triplicate and in three separate gels; each sample from each experimental group was thus present in every single gel.

#### *2.2.7) Drug administration*

A different group of long-term monocularly-deprived rats were subjected to eyelid reopening as described before. Then, under anaesthesia, an osmotic minipump (model 2002; Alzet, Palo Alto, CA), connected via PE tubing to a stainless steel cannula (30 gauge), was implanted in the visual cortex contralateral to the formerly-deprived eye. Osmotic minipumps (flow rate,  $0.25 \mu\text{l hr}^{-1}$ ) were filled up with either U0126 ( $250 \mu\text{M}$ , in DMSO 10%;  $n = 6$  U0-BDNF-bin rats; see 40), LY294002 ( $5\mu\text{g}/\mu\text{l}$ , in DMSO 10%;  $n = 5$  LY-BDNF-bin rats; see 24), or vehicle solution (10% DMSO;  $n = 5$  veh-BDNF-bin rats). Starting one day after surgery, both groups of rats were subjected to BDNF intranasal treatment. Then, after 14 days of treatment, we performed electrophysiological recordings of visual acuity and C/I ratio by VEPs, as previously described.

## Results

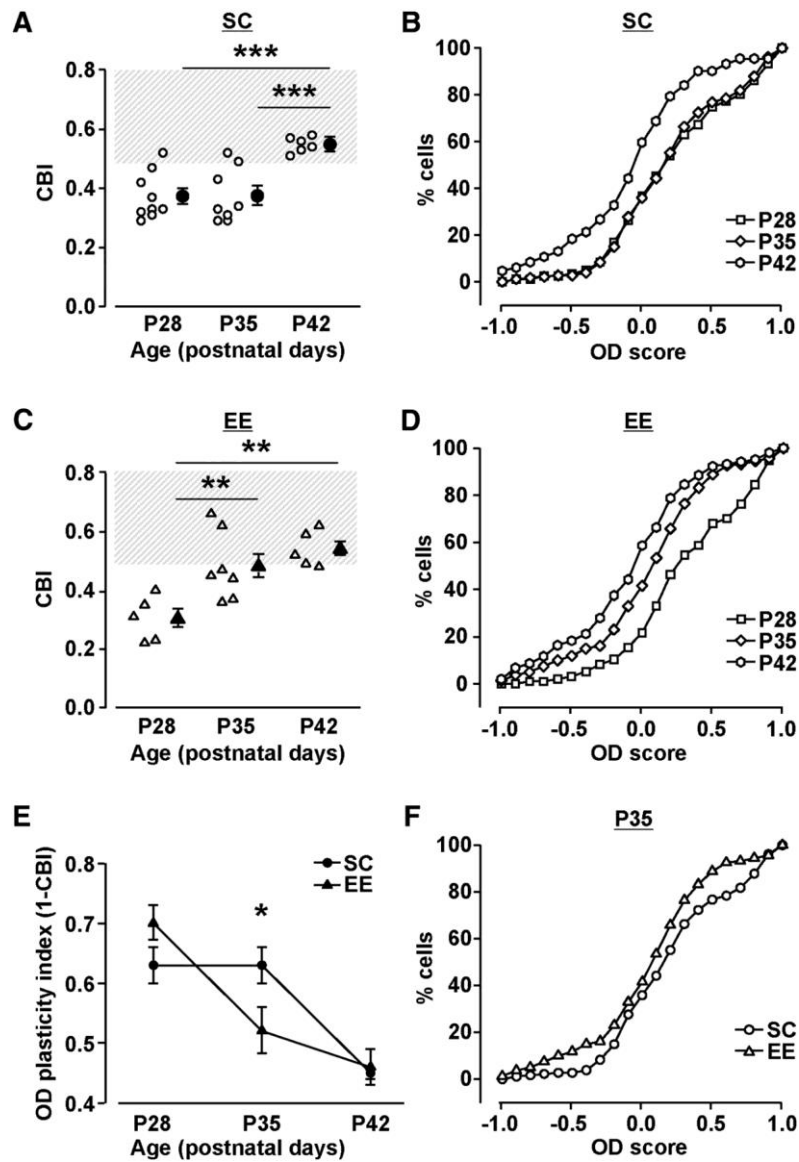
### **3.1) Part one: *Experience affects CP plasticity in the visual cortex through an epigenetic regulation of histone post-translational modifications***

#### *3.1.1) OD plasticity in developing rats*

I first examined, at different postnatal ages, OD plasticity in response to 1 week of MD in rats reared under either SCs or with EE. Single-unit recordings have been used in anesthetized animals to calculate the CBI, which reflects the proportion of cells preferentially driven by the eye contralateral to the brain side from where recordings are made. I found robust and equal levels of OD plasticity in SC animals at P28 (n = 9) and P35 (n = 8; one-way ANOVA, post hoc Holm–Sidak method,  $p = 0.964$ ), with a significant decay in plasticity occurring at P42 (n = 6) with respect to the previous ages (Fig. 1A; one-way ANOVA, post hoc Holm–Sidak method,  $p < 0.001$  for both comparisons).

I also performed a finer comparison of OD distributions, computing a normalized OD score of single neurons. Consistently, the OD score distribution for P28 (202 cells) and P35 SC animals (181 cells) was shifted in favour of the not-deprived eye and did not differ between each other (Kolmogorov–Smirnov test,  $p = 0.924$ ), whereas it was significantly changed in P42 rats (131 cells) with respect to both P28 and P35, as a result of a reduction in the shift of OD in favour of the not-deprived eye (Fig. 1B;  $p < 0.001$  for both comparisons).

The decay of OD plasticity turned out to be accelerated in rats raised with EE. Indeed, I found that the CBI of EE animals was robustly shifted toward the not-deprived eye only at P28 (n = 5), when it significantly differed from that recorded at both P35 (n = 7) and P42 (n = 5; one-way ANOVA, post hoc Holm–Sidak method,  $p < 0.01$  for both comparisons);

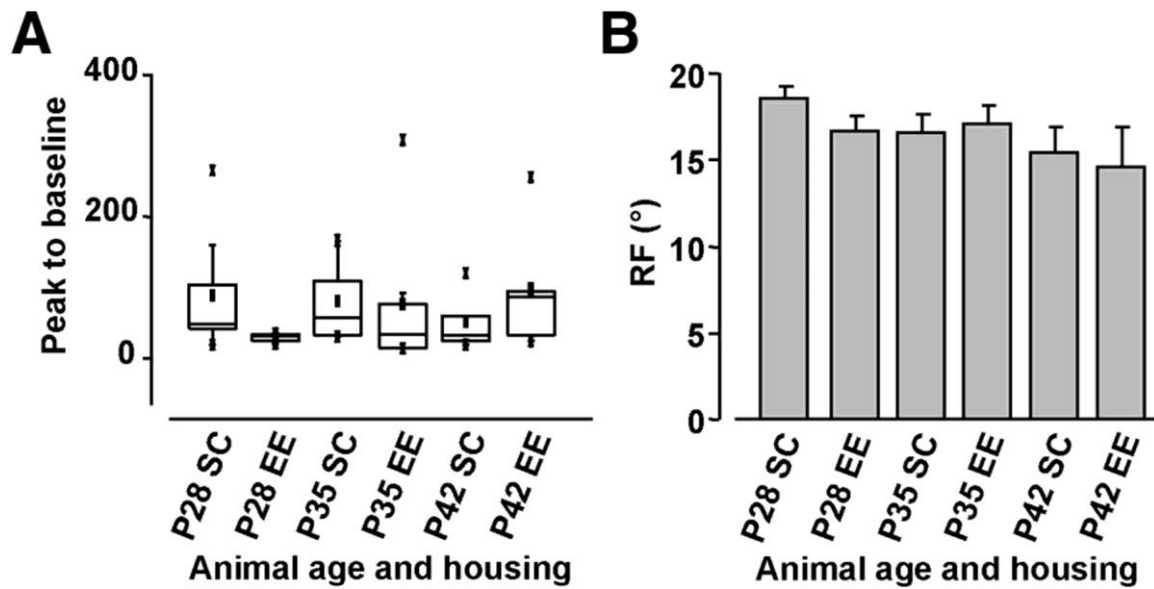


**Figure 1.** Early environmental enrichment accelerates the critical period for OD plasticity. **A**, Filled circles represent the average CBI  $\pm$ SEM for each experimental group; open symbols represent individual CBIs for each animal. At P28 and P35, the CBIs of SC-reared animals were comparable, indicating a clear OD shift toward the open eye (P28:  $n=9$ , CBI= $0.37\pm 0.03$ ; P35:  $n=8$ , CBI= $0.38\pm 0.03$ ; one-way ANOVA, post hoc Holm–Sidak method,  $p=0.964$ ); at P42, the CBI of SC-reared rats differed from those of the previous two ages ( $n=6$ , CBI= $0.55\pm 0.01$ ;  $p<0.001$  for both comparisons) as a result of a reduced OD plasticity. The grey box denotes the CBI range in adult normal animals calculated as the mean  $\pm 2$ \*SD from recordings of adult naive rats. **B**, Analogously to CBI, the OD score distributions for P28 animals ( $n=9$ ; 202 cells) and P35 animals ( $n=8$ , 181 cells) did not differ (Kolmogorov–Smirnov test,  $p=0.924$ ), whereas the OD score distribution for P42 rats ( $n=6$ , 131 cells) was different from those of the two other ages ( $p<0.001$  for both comparisons). **C**, The CBI of enriched animals was shifted toward the not-deprived eye only at P28, when it significantly differed from that at both P35 and P42 (P28:  $n=5$ , CBI= $0.30\pm 0.03$ ; P35:  $n=7$ , CBI= $0.48\pm 0.04$ ; P42:  $n=5$ , CBI= $0.54\pm 0.03$ ; one-way ANOVA, post hoc Holm–Sidak method,  $p<0.01$  for both comparisons); starting from P35, enriched rats displayed an earlier closure of OD plasticity, as indicated by their higher CBI, which did not differ from that recorded at P42 ( $p=0.299$ ). The grey box denotes the CBI range in adult normal animals calculated as the mean  $\pm 2$ \*SD from recordings of adult naive rats. **D**, The results obtained with the CBI were confirmed by those with computation of OD score in enriched animals: indeed, the OD score distribution for P28 animals ( $n=8$ ; 97 cells) differed from that at both P35 ( $n=7$ , 161 cells) and P42 ( $n=5$ , 104 cells; Kolmogorov–Smirnov test,  $p<0.01$  for P28 vs P35;  $p<0.001$  for P28 vs P42). The OD score distributions for P35 and P42 groups were not different ( $p=0.088$ ). **E**, A plasticity index for each animal was calculated as  $1-\text{CBI}$ . Two-way ANOVA showed an interaction between age and environmental housing conditions ( $p<0.05$ ). A post hoc Holm–Sidak test revealed a difference (\*) at P35 between SC and EE groups ( $p<0.05$ ). **F**, OD score cumulative distributions for the P35 SC and EE groups differed (Kolmogorov–Smirnov test,  $p=0.05$ ). The asterisks indicates statistical significance: \* $p<0.05$ ; \*\* $p<0.01$ ; \*\*\* $p<0.001$ . Data are expressed as the mean  $\pm$  SEM.

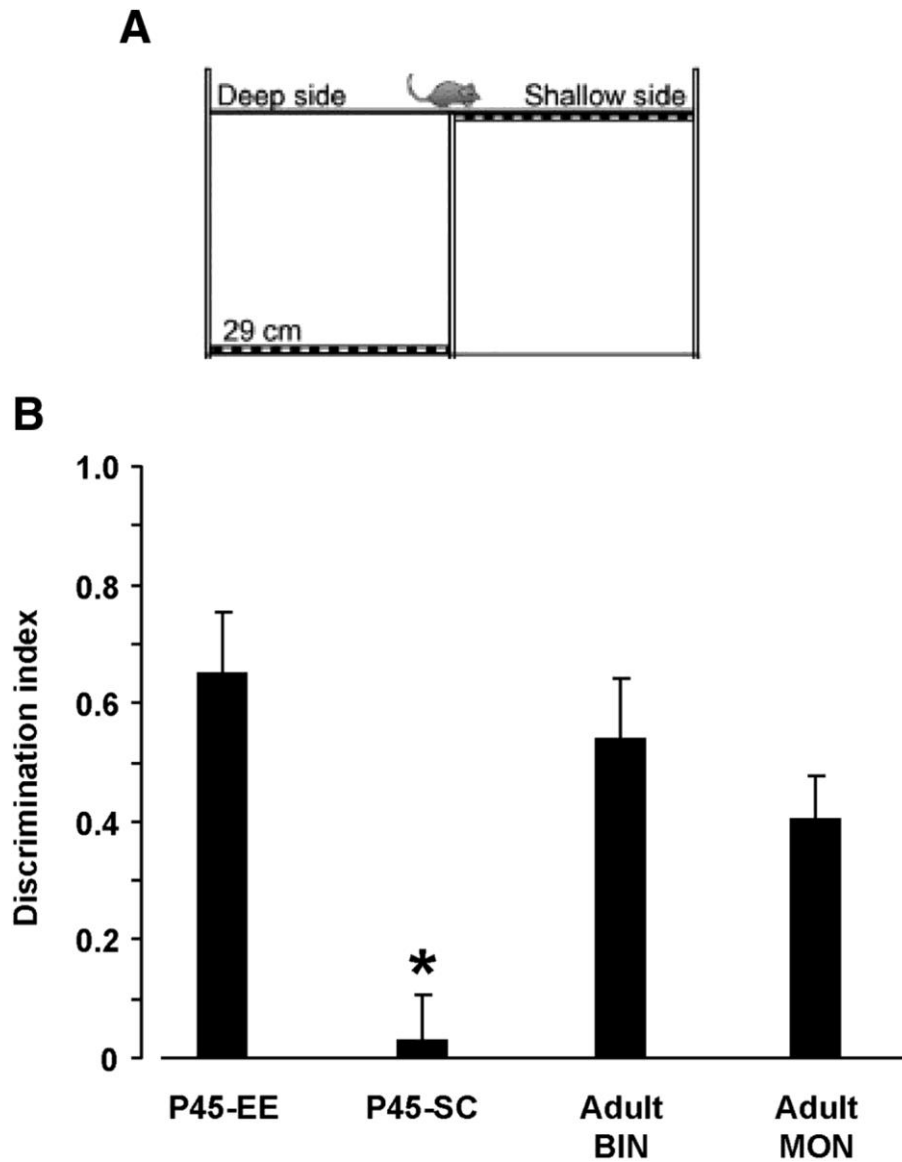
differently from SC rats, the CBI computed for EE rats already appeared to be significantly increased at P35, with levels comparable to those recorded 1 week later, at P42, reflecting a 1 week acceleration in the decay of visual cortex plasticity (Fig. 1C;  $p = 0.299$ ). This result was confirmed by the OD score distribution, which at P28 (97 cells) was found to differ from that at both P35 (161 cells) and P42 (104 cells) EE animals (Kolmogorov–Smirnov test:  $p < 0.01$  for P28 vs P35;  $p < 0.001$  for P28 vs P42), while no difference in OD score distribution was detected between P35 and P42 (Fig. 1D;  $p = 0.088$ ).

To better compare OD plasticity between rats raised under SCs and with EE, I calculated for each animal a plasticity index, expressed as  $1 - \text{CBI}$ , a parameter that is thus directly proportional to OD plasticity levels (Fig. 1E). A two-way ANOVA of this parameter showed an interaction between age and environmental housing condition ( $p < 0.05$ ). A post hoc Holm-Sidak test revealed a difference between the SC and EE groups only at P35 ( $p < 0.05$ ), when the OD score cumulative distribution was also found to differ between the two groups (Fig. 1F; Kolmogorov–Smirnov test,  $p < 0.05$ ). No difference was found between SC and EE animals in terms of either cell responsiveness (two-way ANOVA on ranks,  $p = 0.243$ ) or receptive field size distribution (Fig. 2A,B; two-way ANOVA,  $p = 0.474$ ). Thus, these data demonstrate that an early exposure to EE accelerates the closure of the CP for OD plasticity in the primary visual cortex.

It has been previously suggested that altering the timing of the CP for OD plasticity might result in abnormal visual development [344]. Thus, I also evaluated whether rearing in EE from birth affected stereopsis abilities at P45 (i.e., at the end of the CP). Not deprived animals raised under SCs and with EE have been tested in the visual cliff task, evaluating their spontaneous preference for the deep side or the shallow side of the arena ([324], Fig. 3A). I found that both groups of animals displayed a clear preference for the shallow side of



**Figure 2.** Similar basic cell properties in enriched and standard-reared animals. We analysed cell responsiveness and receptive field (RF) size in the same animals in which OD evaluation was performed. **A**, Cell responsiveness for each unit was expressed as the ratio between the peak response and the mean baseline activity obtained by optimal stimulation of the preferred eye. Data are represented as box charts. For each box chart, the central horizontal line represents the median value, and the other two horizontal lines are the 25th and 75th percentiles; error bars denote the 5th and 95th percentiles; square symbols denote the mean value. No statistical difference in cell responsiveness was found among the experimental groups (two-way ANOVA on ranks,  $p=0.243$ ). **B**, RF size for each cell was calculated on the basis of the peristimulus time histogram obtained by optimal stimulation of the preferred eye and was expressed in degrees ( $^{\circ}$ ) of visual angle. No differences in receptive field size distribution were detected among the experimental groups (mean RF size: P28-SC= $18.6^{\circ}\pm 0.7^{\circ}$ ; P28-EE= $16.7^{\circ}\pm 0.9^{\circ}$ ; P35-SC= $16.6^{\circ}\pm 1.1^{\circ}$ ; P35-EE= $17.1^{\circ}\pm 1.1^{\circ}$ ; P42-SC= $15.5^{\circ}\pm 1.5^{\circ}$ ; P42-EE= $14.6^{\circ}\pm 2.3^{\circ}$ ; two-way ANOVA,  $p=0.474$ ). Data are expressed as the mean  $\pm$  SEM.



**Figure 3.** Depth perception abilities are not altered by the acceleration of critical period timing in enriched rats. **A**, In an explorative version of the visual cliff task, P45 SC animals ( $n=17$ , exploration index= $0.401 \pm 0.073$ ) and EE animals ( $n=12$ , exploration index= $0.541 \pm 0.100$ ) displayed a preference for the shallow side, thus revealing the maturation of proper stereopsis abilities. **B**, The discrimination index scores did not differ between the two experimental groups (one-way ANOVA, post hoc Holm–Sidak method,  $p=0.409$ ), and, importantly, it was also not significantly different from that recorded in adult naive animals with normal binocular vision (adult BIN:  $n=9$ , exploration index= $0.652 \pm 0.101$ ; one-way ANOVA, post hoc Holm–Sidak method,  $p=0.147$  and  $p=0.409$  respectively); instead, adult rats with one eye closed through eyelid suture exhibited a prominent change in their discrimination index (adult MON:  $n=9$ ; exploration index= $0.028 \pm 0.077$ ), equally exploring the deep and the shallow sides of the arena (one-way ANOVA, post hoc Holm–Sidak method,  $p < 0.05$  for all comparison with adult MON animals). The asterisk indicates statistical significance:  $*p < 0.05$ . Data are expressed as the mean  $\pm$  SEM

the arena (SC, n = 17; EE, n = 12) without a significant difference between them (one way ANOVA, post hoc Holm–Sidak method, p = 0.409; Fig. 3B). Moreover, the two groups of animals did not differ in their respective exploration index from adult naive rats (adult BIN, n = 9; one way ANOVA, post hoc Holm–Sidak method, p = 0.147 and p = 0.409 respectively), which instead exhibited a marked difference compared with monocularly deprived animals in which stereopsis abilities were hampered by imposing single eye vision through an eyelid suture (adult monocular vision (MON), n = 9; one-way ANOVA, post hoc Holm–Sidak method, p < 0.05 for all comparisons with adult MON animals).

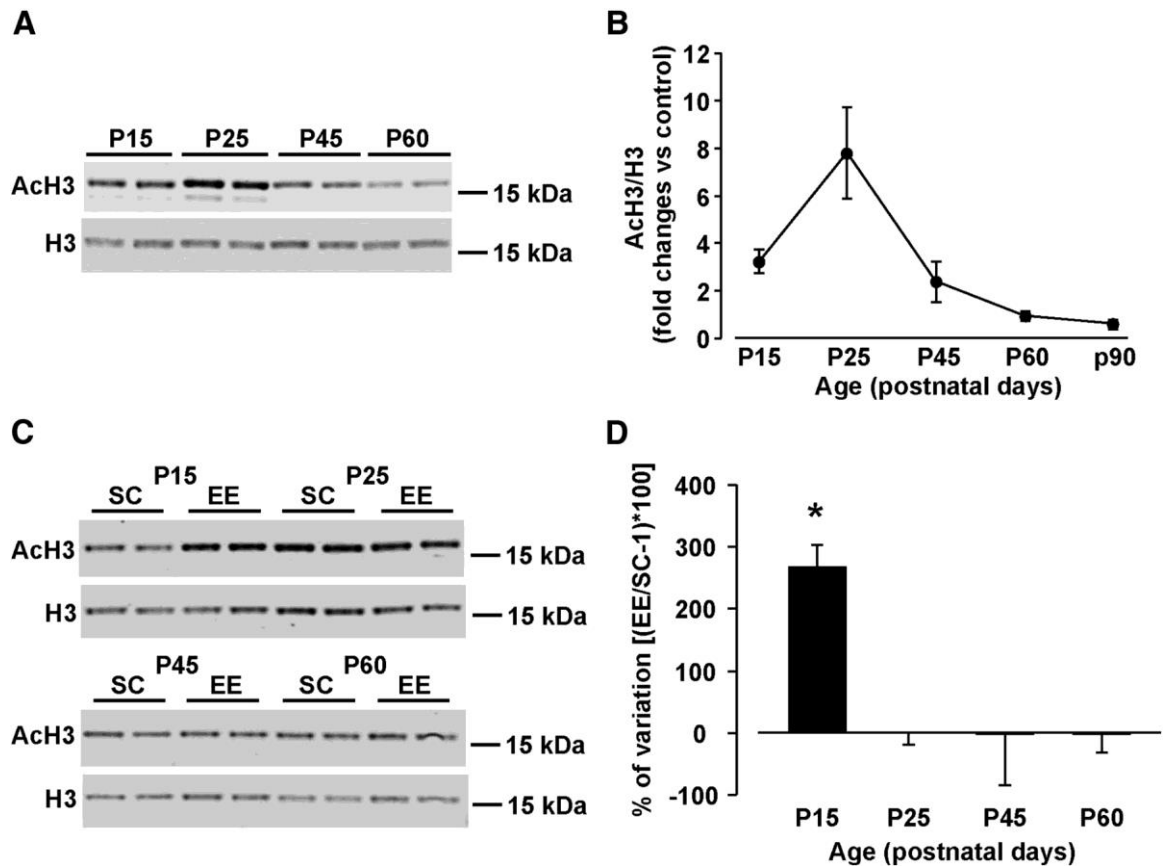
Together, these results indicate that an accelerated CP time course did not alter the maturation of visual depth perception abilities in EE rats.

### 3.1.2 *EE enhances histone acetylation in the primary visual cortex*

Since changes in the functional state of chromatin have been reported to be involved in visual system developmental plasticity [171], I measured, in the visual cortex of pups reared either with EE or under SCs, the levels of acetylated histone H3-Lys 9 (ACh3), expressing the data as the ratio between the intensity of the Western blot bands of ACh3 and total H3 (ACh3/H3 ratio; SC: n(P15)=11, n(P25)=11, n(P45)=9, n(P60)=9, n(P90)=8; EE: n(P15)=8; n(P25)=6; n(P45)=7; n(P60)=6).

First, I found a developmental regulation of ACh3 levels in the primary visual cortex of SC rats, with low levels detectable at P15, a peak at P25, and then a progressive decay up to P90 (Fig. 4A,B; effect of age, p < 0.001, Kruskal–Wallis one-way ANOVA on ranks). Then, I measured developmental changes in the ACh3/H3 ratio between SC and EE rats (Fig. 4C,D). I found that the ACh3/H3 ratio was significantly increased in the visual cortex of EE animals at P15 (Mann–Whitney rank sum test, p < 0.05); no difference between SC and EE groups was





**Figure 4.** Developmental regulation of histone acetylation in V1: effects of exposure to EE. The ratio between the intensity of the bands of acetyl-H3 and total H3 (AcH3/H3 ratio) was used as an index for measuring the amount of acetylated H3. **A**, A representative immunoblot showing protein levels in the visual cortex of SC-reared rats at P15 (n=11), P25 (n=11), P45 (n=9), P60 (n=9), and P90 (n=8). In each gel, the AcH3/H3 ratio measured for all samples was normalized to the mean ratio calculated for the control group (P60 animals). **B**, Quantification of acetylated H3 levels showed a developmental regulation in the primary visual cortex of SC-reared rats. A Kruskal–Wallis one-way ANOVA on ranks revealed a significant effect of age ( $p < 0.001$ ). **C**, A representative Western blot gel displaying protein levels in the visual cortex of P15, P25, P45, and P60 animals reared either in SC or EE conditions (EE-P15, n=4; EE-P25, n=6; EE-P45, n=7; EE-P60, n=6). In each gel, the AcH3/H3 ratio measured for all samples was normalized to the mean ratio calculated for the control group (SC animals for each age). **D**, Percentage of variation of AcH3/H3 ratio in the visual cortices of rats reared under SCs and with EE computed as  $[(EE/SC-1) \times 100]$  at different ages. Acetylated H3 levels were significantly increased in the visual cortex of EE animals at P15 (Mann–Whitney rank sum test,  $p < 0.05$ ), while they did not differ between SC and EE groups at the other ages tested (t test,  $p = 0.994$  for P25; Mann–Whitney rank sum test,  $p = 0.535$  for P45; t test,  $p = 0.850$  for P60). The asterisk indicates statistical significance: \* $p < 0.05$ . Data are expressed as the mean  $\pm$  SEM.

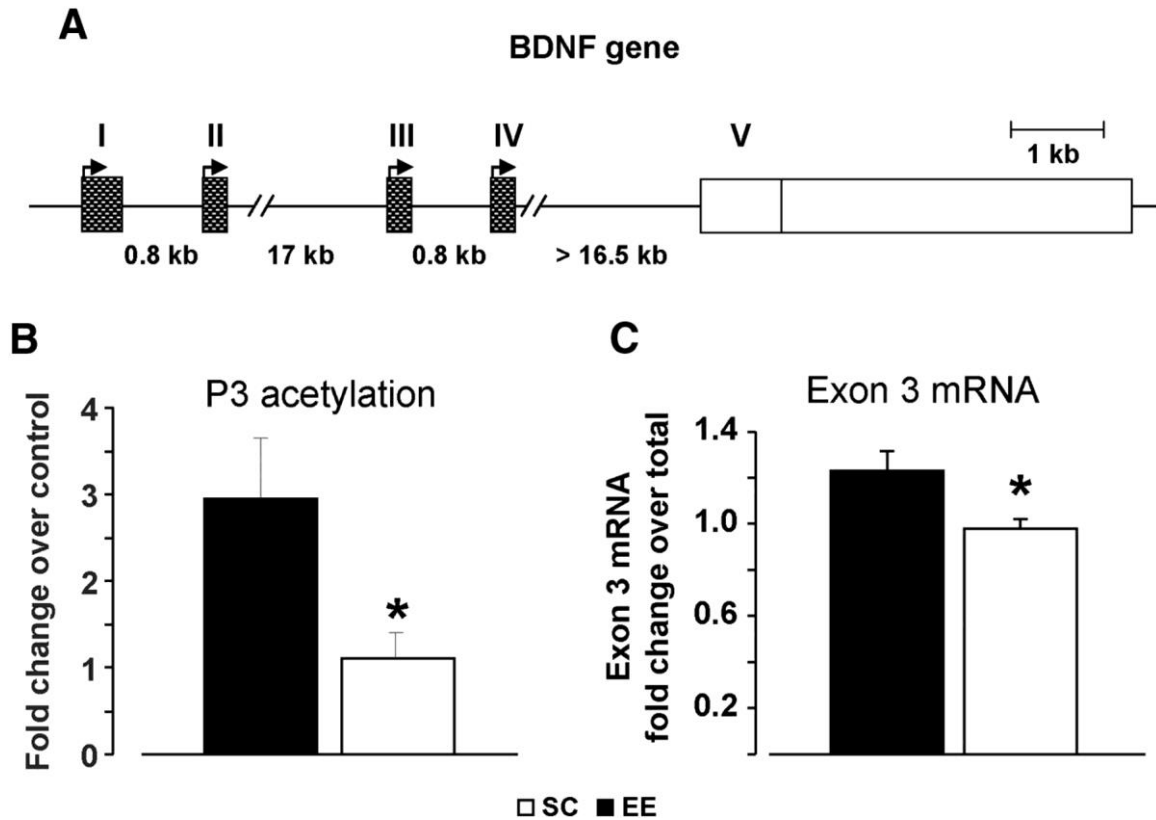
instead detected at the other ages tested (t test,  $p=0.994$  for P25; Mann–Whitney rank sum test,  $p=0.535$  for P45; t test,  $p=0.850$  for P60).

These results indicate a developmental regulation of histone acetylation in the primary visual cortex, with early exposure to EE resulting in an upregulation of Ach3 levels. To evaluate whether the regulation of histone acetylation by EE was specific to the cerebral cortex, Ach3 have been also assessed levels in the hippocampus of EE and SC rats at P15. I found increased Ach3 levels in EE compared with SC animals (t test,  $p < 0.05$ ).

### *3.1.3) Histone acetylation at the BDNF gene promoter*

Chromatin remodelling is a dynamic process that modulates gene expression, with hyperacetylation at promoters usually loosening the structural interactions between DNA and histones, allowing the transcriptional machinery access to the gene promoters, thus resulting in an increase in gene activity. To better understand the molecular factors by which EE exerts its impact on visual cortex plasticity, I studied histone acetylation at the promoters of the gene encoding BDNF, a factor crucially involved in developmental plasticity in the primary visual cortex and in the effects of early exposure to EE [49], [345], [313],[312].

Focusing on P15, the age at which maximal changes in total histone acetylation levels were found between rats reared with EE and under SCs, I specifically examined, via chromatin immunoprecipitation followed by real-time PCR, how histone modifications were altered at both P1 and P3 BDNF gene promoters, two of the four BDNF promoters (P1 through P4) that can differentially regulate BDNF expression in the rat brain[346]. While no difference between the two groups of animals was found for BDNF P1 H3 acetylation (t test,  $p = 0.475$ ), I found a significant increase in BDNF P3 H3 acetylation in the visual cortex of EE subjects ( $n = 4$ ) with respect to SC animals ( $n = 4$ ; Fig. 5B; t test,  $p < 0.05$ ). As control, I also



**Figure 5.** Exposure to EE enhances H3 acetylation at the BDNF promoter 3. **A**, Schematic diagram of the structure of the rat BDNF gene. Boxes represent exons, and lines represent introns. BDNF exons I to IV are shown as chequered boxes and are directed by promoters I to IV, shown as right-angled arrows. **B**, Levels of H3 acetylation at the BDNF promoter 3 in rats reared with EE and under SCs at P15 (n=4 in both groups), calculated as mean fold changes over SC-reared controls. A t test revealed a significant increase in the fold change for EE rats compared with SC-reared animals (  $p < 0.05$ ). **C**, Levels of exon 3 BDNF mRNA normalized over total BDNF mRNA in rats reared with EE and under SCs at P15 (n=4 in both groups). A t test revealed a significant increase in the fold change for EE rats compared with SC-reared animals (  $p < 0.05$ ). The asterisk indicates statistical significance: \* $p < 0.05$ . Data are expressed as the mean  $\pm$  SEM.

measured levels of histone acetylation at the promoter of the  $\beta$ -tubulin gene, which is expressed in the visual cortex, but whose expression levels are expected to be unchanged after exposure to EE. No difference was found in H3 acetylation at the  $\beta$ -tubulin promoter between SC and EE rats ( $n=4$  for both groups; t test,  $p=0.181$ ).

These findings indicate that the observed changes in histone acetylation at the BDNF P3 promoter did not depend on a difference between EE and SC animals in terms of global histone acetylation. Finally, I also measured by real-time PCR the P3/total mRNA ratio for the BDNF gene at P15. Notably, I found that the BDNF P3/total mRNA ratio was significantly increased in EE rats compared with SC rats (Fig. 5C;  $n=4$  for both groups; t test,  $p < 0.05$ ), which is consistent with the increased P3 H3 acetylation.

#### *3.1.4) A causal link between histone acetylation and CP plasticity*

To assess whether the early increase in H3 acetylation detected in the visual cortex of EE rats was functionally linked to the accelerated decline in visual cortical plasticity displayed by the same group, we analysed the effects of MD in SC animals treated with SAHA, a deacetylase inhibitor that has been widely used to increase histone acetylation in the brain [347],[348]. To better mimic the effects of exposure to EE, SAHA was intraperitoneally injected for 7 d starting at P12. Then, OD plasticity was assessed at P35 (MD from P28 to P35).

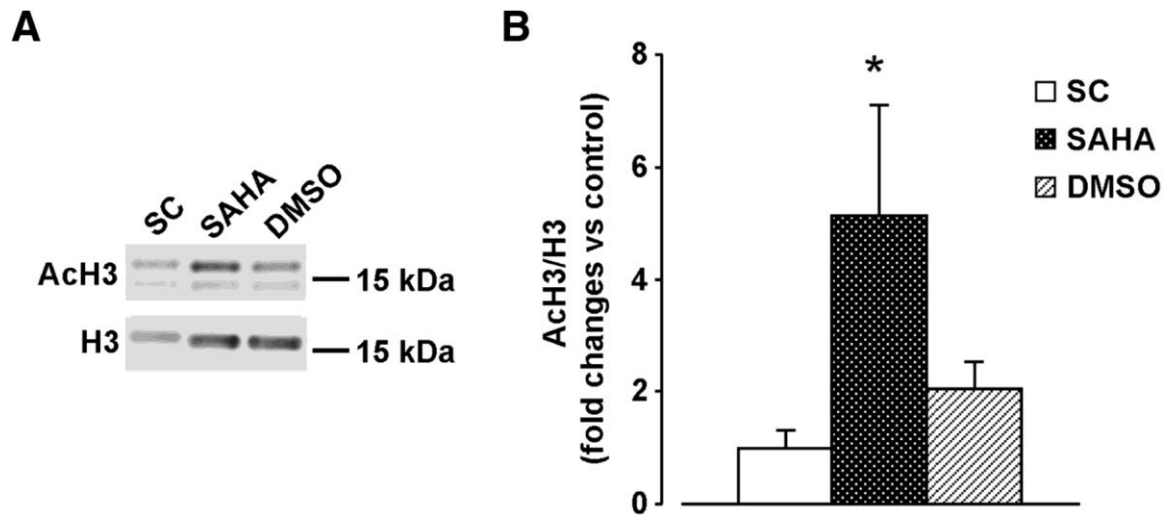
First, I assessed whether treatment with SAHA was able to replicate the early increase in H3 acetylation previously documented in the visual cortex of EE rats. I found that the primary visual cortex of SC-SAHA rats ( $n=6$ ) displayed, at P15, increased amounts of AcH3 compared with SC animals (Kruskal–Wallis one-way ANOVA on ranks vs control, post hoc Dunn's method,  $p<0.05$ ), while histone acetylation levels in vehicle-treated age-matched rats (50% DMSO in saline;  $n = 6$ ) were not different from those of controls (Kruskal–Wallis one-way ANOVA on ranks vs control, post hoc Dunn's method; Fig. 6A,B).

Second, I performed single-unit recordings in SC animals treated with SAHA and subjected to MD starting at P28. Similar to what was found previously in EE animals, I found an earlier decay in OD plasticity in SAHA-treated animals ( $n = 5$ ) with respect to untreated rats raised under SCs (one-way ANOVA vs control, post hoc Holm–Sidak method,  $p < 0.05$ ), while animals to which the vehicle solution (DMSO;  $n=7$ ) was administered showed, as expected, a mean CBI shifted in favour of the not-deprived eye, like rats raised under SCs (Fig. 7A;  $p = 0.422$ ). The comparison of OD score distributions confirmed these results. Indeed, the OD score cumulative distribution did not differ between SC and vehicle treated animals (Kolmogorov–Smirnov test,  $p=0.540$ ), whereas it was statistically different in SAHA-treated rats (Kolmogorov–Smirnov test,  $p < 0.01$ ), further demonstrating that increased levels of histone acetylation induce an acceleration of visual cortex development (Fig. 7B). No difference was found among animals raised under SCs, and those treated with SAHA and DMSO in terms of either cell responsiveness (Fig. 7C; Kruskal–Wallis one-way ANOVA on ranks,  $p=0.939$ ) or receptive field size distribution (Fig. 7D; one-way ANOVA,  $p=0.289$ ).

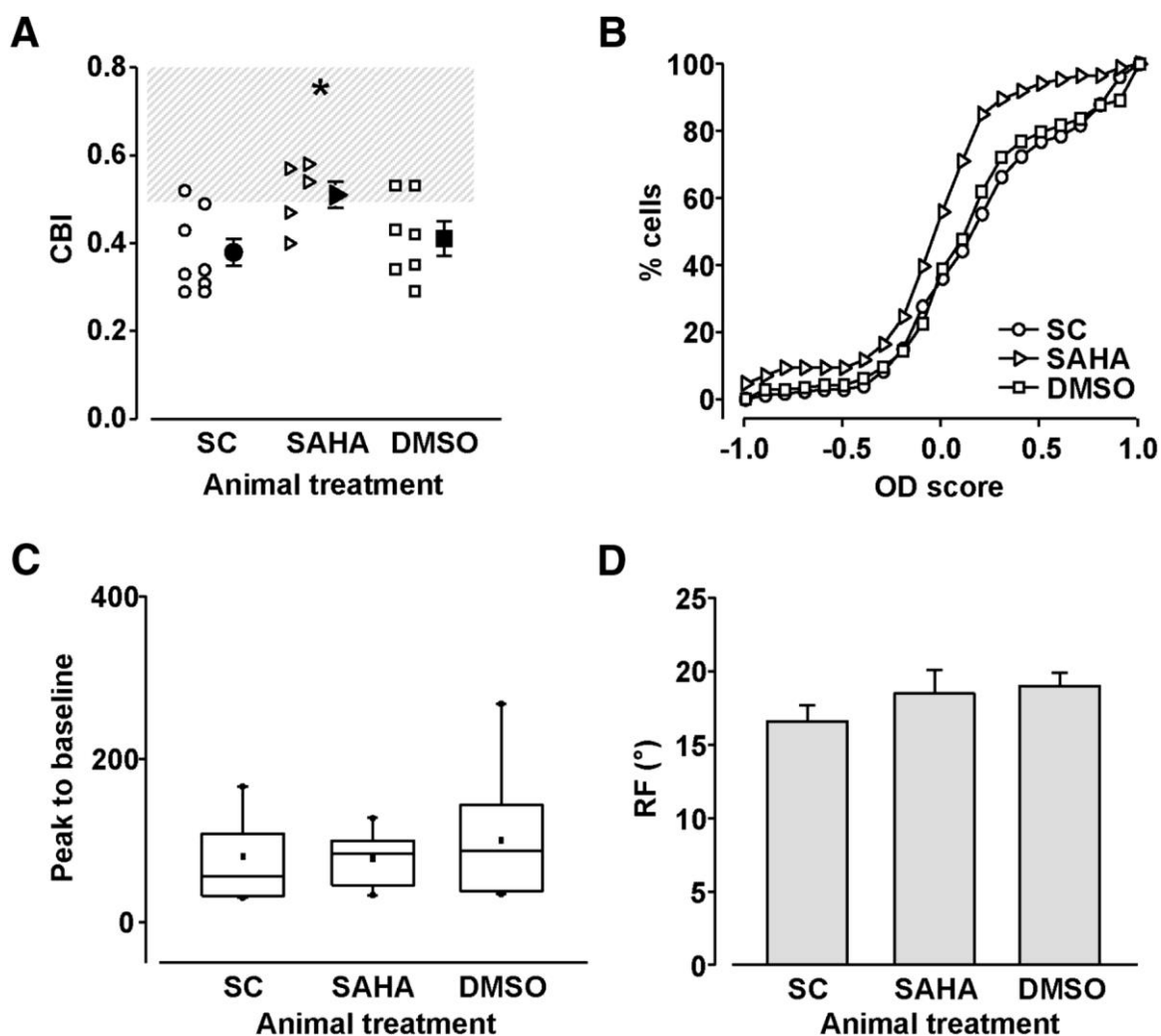
These results together demonstrate that the early increase in H3 acetylation found in rats exposed to EE is causally linked to the accelerated decline of visual cortical plasticity induced by the enriched experience.

### *3.1.5) EE in adulthood increases H3 acetylation and reinstates OD plasticity in the rat visual cortex*

To assess whether the developmental downregulation of experience-dependent histone acetylation could have a functional consequence for visual cortical plasticity, I took



**Figure 6.** Injection of a histone deacetylase inhibitor mimics increased H3 acetylation. **A**, A representative Western blot filter displays protein levels in the visual cortex of animals raised under SCs (n=8), and those treated with SAHA (n=5) and DMSO (n=7) at P15. In each gel, the AcH3/H3 ratio measured for all samples was normalized to the mean ratio calculated for the control group (SC of the same age). **B**, Quantification of acetylated H3 levels showed that acetylation levels were significantly increased in the visual cortex of SAHA animals, while the AcH3/H3 ratio of DMSO rats was not different from that measured in the SC control group (Kruskal–Wallis one-way ANOVA on ranks vs controls, post hoc Dunn’s method). The asterisk indicates statistical significance: \*p<0.05. Data are expressed as the mean ± SEM.



**Figure 7.** Injection of a histone deacetylase inhibitor mimics accelerated closure of OD plasticity. **A**, CBI values for animals raised under SCs ( $n=8$ ,  $0.38 \pm 0.03$ ), and those treated with SAHA ( $n=5$ ,  $0.51 \pm 0.03$ ) and DMSO ( $n=7$ ,  $0.41 \pm 0.04$ ). Filled circles represent the average CBI  $\pm$  SEM for each experimental group; open symbols represent individual CBIs for each animal. At P35, the CBI of DMSO-treated rats was not significantly different from that of SC animals, whereas the visual cortex driving force in SAHA rats remained significantly shifted toward the contralateral (deprived) eye (one-way ANOVA vs control, post hoc Holm–Sidak method). The gray box denotes the CBI range in adult normal animals calculated as the mean  $\pm$  2\*SD from recordings of adult naive rats. **B**, OD score distribution for animals raised under SCs (181 cells) and those treated with DMSO (147 cells) did not significantly differ between each other (Kolmogorov–Smirnov test,  $p=0.540$ ), whereas OD distribution for the SAHA-treated group (109 cells) was significantly shifted in favour of the deprived eye (Kolmogorov–Smirnov test,  $p<0.01$  for both comparisons). **C**, **D**, The functional basic properties of visual cortical neurons were not affected in SAHA- and DMSO-treated animals. The data for cell responsiveness are represented as box charts. No statistical differences were present among all groups for either cell responsiveness (C, Kruskal–Wallis one-way ANOVA on ranks,  $p=0.939$ ) or RF size distribution (mean RF size: SC= $16.6^\circ \pm 1.1^\circ$ ; SAHA= $18.5^\circ \pm 1.6^\circ$ ; DMSO= $19.0^\circ \pm 0.9^\circ$ ; one-way ANOVA,  $p=0.289$ ). The asterisk indicates statistical significance: \* $p<0.05$ . Data are expressed as the mean  $\pm$  SEM.

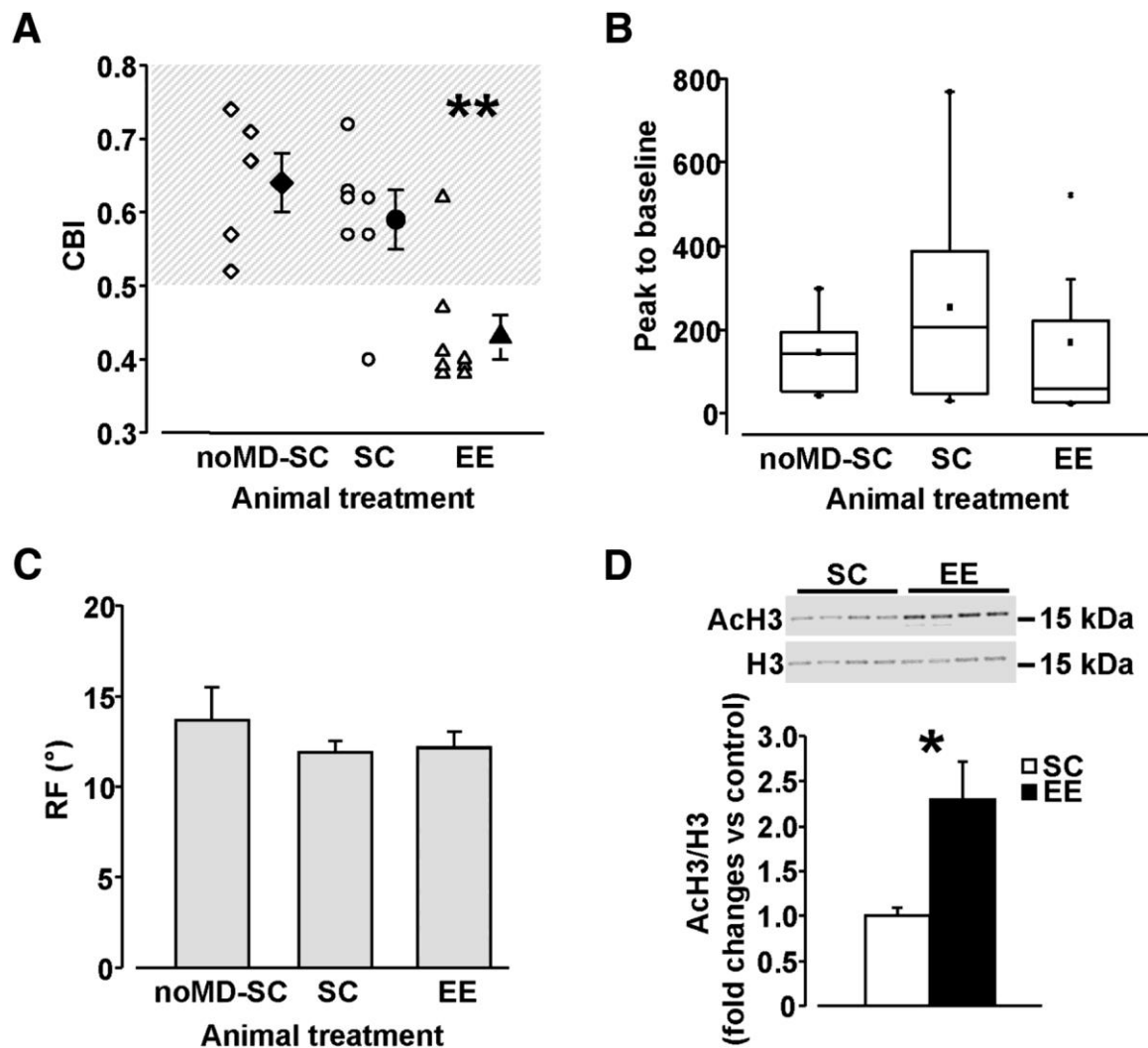
advantage of the remarkable capability of EE to reinstate OD plasticity in adult rats past the end of the CP [136]. After 14 d of differential rearing in adulthood (starting from P60), SC and EE animals were monocularly deprived and maintained under their respective rearing conditions for another 7 d. Then, I recorded single unit activity to assess OD plasticity.

I found that the CBI of EE rats ( $n = 8$ ) was reduced in response to MD with respect to that of both SC rats ( $n = 7$ ) and naive adult rats ( $n = 5$ ; no MD-SC rats), as a result of a plastic change in eye preference displayed by visual cortical neurons (Fig. 8A; one-way ANOVA, post hoc Holm–Sidak method,  $p < 0.01$  for both comparisons). No statistical difference was present among the three experimental groups in terms of cell responsiveness (Fig. 8B; Kruskal–Wallis one-way ANOVA on ranks,  $p = 0.749$ ) or receptive field size distribution of the cell population (Fig. 8C; one-way ANOVA,  $p = 0.515$ ).

Finally, I investigated whether the reopening of CP plasticity in adult EE rats was accompanied by changes at the histone acetylation level. I found an upregulation of Ach3 in the primary visual cortex of not-deprived animals exposed to EE for 14 d ( $n = 7$ ) compared with controls raised under SCs ( $n = 8$ ; Fig. 8D; ttest,  $p < 0.05$ ). An increased Ach3 acetylation was also found in the primary visual cortex of MD-EE rats ( $n = 8$ ) compared with MD-SC controls ( $n=8$ ; t test,  $p < 0.05$  in both cases).

Thus, exposure to EE is able to raise histone acetylation in the adult visual cortex, leading to a reopening of the CP for OD plasticity.





**Figure 8.** Environmental enrichment restores OD plasticity in adulthood and increases H3 acetylation in the visual cortex of adult animals. **A**, Filled symbols represent the average CBI±SEM for each experimental group; open symbols represent the CBI of each individual recorded. After 7 d of MD, the CBI of SC rats was completely comparable to that of noMD-SC animals (SC rats: n=7, CBI=0.59±0.04; noMD-SC: n=5, CBI=0.64±0.04; one-way ANOVA, post hoc Holm–Sidak method, p=0.342), whereas the CBI of EE rats (n=8; CBI=0.43±0.03) significantly differed from those of the two control groups (one-way ANOVA, post hoc Holm–Sidak method, p < 0.01 for both comparisons). **B**, No statistical difference in cell responsiveness was present among noMD-SC (132 cells), SC (189 cells), and EE animals (211 cells; Kruskal–Wallis one-way ANOVA on ranks, p=0.749). Data are represented as box charts. **C**, EE exposure did not alter the receptive field size distribution of the cell population (mean RF size: noMD-SC = 13.7° ± 1.8°; SC = 11.9°±0.6°; EE = 12.1°±0.9°; one-way ANOVA, p=0.515). **D**, Inset, A representative immunoblot showing protein levels in the visual cortex of animals of different experimental groups (animals reared under SCs and animals reared with EE for 14 d). In each gel, the AcH3/H3 ratio measured for all samples was normalized to the mean ratio calculated for the control group (SCs). Quantification of acetylated H3 levels revealed that EE conditions significantly increased histone acetylation in the adult visual cortex (SCs: n=8; EE: n=7; t test, p<0.05). Data are expressed as the mean ± SEM. The asterisks denote significant differences: \*p<0.05; \*\*p<0.01.

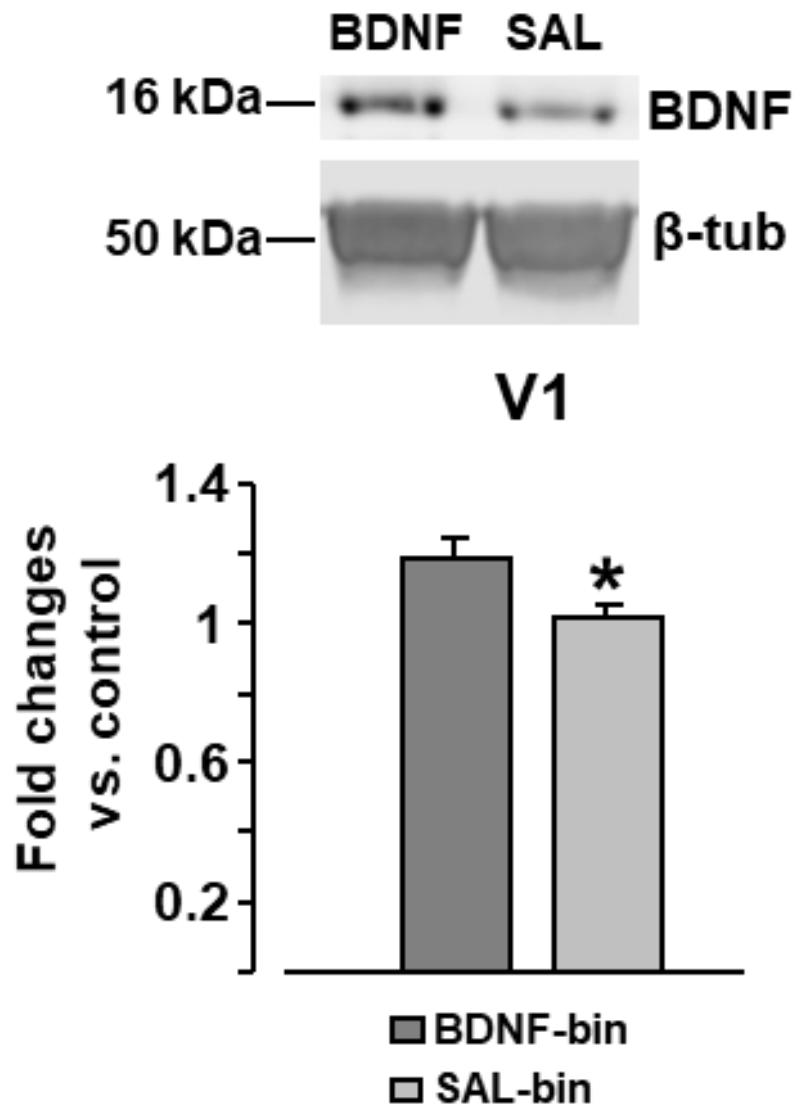
### **3.2) Part two: *Intranasal BDNF administration promotes visual function recovery in adult amblyopic subjects***

#### *3.2.1) Evaluation of BDNF pro-convulsive side effects*

Since previous studies in animal models have reported possible pro-convulsive effects of BDNF treatment [349],[350] I started this study by performing a preliminary analysis aimed at evaluating rat survival and seizure susceptibility. Behavioural observations began the second day of treatment and were performed every other day until the end of the protocol. Rat survival and the presence of seizures were qualitatively inspected during observation sessions of 120 min each, assigning a score in a rating scale for seizures every 5 minutes [351]. In both BDNF-treated and saline-treated animals, we did not observe any case of death or tonic clonic seizure (n = 5 for both groups; Two-Way RM ANOVA, effect of treatment , F = 1.136, p = 0.720318; treatment x day interaction, F = 0.631, p = 0.582677), and the behaviour of both groups of animals were always classifiable in the lowest risk classes of the rating scale.

#### *3.2.2) Intranasal BDNF infusion increases BDNF levels in V1*

I investigated levels of BDNF protein content via Western blot analysis, focusing on the primary visual cortex (V1). I found that BDNF levels were significantly increased in BDNF-treated rats with comparison to saline-treated animals (n = 7 in both groups) (Fig. 9), demonstrating that intranasal BDNF infusion was able to enhance BDNF availability in V1.



**Figure 9.** Quantification of mature BDNF expression in the visual cortex of rats treated with either intranasal BDNF or intranasal saline. BDNF expression was significantly increased in the visual cortex of BDNF-treated animals (t-test,  $p < 0.05$ ). (a, inset) Representative immunoblotting of BDNF expression in the two groups of animals. \*, statistical significance. Error bars, s.e.m.

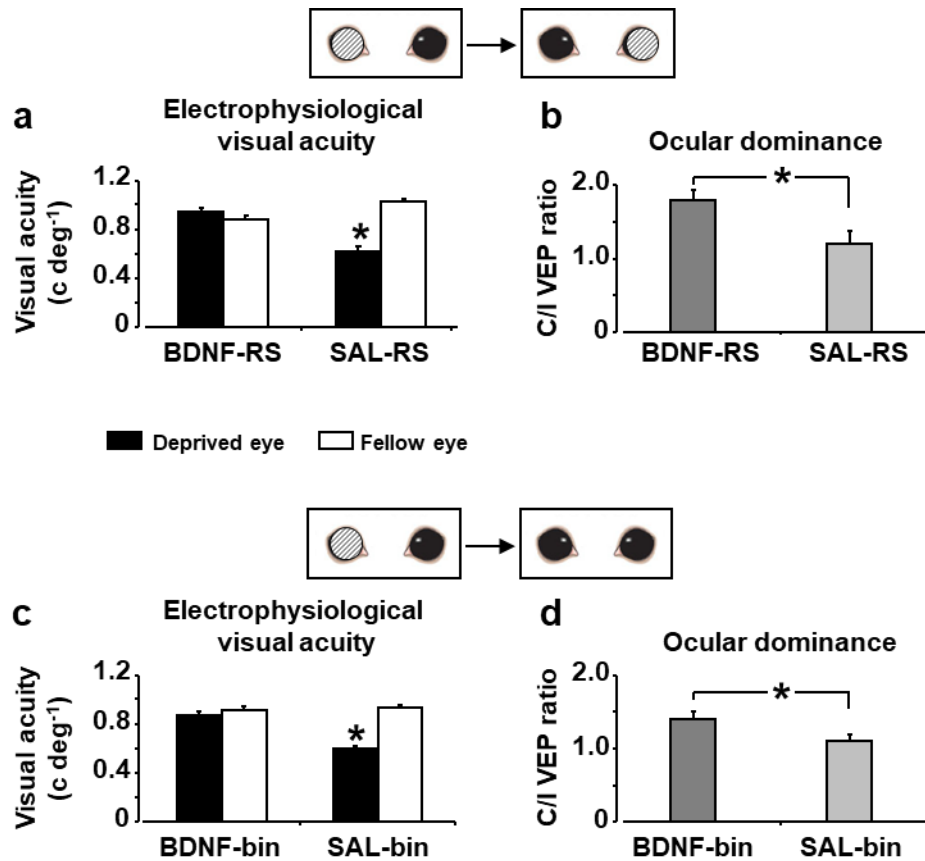
### 3.2.3) *Visual function recovery in adult rats with reverse-occlusion*

To investigate the effects of intranasal BDNF delivery on visual function recovery in amblyopic rats I first assessed the effects of BDNF administration (1  $\mu$ M in saline) in adult animals that were rendered amblyopic by long-term monocular deprivation (MD) started during the CP, and then subjected to reverse suture (reopening of the deprived eye, closure of the fellow eye, BDNF-RS, n = 7). Visual acuity was measured using electrophysiological recordings of visual evoked potentials (VEPs) from V1.

In control rats treated with intranasal saline after RS (SAL-RS, n = 7), visual acuity of the deprived eye remained significantly lower ( $0.62 \pm 0.04$  cycles per degree, c deg<sup>-1</sup>) with respect to the other eye ( $1.03 \pm 0.03$  c deg<sup>-1</sup>) (Fig. 10a). In contrast, I found a full visual acuity recovery in BDNF-RS rats ( $0.95 \pm 0.03$  c deg<sup>-1</sup>, see Fig. 10a).

To determine ocular dominance (OD), I measured the contralateral to ipsilateral (C/I) VEP ratio in response to a low spatial frequency grating (0.1 c deg<sup>-1</sup>) in the same animals in which I assessed visual acuity recovery. As previously reported, MD effects are maximal for cells located close to the vertical meridian 20. Therefore, in order to analyse recovery of OD at cortical locations corresponding to the representation of the vertical meridian, I used the procedure described by Pizzorusso and colleagues [171]: I first stimulated with a narrow grating (width of 20 deg) that could be positioned at different azimuths in the visual field and then measured the C/I VEP ratio at the electrode location for which the response to the narrow grating was maximal with the stimulus positioned around the vertical meridian. In SAL-RS rats, there was no recovery of OD in the visual cortex contralateral to the formerly deprived eye (C/I VEP ratio =  $1.20 \pm 0.20$ , Fig. 10b). In contrast, BDNF-RS rats showed a marked rescue of OD, with a C/I VEP ratio of  $1.79 \pm 0.13$ .

Amblyopia results in marked deterioration not only in the visual acuity domain, but also in stereopsis abilities, which, albeit being independent from visual acuity performance, are very rarely analysed in the animal model literature on amblyopia. Therefore, I investigated whether BDNF treatment affected stereopsis abilities in the visual cliff task, evaluating the spontaneous preference of amblyopic rats for the deep side or the shallow side of the arena. I found no preference for the shallow side of the arena in SAL-RS rats ( $n = 5$ ), whose exploration index ( $-0.04 \pm 0.08$ ) was markedly lower than that of naïve rats with normal binocular sight vision ( $0.65 \pm 0.10$ ) (Fig. 11). BDNF-RS rats ( $n = 8$ ) displayed a clear preference towards the shallow side of the arena, with an exploration index ( $0.31 \pm 0.09$ ) much higher than that of SAL-RS rats (Fig. 11); the rescue of stereopsis in BDNF-treated rats, albeit remarkable, was not complete, as their exploration index remained lower than that of naïve rats with normal binocular sight vision.



**Figure 10.** Electrophysiological assessment of visual acuity and ocular dominance through VEPs' recordings in reversed sutured rats (**a, b**) and in rats with unrestricted binocular vision (**c, d**). One-way ANOVA showed a full rescue of visual acuity in both BDNF-RS and BDNF-bin rats, with levels statistically not different from those of adult naïve rats (Holm-Sidak method,  $p = 0.487$  and  $0.197$ , respectively); in contrast, SAL-RS and SAL-bin rats displayed visual acuity values significantly lower than those of adult naïve rats (Holm-Sidak method,  $p < 0.05$  in both cases). Visual acuity was lower in the formerly deprived eye than in the fellow eye in SAL-RS and SALbin rats (paired t-test,  $p < 0.05$ ), but not in BDNF-bin animals (paired t-test,  $p = 0.140$ ); in BDNFRS rats, visual acuity of the formerly deprived eye became even better than that of the other eye (paired t-test,  $p < 0.05$ ). The C/I VEP ratio was significantly higher in BDNF-RS than in SAL-RS rats, and in BDNF-bin than SAL-bin rats (One-way ANOVA, Holm-Sidak method,  $p < 0.05$ ). \*, statistical significance. Error bars, s.e.m.

#### 3.2.4) *Visual function recovery in adult rats with unrestricted binocular sight*

With the aim to maximize the translational impact of our research, I assessed visual function recovery in a separate group of adult amblyopic rats subjected to eyelid reopening at the beginning of BDNF administration, with no RS (normal binocular sight rats: BDNF-bin, n = 10).

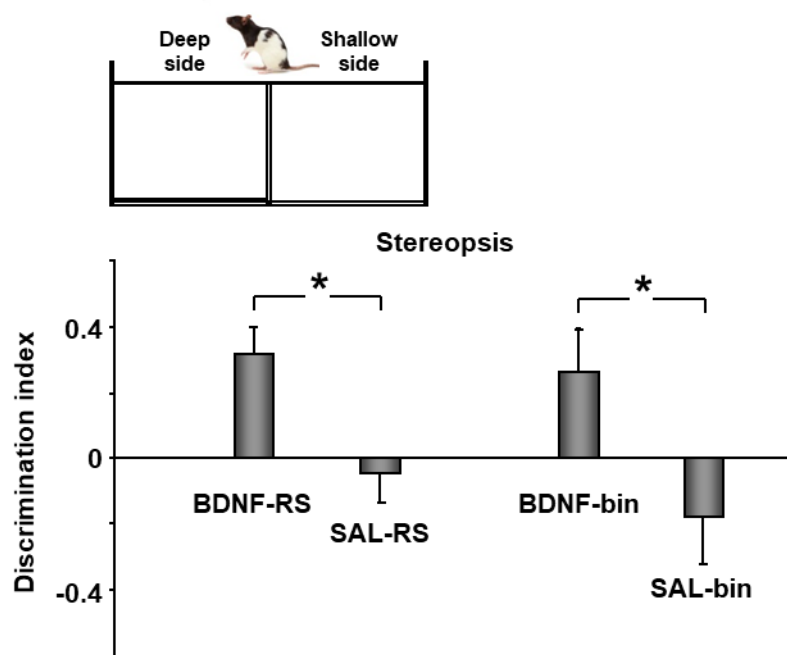
BDNF-bin rats displayed a marked rescue of both visual acuity ( $0.87 \pm 0.03$  c deg<sup>-1</sup>) and ocular dominance (C/I VEP ratio:  $1.41 \pm 0.09$ ), in comparison with a total lack of rescue in control rats treated with saline after eyelid reopening (SAL-bin rats, n = 7; visual acuity:  $0.60 \pm 0.02$  c deg<sup>-1</sup>; C/I VEP ratio:  $1.10 \pm 0.09$ ) (Fig. 10c, d). In the visual cliff task, stereopsis turned out to remain severely impaired in SAL-bin rats (n = 6; exploration index:  $-0.17 \pm 0.14$ ), while a marked recovery was displayed by BDNF-bin animals, which displayed a preference toward the shallow side of the arena (n = 7; exploration index:  $0.26 \pm 0.13$ ) (Fig. 11).

In these groups of animals with binocular vision, a behavioural measure of visual acuity through the visual water-box task was also performed[343],[138]. This task challenges the animal's ability to distinguish a grating from homogeneous grey. Rats were first conditioned to distinguish a low spatial frequency grating from the grey, with high reliability (training phase). Each animal's ability limit was then assessed at higher spatial frequencies (visual acuity measurement). I took advantage of the possibility to use the visual water box task to follow visual acuity recovery longitudinally in the same individuals. This allowed me to measure visual acuity through the amblyopic eye three times, i.e. immediately before treatment, immediately at the end of BDNF infusion, and after one month past the end of the treatment. The behavioural assessment fully confirmed the electrophysiological data: a full recovery of visual acuity was evident in BDNF-bin animals (n = 6) immediately after the

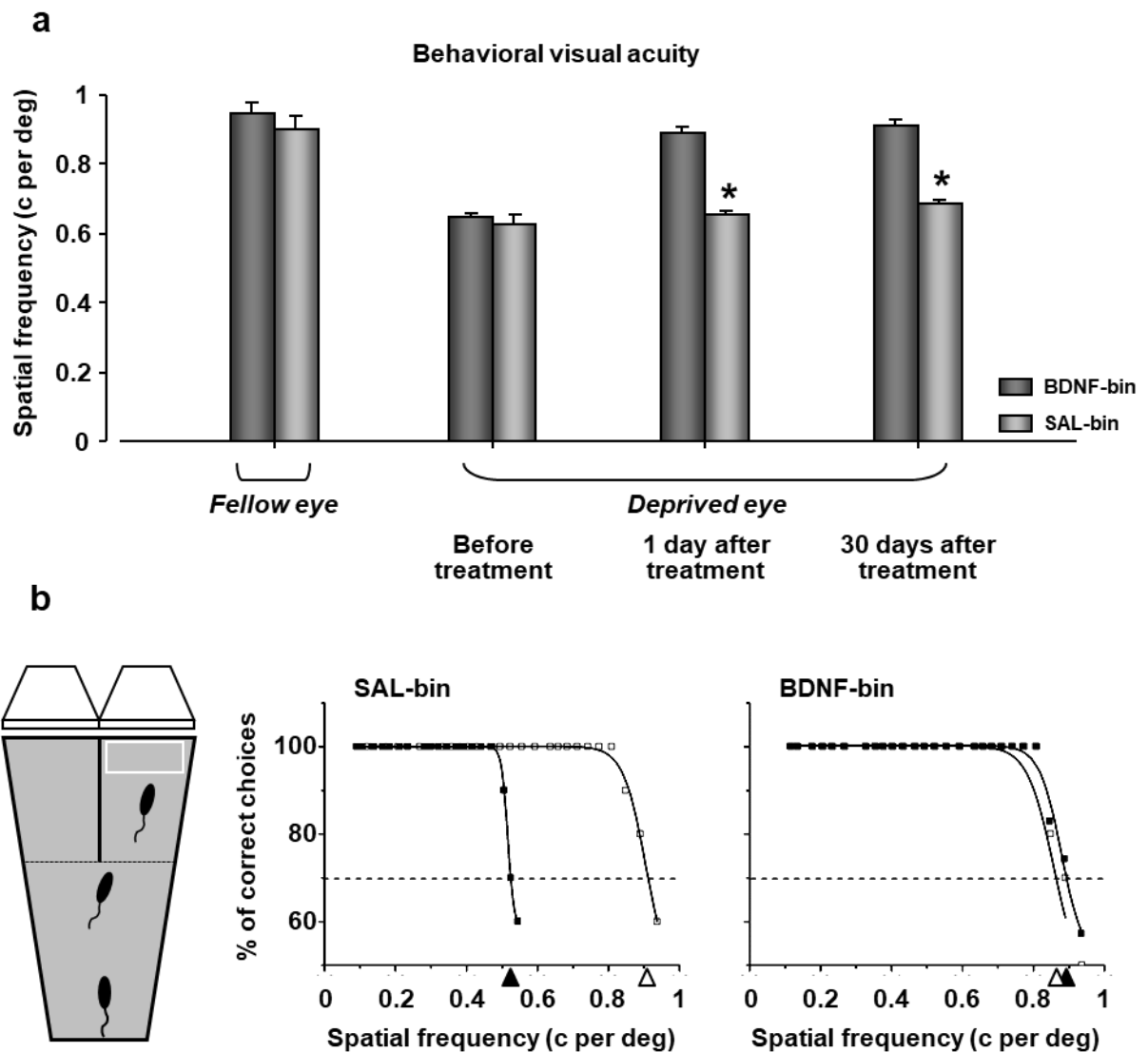
end of the treatment (amblyopic eye:  $0.89 \pm 0.02$  c deg<sup>-1</sup>; fellow eye:  $0.94 \pm 0.03$  c deg<sup>-1</sup>), while recovery was totally absent in SAL-bin rats (n = 6; amblyopic eye:  $0.65 \pm 0.01$  c deg<sup>-1</sup>; fellow eye:  $0.90 \pm 0.04$  c deg<sup>-1</sup>) (Fig. 12 a,b). Importantly, visual acuity recovery in the formerly amblyopic eye turned out to persist unaltered 4 weeks after the end of the BDNF treatment ( $0.91 \pm 0.02$  c deg<sup>-1</sup>) (Fig. 12a).

Moreover, I also exploited the visual water task to assess, in the two groups of animals, visual learning abilities through the amblyopic eye, before and after intranasal treatment. While, before treatment, no difference was present in visual learning abilities between BDNF-bin and SAL-bin rats to learn the task with the amblyopic eye (Fig. 13a), a significant improvement in learning performance was found in BDNF-bin rats compared to SAL-bin controls at the end of the treatment, when the animals were subjected to new training sessions in the visual water task to reassess visual functions through the amblyopic eye (Fig. 13b).

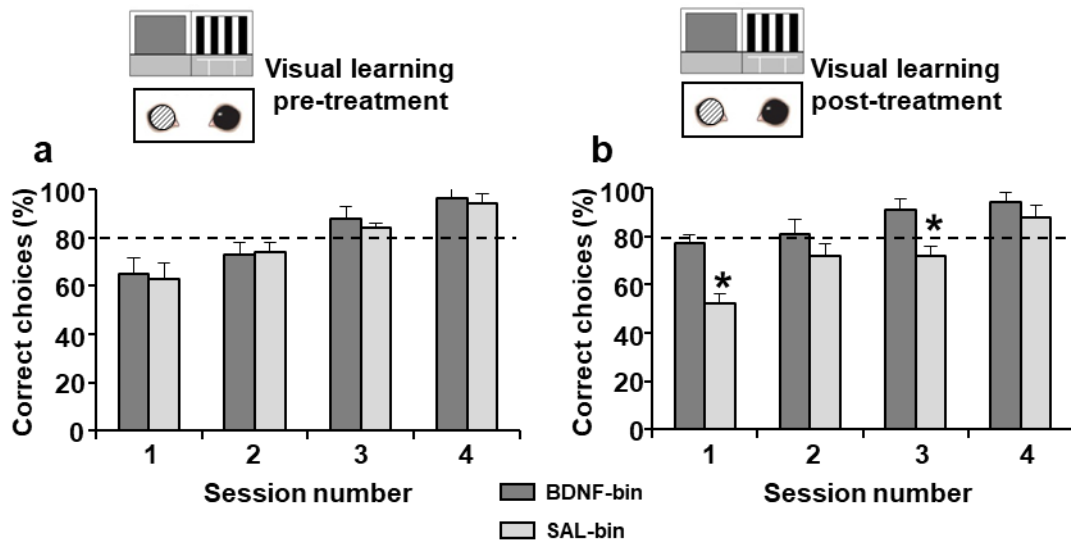




**Figure 11.** Exploration preference for the shallow and depth side of the arena in the visual cliff task (**inset**). One-way ANOVA showed a significant preference for the shallow side in BDNF-RS and in BDNFbin animals, which exhibited an exploration index statistically higher, respectively, than that of SAL-RS and SAL-bin rats (Holm-Sidak method,  $p < 0.05$ ). All animals were tested after restoration of binocular vision. \*, statistical significance. Error bars, s.e.m.



**Figure 12. a**, Visual acuity of both the long-term deprived and the fellow eye was measured using the visual water box task. At the end of the BDNF treatment, visual acuity of the previously deprived eye was different from that of the fellow eye in SAL-bin rats (paired t-test,  $p < 0.001$ ), but not in BDNF-bin animals (paired t-test,  $p = 0.219$ ). Two-Way RM ANOVA revealed that, in BDNF-bin rats, the visual acuity of the previously deprived eye measured immediately after BDNF treatment was significantly increased with respect to that measured before treatment (Holm-Sidak method,  $p < 0.001$ ) and remained unaltered 4 weeks after the end of BDNF intranasal infusion ( $p = 0.286$ ). In contrast, the visual acuity of the long-term deprived eye did not change throughout the study in SAL-bin rats (Two-Way RM ANOVA with Holm-Sidak method, pre-treatment vs. post-treatment,  $p = 0.731$ ; pre-treatment vs. 1 month after treatment,  $p = 0.350$ ). **b**, Visual acuity was obtained by extrapolation to 70% of correct choices on the sigmoidal function fitting the psychometric function in which the percentage of correct choices is plotted against spatial frequency (black triangles: deprived eye; white triangles: fellow eye). \*, statistical significance. Error bars, s.e.m.

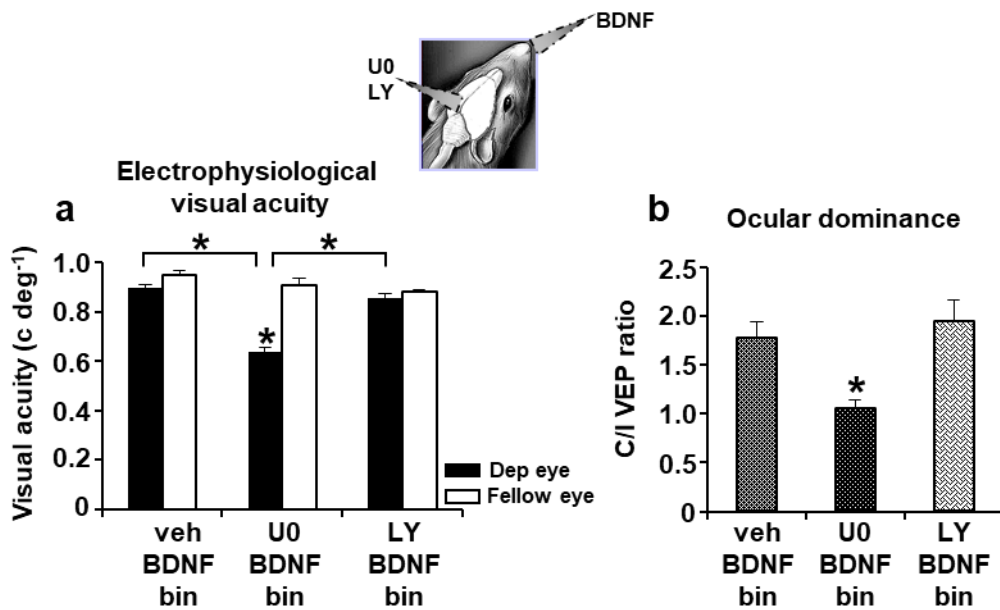


**Figure 13.** Percentage of correct responses during the training sessions of the visual water task in BDNF treated and saline-treated rats. The animals first learned the task with their amblyopic eye before treatment **a**, and then, after the end of either BDNF or saline intranasal infusion (14 days of treatment), they were required to re-learn the same task with the same eye. As expected, the behaviour of the two experimental groups was not different in the pre-treatment sessions (Two-Way RM ANOVA,  $p = 0.738$ ); post-treatment learning curves were instead different between the two groups of rats (Two-Way RM ANOVA,  $p < 0.01$ ), with BDNF-bin rats displaying a significantly improved performance compared to SAL-bin rats at both the first and third learning sessions (Holm-Sidak method,  $p < 0.05$ ). The bars indicate SEM. The asterisk indicates that the groups are statistically different. \*, statistical significance. Error bars, s.e.m.

### *3.2.5) Blockade of BDNF signalling in V1 prevents recovery from amblyopia in BDNF-treated rats*

Finally, I tested whether the enhanced activation of the BDNF pathway achieved by intranasal BDNF infusion was causally linked to the recovery of visual functions in BDNF-treated animals. I focused on two distinct major molecules in the signaling pathways downstream of BDNF binding to its primary TrkB receptor, i.e. the extracellular signal-regulated kinase 1,2 (ERK)[352], and the phosphoinositide 3-kinase (PI3K)[353]. For the entire length of intranasal BDNF administration I continuously infused (via osmotic minipumps) two different groups of BDNF-bin rats with either U0126 (UO, 1  $\mu$ M; n = 5), an inhibitor of ERK, or with LY294002 (LY, 5 $\mu$ g/ $\mu$ l; n = 5), an inhibitor of the Akt/PI3K pathway. Cortical UO administration totally prevented the BDNF-induced recovery effect, both at the level of visual acuity and of the C/I VEP ratio (amblyopic eye:  $0.64 \pm 0.02$  c deg<sup>-1</sup>; fellow eye:  $0.91 \pm 0.03$  c deg<sup>-1</sup>; C/I VEP ratio:  $1.06 \pm 0.09$ ) (Fig. 14 a, b).

In contrast, both BDNF-bin animals treated with LY and control BDNF-bin rats infused with vehicle solution (n = 5) displayed a rescue of visual acuity and OD (LY-BDNF-bin: amblyopic eye,  $0.87 \pm 0.01$  c deg<sup>-1</sup>; fellow eye,  $0.87 \pm 0.02$  c deg<sup>-1</sup>; C/I VEP ratio,  $2.06 \pm 0.12$ ; veh-BDNF-bin: amblyopic eye,  $0.90 \pm 0.01$  c deg<sup>-1</sup>; fellow eye,  $0.95 \pm 0.02$  c deg<sup>-1</sup>; C/I VEP ratio,  $1.78 \pm 0.16$ ) (Fig. 14 a, b). These results indicate that intranasal BDNF administration results in enhanced BDNF signalling activation in V1 and that the BDNF-induced rescue effect is specific to the ERK pathway activation.



**Figure 14.** Infusion of the ERK inhibitor U0126 into the visual cortex prevented recovery of vision in BDNF-treated rats. **a**, Electrophysiological visual acuity was statistically different between the formerly deprived eye and the fellow eye in BDNF-bin rats infused with U0 (U0-BDNF-bin; paired t-test,  $p < 0.05$ ), but not in either BDNF-bin rats infused with LY294002 (LY-BDNF-bin; paired t-test,  $p = 0.875$ ) or in control BDNF-bin rats infused with vehicle solution (veh-BDNF-bin; paired t-test,  $p = 0.208$ ). Accordingly, visual acuity in the formerly deprived eye was significantly higher in LY-BDNF-bin and in veh-BDNF-bin rats than in U0-BDNF-bin animals (One-Way ANOVA with Holm-Sidak method,  $p < 0.001$  in both cases) **b**, In agreement with the results for visual acuity, the C/I VEP ratio was statistically lower in U0-BDNF-bin than in either LY-BDNF-bin and veh-BDNF-bin rats (t-test,  $p < 0.05$ ) \*, statistical significance.

## Discussion

### **4.1) Part one: *Experience affects CP plasticity in the visual cortex through an epigenetic regulation of histone post-translational modifications***

It has been previously demonstrated that an epigenetic remodelling of chromatin controls developmental plasticity in the visual cortex [171]. To date, however, three main questions have remained open in this fundamental field. Which is the physiological time course of histone post-translational modifications during the CP? Is it possible, by manipulating the developmental epigenetic state of chromatin in the visual cortex, to modulate plasticity levels during the CP? How can we change histone acetylation levels in the adult brain in a non-invasive manner, reopening plasticity windows through procedures suitable for human application?

Here, I provided answers to these still open issues. First, I demonstrated that CP plasticity in the rat primary visual cortex is controlled at an epigenetic level through histone posttranslational modifications. Indeed, I showed that H3 histone acetylation is developmentally regulated in the visual cortex during the CP, with a peak at 1 week before that for OD plasticity. Second, a physiological paradigm used to cause an increase in H3 histone acetylation (i.e., exposure to early EE) turned out to accelerate the closure of the plastic window, suggesting a causal link between these two events. A direct demonstration of this hypothesis has been provided, showing that treatment with the histone deacetylase inhibitor SAHA increased H3 acetylation in rats raised under SCs and was sufficient to determine a faster closure of the CP in the same group. These results are in agreement with previous findings obtained in the mouse, which showed that exposure to early EE results in an earlier binocular matching of orientation preference, likely mediated by increased histone acetylation in the visual cortex [354]. As suggested by Wang et al. [354], a precocious plastic

window might negatively affect visual function development when the earlier time course for plasticity is not matched by an earlier maturation of visual functions. Here I showed that the accelerated time course for OD plasticity induced by EE did not result in any detrimental effect for visual function maturation, as indicated by similar visual depth perception abilities in animals raised with EE and under SCs at the CP end and by our previous data concerning visual acuity maturation [312].

I also showed that EE induced a pronounced increase in H3 acetylation in the developing hippocampus, demonstrating that EE effects were not confined to the visual cortex.

These results also suggest that histone acetylation changes might act as the link between sensory experience and the activity modulation of genes involved in brain plasticity, like BDNF. BDNF exerts a profound influence on visual system development. Mice overexpressing BDNF exhibit a pronounced acceleration in both the development of visual acuity and the time course of OD plasticity [49], and Sale et al. [355] has previously provided evidence that, regarding visual acuity maturation, changes very similar to these can also be induced by early exposure to EE, which is associated with early increases in V1 BDNF protein levels. Now, I further explored the capability of EE to impact on developmental plasticity in the primary visual cortex, showing that EE also leads to an earlier closure of the CP for the effects of MD and an up-regulation of histone H3 acetylation at the level of the P3 BDNF gene promoter. The increased acetylation at P3 was paralleled by an increased relative amount of P3 BDNF mRNA relative to total BDNF mRNA, suggesting a positive effect of histone acetylation on activity-dependent BDNF gene expression. While the regulation of BDNF promoter acetylation in the cerebral cortex has been investigated in response to various pharmacological treatments or, in exercised animals, in the hippocampus [356],

[357], to my knowledge, this is the first time that a specific epigenetic control at the BDNF gene promoter is reported as a likely underlying mechanism for visual cortical plasticity during the CP.

One of the possible contributing factors for the early effects induced by EE might be maternal care, one major regulator for the programming of individual differences. Interestingly, it has been demonstrated that maternal care levels are implicated in the early EE effects, resulting in an accelerated maturation of visual system functions and visual cortical plasticity decline, due to a faster maturation of the GABAergic transmission[313]. Weaver et al. [358] have shown that offspring differences in stress response depend on epigenetic changes in the promoter of the glucocorticoid receptor gene, under the direct control of maternal care levels received by pups during the first postnatal week. Moreover, it was previously reported [313] that enriched pups receive higher levels of maternal and non-maternal licking and grooming. The EE-dependent acceleration of visual acuity maturation is mimicked in rat pups raised under SCs by a cycle of artificial massages reproducing sustained maternal behaviour [319]. Thus, higher maternal care levels received by enriched pups might also have an effect on H3 acetylation levels, even if a direct influence of enriched stimuli on the developing subject is not negligible, once pups start exploring the surrounding area after eye opening.

Finally, I focused on adult rats, showing that 2 weeks of EE is a sufficient length of time to induce an increase of histone acetylation in the visual cortex. This finding suggests that EE drives a transitory epigenetic remodelling of chromatin structure that underlies the reinstatement of juvenile-like plasticity in the visual system, with possible implications for the treatment of visual deficits, like amblyopia. It has been shown that an increase in histone acetylation might be used to promote functional recovery in adult amblyopic rats [322], but



how to translate these invasive treatments in manipulations useful for amblyopic humans remained unclear. The results here reported, suggest that an increment in sensory-motor stimulation might be used to impact on the chromatin functional state, promoting plasticity in the adult visual cortex [138].

These results, together with those previously reported concerning GABAergic inhibition levels in enriched animals, show that the same perturbation (EE) is associated with an increase of histone acetylation and BDNF in the visual cortex, with opposite effects occurring in the juvenile or adult brain (i.e., respectively increased inhibition paralleled by earlier closure of the plasticity window [317], [318], [345] or reduced inhibition accompanied by OD plasticity reinstatement [136], [325], [326], [359]–[361]). In the developing visual system, it has been well documented that BDNF directly promotes the maturation of GABAergic neurotransmission both at the presynaptic and postsynaptic level, leading to the stabilization of inhibitory synapses [32], [133], [362]. Thus, the early increase in BDNF detected in developing pups reared from birth in an enriched environment [313], [345] might be the prime trigger factor guiding the development of inhibitory circuitry in the immature brain, when GABAergic interneurons are particularly sensitive to the trophic action of this neurotrophin. In the adult visual system, once the mature level of GABAergic inhibition has been reached and the critical period is closed in the primary visual cortex, the increase in BDNF prompted by a histone acetylation enhancement might lead to a suppression of GABAergic inhibition, as reported for other brain regions [363]–[365], with BDNF inducing a reduction in postsynaptic GABA<sub>A</sub> receptor numbers that is responsible for the decline in GABAergic mIPSC amplitudes [366]. The direction of BDNF effects on inhibitory synapses could depend both on the preferred postsynaptic signalling cascade activated by the TrkB receptor [367] and the phosphorylation state of the GABA receptors [368], and has

been previously reported to be dependent of age, which is in agreement with our model [367].

It is also worth noting that visual cortex infusion of BDNF antisense oligonucleotides only partially blocks the OD shift in response to MD in adult animals in which the CP has been reopened by exposure to EE [136]. This suggests that BDNF is one player, but not the only one, in the restoration of adult plasticity, likely acting in parallel with other molecular factors. The reduction of intracortical inhibition in adult enriched animals, indeed, might be prompted also by increased serotonin levels [136], [369] or enhanced IGF-1 signalling [370].

Outside the visual system, it has been shown that EE is able to rescue memory abilities in a mouse model of brain degeneration, acting through the enhancement of histone acetylation [285]. Results here reported extend this view to include the visual cortex, suggesting that optimization of external inputs elicits neural plasticity in the whole brain, leading to functional recovery in a number of different neural dysfunctions. Alone or in combination with suitable pharmacological treatments, exposure to procedures akin to EE might thus emerge as a fundamental route toward endogenous pharmacotherapy [371], a process by which brain circuits, appropriately fed with enhanced sensory inputs, are led to repair by unfolding their residual potential for plasticity.

#### **4.2) Part two: *Intranasal BDNF administration promotes visual function recovery in adult amblyopic subjects***

The findings here reported show that intranasal BDNF treatment promotes a marked recovery of visual functions in adult amblyopic animals. To my knowledge, this is the first time that intranasal infusion of BDNF is used in the visual system and that this treatment is shown to promote recovery from amblyopia in adult subjects. Importantly, the recovery

effect induced by BDNF was clearly evident not only in reverse-sutured animals, as reported with other kind of treatments in most papers published so far in adult amblyopic rats [138], [352], [372], [373], but also in animals with a normal binocular sight. This significantly strengthens the chance for clinical application to human patients, where a careful sparing of proper binocular interactions is increasingly considered instrumental for maximizing the amount of visual function recovery in adult subjects [186], [374].

The results in animals with binocular sight without reverse occlusion are particular relevant for stereopsis. Impaired stereoscopic depth perception is the most common deficit associated with amblyopia [375], appearing as a major impediment in the normal everyday life [186]. A recent work by Mitchell's lab found that using exposure to total darkness as a boost for adult visual cortical plasticity, visual acuity for the amblyopic eye was increased but measurements of depth perception indicated that despite possessing normal visual acuity in both eyes, only about a quarter of animals showed evidence of having attained normal stereoscopic vision [376]. Thus, besides visual acuity, measuring stereopsis is a necessary step in clinical and basic research studies with amblyopic subjects. I demonstrated that intranasal BDNF treatment results in a marked recovery of stereopsis abilities in adult amblyopic rats, which Baroncelli et al. [324] previously showed to be severely impaired in the visual cliff task after a period of long-lasting eye occlusion started during the CP.

In recent years, the rodent adult visual cortex has been shown to exhibit several forms of response modification sub-serving higher brain functions such as stimulus familiarity, reward-timing prediction, and spatiotemporal sequence learning [377]. Moreover, the visual cortex is currently considered the prime site for visual perceptual learning, one of the most promising active strategies for treating amblyopia in adulthood [184], [185], [334]. I report that, after BDNF intranasal treatment, there was an

improvement in the learning abilities displayed by adult amblyopic rats in the visual water box task when using their previously amblyopic eye. In this task, the event represented by the finding of the submerged platform is associated with a low spatial frequency value of the stimulus, an association that forms the basis for further comparisons performed by the animal during subsequent expositions to higher spatial frequencies during visual acuity assessment. It is likely that this learning variable might contribute, in parallel to a pure sensory deficit, to visual perceptual dysfunctions displayed by amblyopic subjects and, accordingly, improving visual learning abilities may eventually benefit visual discrimination performance in amblyopic subjects exposed to intranasal BDNF treatment.

Importantly, visual function recovery in BDNF-treated animals appeared long-lasting, persisting at least 4 weeks after the end of the pharmacological treatment. It has been estimated that, in adulthood, every day of a rat's life is approximately equivalent to 34.8 human days [378]. Thus, in the timescale of human life, the result here reported results appear quite strong, suggesting the possibility for visual acuity to remain unaltered for at least three human years.

BDNF administered into the nasal cavity might reach the central nervous system through four independent, parallel routes: olfactory nerve pathway, trigeminal nerve pathway, perivascular pathway and cerebrospinal fluid pathway. The most accredited route to reach the posterior areas of the brain is along the peripheral ophthalmic branch of trigeminal nerves, emerging from the semilunar Gasser ganglion, which innervates the respiratory and olfactory epithelium, and whose central processes enter the CNS, from the Gasser ganglion at the level of the pons. Collaterals from the trigeminal nerve also reach the olfactory bulb. Molecules could travel along trigeminal axons through paracellular channel mechanisms.[379]. Here, I demonstrated for the first time that intranasal BDNF results in

increased BDNF content in the visual cortex of BDNF-treated rats compared to controls. Moreover, I also provided evidence for an involvement of the intracellular BDNF signalling pathway in the recovery effect induced by the treatment. Indeed, I focused on ERK and PI3K, two independent key protein kinases downstream of BDNF binding to its primary TrkB receptor [353], [380]–[383]. Akt/PI3 was an interesting candidate to mediate amblyopia recovery, since it has been demonstrated to be regulated by monocular deprivation [384] and recognised as a candidate gene in the regulation of OD plasticity [385]. Di Cristo and colleagues [386] showed that OD plasticity can be prevented by inhibiting ERK. While blockade of PI3K did not display any effect in BDNF-treated animals, blockade of ERK by intracortical infusion of the inhibitor U0 was sufficient to prevent recovery of visual functions in BDNF-treated rats. The lack of any effect observed when blocking PI3K could be explained by the fact that, in the visual cortex, its activation has been mainly related to the action of IGF-1, rather than BDNF [384]. Thus, the data here reported results underscore a direct involvement of the BDNF-ERK in the recovery of visual functions in amblyopia. This in agreement with previous findings reporting a pivotal role for ERK in another form of visual cortex plasticity, i.e. the ocular dominance shift in binocular cell activation in response to monocular deprivation during the CP [381], [386].

Outside the visual system, BDNF attracts huge interest for its remarkable ability to promote neural plasticity and functional recovery, suggesting a potential role for this molecule in the pathogenesis and treatment of both neurological and psychiatric disorders [342]. The results here reported highlight the possibility to replace invasive BDNF central administration with a safe procedure of potential great interest in a number of currently still cureless central nervous system pathologies.

## **Appendix: Scientific Production during the PhD**

### **Articles**

"Pterostilbene improves cognitive performance in aged rats: an in vivo study"

Martina La Spina\*, **Gabriele Sansevero**\*, Lucia Biasutto, Mario Zoratti, Nicoletta Berardi, Alessandro Sale, Michele Azzolini.

Oxidative Medicine and Cellular Longevity, under review. \*Equal contribution authorship

"Intranasal BDNF administration promotes visual function recovery in adult amblyopic subjects"

**Gabriele Sansevero**\*, Laura Baroncelli\*, Alessandro Sale.

Neuropharmacology (I.F. 5,012), 2018 Feb. \*Equal contribution authorship

"Early impoverished environment delays the maturation of cerebral cortex"

Roberta Narducci, Laura Baroncelli, **Gabriele Sansevero**, Tatjana Begenisic, Concetta Prontera, Alessandro Sale, Maria Cristina Cenni, Nicoletta Berardi, and Lamberto Maffei  
Scientific Reports (I.F. 4,259), 2018 Jan.

"Environment as therapy: neuroscience for intellectual disability and dementia"

**Gabriele Sansevero** and Alessandro Sale

Oncotarget (I.F. 5,168), 2017 Jan.

"Experience-dependent reduction of soluble  $\beta$ -amyloid oligomers and rescue of cognitive abilities in middle-age Ts65Dn mice, a model of Down Syndrome."

**Gabriele Sansevero**\*, Tatjana Begenisic\*, Marco Mainardi, and Alessandro Sale.

Experimental Neurology (I.F. 4,706), 2016 Sep. \*Equal contribution authorship

"Experience affects critical period plasticity in the visual cortex through an epigenetic regulation of histone post-translational modifications"

Laura Baroncelli\*, Manuela Scali\*, **Gabriele Sansevero**\*, Francesco Olimpico, Ilaria Manno, Mario Costa, and Alessandro Sale.

J Neurosci (I.F. 5,988), 2016 Mar. \*Equal contribution authorship

"Early environmental therapy rescues brain development in a mouse model of Down syndrome."

Tatjana Begenisic\*, **Gabriele Sansevero**\*, Laura Baroncelli, Giovanni Cioni, and Alessandro Sale.

Neurobiol. Disease (I.F. 5,020), 2015 Jul. \*Equal contribution authorship

### **Meetings and posters**

"Intranasal BDNF administration promotes visual function recovery in adult amblyopic subjects"

**Gabriele Sansevero**.

(24/5/2017, Florence, PhD Day8, University of Florence). Oral Presentation.

“Intranasal BDNF administration promotes visual function recovery in adult amblyopic subjects”

**Gabriele Sansevero**, Laura Baroncelli, Alessandro Sale.  
(13-16/9/2016, Padua, CNR IN Retreat). Poster.

“Pterostilbene and cognitive performance in the aged rat model: molecular and behavioral effects”

Michele Azzolini, Martina La Spina, **Gabriele Sansevero**, Giulietta Di Benedetto, Silvia Morea, Laura Baroncelli, Alessandro Sale, Nicoletta Berardi, Mario Zoratti, Lucia Biasutto.  
(13-16/9/2016, Padua, CNR IN Retreat). Poster.

“Early environmental therapy rescues brain development in a mouse model of Down syndrome.”

**Gabriele Sansevero**, Tatjana Begenisic, Laura Baroncelli, Giovanni Cioni, and Alessandro Sale.  
(27/5/2015, Florence, PhD Day6, University of Florence). Poster.

“Early environmental therapy rescues brain development in a mouse model of Down syndrome.”

**Gabriele Sansevero**, Tatjana Begenisic, Laura Baroncelli, Giovanni Cioni, and Alessandro Sale.  
(13-15/05/2015, Pisa, CNR IN Retreat). Poster.

## References

- [1] C. Darwin, "On the origin of the species," Nov. 1859.
- [2] N. Berardi, T. Pizzorusso, and L. Maffei, "Critical periods during sensory development," *Curr. Opin. Neurobiol.*, vol. 10, no. 1, pp. 138–145, 2000.
- [3] M. Fagiolini, C. L. Jensen, and F. A. Champagne, "Epigenetic influences on brain development and plasticity," *Curr. Opin. Neurobiol.*, vol. 19, no. 2, pp. 207–212, Apr. 2009.
- [4] T. K. Hensch, "Critical period plasticity in local cortical circuits," *Nat. Rev. Neurosci.*, vol. 6, no. 11, pp. 877–888, Nov. 2005.
- [5] E. I. Knudsen, "Sensitive periods in the development of the brain and behavior," *J Cogn Neurosci*, vol. 16, no. 8, pp. 1412–1425, 2004.
- [6] C. N. Levelt and M. Hübener, "Critical-period plasticity in the visual cortex," *Annu Rev Neurosci*, vol. 35, pp. 309–330, 2012.
- [7] T. K. Hensch, "CRITICAL PERIOD REGULATION," *Annu. Rev. Neurosci.*, vol. 27, no. 1, pp. 549–579, Jul. 2004.
- [8] T. N. Wiesel and D. H. Hubel, "Responses in Striate Deprived of Vision Cortex of One Eye1," *J. Neurophysiol.*, vol. 26, pp. 1003–1017, 1963.
- [9] D. H. Hubel and T. N. Wiesel, "The period of susceptibility to the physiological effects of unilateral eye closure in kittens.," *J. Physiol.*, vol. 206, no. 2, pp. 419–436, 1970.
- [10] C. R. Olson and R. D. Freeman, "Progressive changes in kitten striate cortex during monocular vision.," *J. Neurophysiol.*, vol. 38, no. 1, pp. 26–32, Jan. 1975.
- [11] J. A. Movshon and M. R. Dürsteler, "Effects of brief periods of unilateral eye closure on the kitten's visual system.," *J. Neurophysiol.*, vol. 40, no. 6, pp. 1255–1265, Nov.



1977.

- [12] C. R. Olson and R. D. Freeman, "Profile of the sensitive period for monocular deprivation in kittens.," *Exp. brain Res.*, vol. 39, no. 1, pp. 17–21, 1980.
- [13] T. N. WIESEL and D. H. HUBEL, "EFFECTS OF VISUAL DEPRIVATION ON MORPHOLOGY AND PHYSIOLOGY OF CELLS IN THE CATS LATERAL GENICULATE BODY.," *J. Neurophysiol.*, vol. 26, pp. 978–93, Nov. 1963.
- [14] S. M. Sherman and J. Stone, "Physiological normality of the retinal in visually deprived cats.," *Brain Res.*, vol. 60, no. 1, pp. 224–30, Sep. 1973.
- [15] K. E. Kratz, S. C. Mangel, S. Lehmkuhle, and M. Sherman, "Retinal X- and Y-cells in monocularly lid-sutured cats: normality of spatial and temporal properties.," *Brain Res.*, vol. 172, no. 3, pp. 545–51, Aug. 1979.
- [16] J. A. Baro, S. Lehmkuhle, and K. E. Kratz, "Electroretinograms and visual evoked potentials in long-term monocularly deprived cats," *Investig. Ophthalmol. Vis. Sci.*, vol. 31, no. 7, pp. 1405–1409, Jul. 1990.
- [17] T. N. Wiesel and D. H. Hubel, "Extent of recovery from the effects of visual deprivation in kittens.," *J. Neurophysiol.*, vol. 28, no. 6, pp. 1060–72, Nov. 1965.
- [18] J. A. Movshon, "Reversal of the physiological effects of monocular deprivation in the kitten's visual cortex.," *J. Physiol.*, vol. 261, no. 1, pp. 125–74, Sep. 1976.
- [19] R. C. van Sluyters, "Reversal of the physiological effects of brief periods of monocular deprivation in the kitten.," *J. Physiol.*, vol. 284, pp. 1–17, Nov. 1978.
- [20] C. Blakemore, F. Vital-Durand, and L. J. Garey, "Recovery from monocular deprivation in the monkey. I. Reversal of physiological effects in the visual cortex.," *Proc. R. Soc. London. Ser. B, Biol. Sci.*, vol. 213, no. 1193, pp. 399–423, Nov. 1981.
- [21] A. Antonini, D. C. Gillespie, M. C. Crair, and M. P. Stryker, "Morphology of single

- geniculocortical afferents and functional recovery of the visual cortex after reverse monocular deprivation in the kitten.," *J. Neurosci.*, vol. 18, no. 23, pp. 9896–909, Dec. 1998.
- [22] L. C. Katz and C. J. Shatz, "Synaptic activity and the construction of cortical circuits.," *Science*, vol. 274, no. 5290, pp. 1133–8, Nov. 1996.
- [23] J. T. Trachtenberg and M. P. Stryker, "Rapid anatomical plasticity of horizontal connections in the developing visual cortex.," *J. Neurosci.*, vol. 21, no. 10, pp. 3476–82, May 2001.
- [24] S. Oray, A. Majewska, and M. Sur, "Dendritic spine dynamics are regulated by monocular deprivation and extracellular matrix degradation.," *Neuron*, vol. 44, no. 6, pp. 1021–30, Dec. 2004.
- [25] U. C. Dräger, "Observations on monocular deprivation in mice.," *J. Neurophysiol.*, vol. 41, no. 1, pp. 28–42, Jan. 1978.
- [26] M. Fagiolini, T. Pizzorusso, N. Berardi, L. Domenici, and L. Maffei, "Functional postnatal development of the rat primary visual cortex and the role of visual experience: Dark rearing and monocular deprivation," *Vision Res.*, vol. 34, no. 6, pp. 709–720, Mar. 1994.
- [27] J. A. Gordon and M. P. Stryker, "Experience-dependent plasticity of binocular responses in the primary visual cortex of the mouse.," *J. Neurosci.*, vol. 16, no. 10, pp. 3274–86, May 1996.
- [28] A. Antonini, M. Fagiolini, and M. P. Stryker, "Anatomical correlates of functional plasticity in mouse visual cortex.," *J. Neurosci.*, vol. 19, no. 11, pp. 4388–406, Jun. 1999.
- [29] N. Mataga, Y. Mizuguchi, and T. K. Hensch, "Experience-dependent pruning of

- dendritic spines in visual cortex by tissue plasminogen activator.," *Neuron*, vol. 44, no. 6, pp. 1031–41, Dec. 2004.
- [30] S. M. Sherman and P. D. Spear, "Organization of visual pathways in normal and visually deprived cats.," *Physiol. Rev.*, vol. 62, no. 2, pp. 738–855, Apr. 1982.
- [31] M. C. Crair, D. C. Gillespie, and M. P. Stryker, "The role of visual experience in the development of columns in cat visual cortex.," *Science*, vol. 279, no. 5350, pp. 566–70, Jan. 1998.
- [32] L. Gianfranceschi *et al.*, "Visual cortex is rescued from the effects of dark rearing by overexpression of BDNF.," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 100, no. 21, pp. 12486–91, Oct. 2003.
- [33] T. Pizzorusso, M. Fagiolini, V. Porciatti, and L. Maffei, "Temporal aspects of contrast visual evoked potentials in the pigmented rat: effect of dark rearing.," *Vision Res.*, vol. 37, no. 4, pp. 389–95, Feb. 1997.
- [34] F. Valverde, "Rate and extent of recovery from dark rearing in the visual cortex of the mouse.," *Brain Res.*, vol. 33, no. 1, pp. 1–11, Oct. 1971.
- [35] W. Wallace and M. F. Bear, "A morphological correlate of synaptic scaling in visual cortex.," *J. Neurosci.*, vol. 24, no. 31, pp. 6928–38, Aug. 2004.
- [36] P. Buisseret, E. Gary-Bobo, and M. Imbert, "Plasticity in the kitten's visual cortex: effects of the suppression of visual experience upon the orientational properties of visual cortical cells," *Dev. Brain Res.*, vol. 4, no. 4, pp. 417–426, Aug. 1982.
- [37] P. Buisseret, E. Gary-Bobo, and M. Imbert, "Ocular motility and recovery of orientational properties of visual cortical neurones in dark-reared kittens.," *Nature*, vol. 272, no. 5656, pp. 816–7, Apr. 1978.
- [38] B. M. Hooks and C. Chen, "Critical Periods in the Visual System: Changing Views for a

- Model of Experience-Dependent Plasticity," *Neuron*, vol. 56, no. 2, pp. 312–326, Oct. 2007.
- [39] A. Antonini and M. P. Stryker, "Effect of sensory disuse on geniculate afferents to cat visual cortex.," *Vis. Neurosci.*, vol. 15, no. 3, pp. 401–9, 1998.
- [40] B. Chapman, M. D. Jacobson, H. O. Reiter, and M. P. Stryker, "Ocular dominance shift in kitten visual cortex caused by imbalance in retinal electrical activity.," *Nature*, vol. 324, no. 6093, pp. 154–6, Nov. 1986.
- [41] H. O. Reiter, D. M. Waitzman, and M. P. Stryker, "Cortical activity blockade prevents ocular dominance plasticity in the kitten visual cortex.," *Exp. brain Res.*, vol. 65, no. 1, pp. 182–8, 1986.
- [42] H. O. Reiter and M. P. Stryker, "Neural plasticity without postsynaptic action potentials: less-active inputs become dominant when kitten visual cortical cells are pharmacologically inhibited.," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 85, no. 10, pp. 3623–7, May 1988.
- [43] Y. Hata and M. P. Stryker, "Control of thalamocortical afferent rearrangement by postsynaptic activity in developing visual cortex.," *Science*, vol. 265, no. 5179, pp. 1732–5, Sep. 1994.
- [44] Y. Hata, T. Tsumoto, and M. P. Stryker, "Selective pruning of more active afferents when cat visual cortex is pharmacologically inhibited.," *Neuron*, vol. 22, no. 2, pp. 375–81, Feb. 1999.
- [45] K. D. Miller, J. B. Keller, and M. P. Stryker, "Ocular dominance column development: analysis and simulation.," *Science*, vol. 245, no. 4918, pp. 605–15, Aug. 1989.
- [46] A. E. Harris, G. B. Ermentrout, and S. L. Small, "A model of ocular dominance column development by competition for trophic factor.," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 94,

no. 18, pp. 9944–9, Sep. 1997.

- [47] L. Maffei, N. Berardi, L. Domenici, V. Parisi, and T. Pizzorusso, “Nerve growth factor (NGF) prevents the shift in ocular dominance distribution of visual cortical neurons in monocularly deprived rats.,” *J. Neurosci.*, vol. 12, no. 12, pp. 4651–62, Dec. 1992.
- [48] N. Berardi and L. Maffei, “From visual experience to visual function: roles of neurotrophins.,” *J. Neurobiol.*, vol. 41, no. 1, pp. 119–26, Oct. 1999.
- [49] Z. J. Huang *et al.*, “BDNF regulates the maturation of inhibition and the critical period of plasticity in mouse visual cortex.,” *Cell*, vol. 98, no. 6, pp. 739–55, Sep. 1999.
- [50] M. Y. Frenkel and M. F. Bear, “How Monocular Deprivation Shifts Ocular Dominance in Visual Cortex of Young Mice,” *Neuron*, vol. 44, no. 6, pp. 917–923, Dec. 2004.
- [51] T. D. Mrsic-Flogel, S. B. Hofer, K. Ohki, R. C. Reid, T. Bonhoeffer, and M. Hübener, “Homeostatic regulation of eye-specific responses in visual cortex during ocular dominance plasticity.,” *Neuron*, vol. 54, no. 6, pp. 961–72, Jun. 2007.
- [52] M. Kaneko, D. Stellwagen, R. C. Malenka, and M. P. Stryker, “Tumor Necrosis Factor- $\alpha$  Mediates One Component of Competitive, Experience-Dependent Plasticity in Developing Visual Cortex,” *Neuron*, vol. 58, no. 5, pp. 673–680, Jun. 2008.
- [53] M. Kaneko, J. L. Hanover, P. M. England, and M. P. Stryker, “TrkB kinase is required for recovery, but not loss, of cortical responses following monocular deprivation,” *Nat. Neurosci.*, vol. 11, no. 4, pp. 497–504, Apr. 2008.
- [54] G. B. Smith, A. J. Heynen, and M. F. Bear, “Bidirectional synaptic mechanisms of ocular dominance plasticity in visual cortex.,” *Philos. Trans. R. Soc. Lond. B. Biol. Sci.*, vol. 364, no. 1515, pp. 357–67, Feb. 2009.
- [55] X. F. Wang and N. W. Daw, “Long term potentiation varies with layer in rat visual cortex.,” *Brain Res.*, vol. 989, no. 1, pp. 26–34, Oct. 2003.

- [56] A. J. Heynen and M. F. Bear, "Long-term potentiation of thalamocortical transmission in the adult visual cortex in vivo.," *J. Neurosci.*, vol. 21, no. 24, pp. 9801–13, Dec. 2001.
- [57] N. B. Sawtell, M. Y. Frenkel, B. D. Philpot, K. Nakazawa, S. Tonegawa, and M. F. Bear, "NMDA receptor-dependent ocular dominance plasticity in adult visual cortex.," *Neuron*, vol. 38, no. 6, pp. 977–85, Jun. 2003.
- [58] M. Y. Frenkel, N. B. Sawtell, A. C. M. Diogo, B. Yoon, R. L. Neve, and M. F. Bear, "Instructive Effect of Visual Experience in Mouse Visual Cortex," *Neuron*, vol. 51, no. 3, pp. 339–349, Aug. 2006.
- [59] J. H. Yoon *et al.*, "Diminished Orientation-Specific Surround Suppression of Visual Processing in Schizophrenia," *Schizophr. Bull.*, vol. 35, no. 6, pp. 1078–1084, Nov. 2009.
- [60] J. H. Yoon *et al.*, "GABA Concentration Is Reduced in Visual Cortex in Schizophrenia and Correlates with Orientation-Specific Surround Suppression," *J. Neurosci.*, vol. 30, no. 10, pp. 3777–3781, Mar. 2010.
- [61] R. A. Crozier, Y. Wang, C.-H. Liu, and M. F. Bear, "Deprivation-induced synaptic depression by distinct mechanisms in different layers of mouse visual cortex.," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 104, no. 4, pp. 1383–8, Jan. 2007.
- [62] T. K. Hensch, M. Fagiolini, N. Mataga, M. P. Stryker, S. Baekkeskov, and S. F. Kash, "Local GABA circuit control of experience-dependent plasticity in developing visual cortex.," *Science*, vol. 282, no. 5393, pp. 1504–8, Nov. 1998.
- [63] S.-Y. Choi, B. Morales, H.-K. Lee, and A. Kirkwood, "Absence of long-term depression in the visual cortex of glutamic Acid decarboxylase-65 knock-out mice.," *J. Neurosci.*, vol. 22, no. 13, pp. 5271–6, Jul. 2002.
- [64] T. K. Hensch, J. A. Gordon, E. P. Brandon, G. S. McKnight, R. L. Idzerda, and M. P.

- Stryker, "Comparison of plasticity in vivo and in vitro in the developing visual cortex of normal and protein kinase A R1beta-deficient mice.," *J. Neurosci.*, vol. 18, no. 6, pp. 2108–17, Mar. 1998.
- [65] Y. Rao, Q. S. Fischer, Y. Yang, G. S. McKnight, A. LaRue, and N. W. Daw, "Reduced ocular dominance plasticity and long-term potentiation in the developing visual cortex of protein kinase A R11alpha mutant mice," *Eur. J. Neurosci.*, vol. 20, no. 3, pp. 837–842, Aug. 2004.
- [66] Q. S. Fischer *et al.*, "Requirement for the R11 Isoform of PKA, But Not Calcium-Stimulated Adenylyl Cyclase, in Visual Cortical Plasticity," *J. Neurosci.*, vol. 24, no. 41, pp. 9049–9058, Oct. 2004.
- [67] T. K. Hensch and M. Fagiolini, "Excitatory–inhibitory balance and critical period plasticity in developing visual cortex," in *Progress in brain research*, vol. 147, 2005, pp. 115–124.
- [68] B. R. Beston, D. G. Jones, and K. M. Murphy, "Experience-dependent changes in excitatory and inhibitory receptor subunit expression in visual cortex.," *Front. Synaptic Neurosci.*, vol. 2, p. 138, 2010.
- [69] M. E. Blue and J. G. Parnavelas, "The formation and maturation of synapses in the visual cortex of the rat. I. Qualitative analysis," *J. Neurocytol.*, vol. 12, no. 4, pp. 599–616, Aug. 1983.
- [70] K. D. Micheva and C. Beaulieu, "Development and plasticity of the inhibitory neocortical circuitry with an emphasis on the rodent barrel field cortex: a review.," *Can. J. Physiol. Pharmacol.*, vol. 75, no. 5, pp. 470–8, May 1997.
- [71] K. M. Murphy, B. R. Beston, P. M. Boley, and D. G. Jones, "Development of human visual cortex: A balance between excitatory and inhibitory plasticity mechanisms,"

- Dev. Psychobiol.*, vol. 46, no. 3, pp. 209–221, Apr. 2005.
- [72] M. Fagiolini *et al.*, “Separable features of visual cortical plasticity revealed by N-methyl-D-aspartate receptor 2A signaling,” *Proc. Natl. Acad. Sci.*, vol. 100, no. 5, pp. 2854–2859, Mar. 2003.
- [73] M. Fagiolini and T. K. Hensch, “Inhibitory threshold for critical-period activation in primary visual cortex,” *Nature*, vol. 404, no. 6774, pp. 183–186, Mar. 2000.
- [74] F. Pouille and M. Scanziani, “Enforcement of Temporal Fidelity in Pyramidal Cells by Somatic Feed-Forward Inhibition,” *Science (80-. )*, vol. 293, no. 5532, pp. 1159–1163, Aug. 2001.
- [75] G. Bi and M. Poo, “Synaptic Modification by Correlated Activity: Hebb’s Postulate Revisited,” *Annu. Rev. Neurosci.*, vol. 24, no. 1, pp. 139–166, Mar. 2001.
- [76] A. Harauzov *et al.*, “Reducing Intracortical Inhibition in the Adult Visual Cortex Promotes Ocular Dominance Plasticity,” *J. Neurosci.*, vol. 30, no. 1, pp. 361–371, Jan. 2010.
- [77] J. F. M. Vetencourt *et al.*, “The Antidepressant Fluoxetine Restores Plasticity in the Adult Visual Cortex,” *Science (80-. )*, vol. 320, no. 5874, pp. 385–388, Apr. 2008.
- [78] J. DeFelipe, “Types of neurons, synaptic connections and chemical characteristics of cells immunoreactive for calbindin-D28K, parvalbumin and calretinin in the neocortex,” *J. Chem. Neuroanat.*, vol. 14, no. 1, pp. 1–19, Dec. 1997.
- [79] P. Somogyi, G. Tamás, R. Lujan, and E. H. Buhl, “Salient features of synaptic organisation in the cerebral cortex,” *Brain Res. Brain Res. Rev.*, vol. 26, no. 2–3, pp. 113–35, May 1998.
- [80] M. Fagiolini, J.-M. Fritschy, K. Löw, H. Möhler, U. Rudolph, and T. K. Hensch, “Specific GABAA Circuits for Visual Cortical Plasticity,” *Science (80-. )*, vol. 303, no. 5664, pp.



1681–1683, Mar. 2004.

- [81] P. Buzás, U. T. Eysel, P. Adorján, and Z. F. Kisvárdy, “Axonal topography of cortical basket cells in relation to orientation, direction, and ocular dominance maps,” *J. Comp. Neurol.*, vol. 437, no. 3, pp. 259–285, Aug. 2001.
- [82] M. Galarreta and S. Hestrin, “Spike Transmission and Synchrony Detection in Networks of GABAergic Interneurons,” *Science (80-. )*, vol. 292, no. 5525, pp. 2295–2299, Jun. 2001.
- [83] M. Galarreta and S. Hestrin, “Electrical synapses between Gaba-Releasing interneurons,” *Nat. Rev. Neurosci.*, vol. 2, no. 6, pp. 425–433, Jun. 2001.
- [84] B. Gao and J. M. Fritschy, “Selective allocation of GABAA receptors containing the alpha 1 subunit to neurochemically distinct subpopulations of rat hippocampal interneurons,” *Eur. J. Neurosci.*, vol. 6, no. 5, pp. 837–53, May 1994.
- [85] G. G. Turrigiano and S. B. Nelson, “Homeostatic plasticity in the developing nervous system,” *Nat. Rev. Neurosci.*, vol. 5, no. 2, pp. 97–107, Feb. 2004.
- [86] G. W. Davis, “HOMEOSTATIC CONTROL OF NEURAL ACTIVITY: From Phenomenology to Molecular Design,” <http://dx.doi.org/10.1146/annurev.neuro.28.061604.135751>, Jun. 2006.
- [87] G. G. Turrigiano, “The self-tuning neuron: synaptic scaling of excitatory synapses,” *Cell*, vol. 135, no. 3, pp. 422–35, Oct. 2008.
- [88] N. S. Desai, “Homeostatic plasticity in the CNS: synaptic and intrinsic forms,” *J. Physiol.*, vol. 97, no. 4–6, pp. 391–402, Jul. 2003.
- [89] W. Zhang and D. J. Linden, “The other side of the engram: experience-driven changes in neuronal intrinsic excitability,” *Nat. Rev. Neurosci.*, vol. 4, no. 11, pp. 885–900, Nov. 2003.

- [90] A. Maffei, S. B. Nelson, and G. G. Turrigiano, "Selective reconfiguration of layer 4 visual cortical circuitry by visual deprivation.," *Nat. Neurosci.*, vol. 7, no. 12, pp. 1353–9, Dec. 2004.
- [91] S. . Kirov, L. . Petrak, J. . Fiala, and K. . Harris, "Dendritic spines disappear with chilling but proliferate excessively upon rewarming of mature hippocampus," *Neuroscience*, vol. 127, no. 1, pp. 69–80, Jan. 2004.
- [92] C. J. Wierenga, M. F. Walsh, and G. G. Turrigiano, "Temporal Regulation of the Expression Locus of Homeostatic Plasticity," *J Neurophysiol*, vol. 96, pp. 2127–2133, 2006.
- [93] E. L. Bienenstock, L. N. Cooper, and P. W. Munro, "THEORY FOR THE DEVELOPMENT OF NEURON SELECTIVITY: ORIENTATION SPECIFICITY AND BINOCULAR INTERACTION IN VISUAL CORTEX1," *J. Neurosci ence*, vol. 2, no. 1, pp. 32–48, 1982.
- [94] W. C. Abraham and M. F. Bear, "Metaplasticity: the plasticity of synaptic plasticity," *Trends Neurosci.*, vol. 19, no. 4, pp. 126–130, Apr. 1996.
- [95] G. G. Turrigiano, K. R. Leslie, N. S. Desai, L. C. Rutherford, and S. B. Nelson, "Activity-dependent scaling of quantal amplitude in neocortical neurons.," *Nature*, vol. 391, no. 6670, pp. 892–896, Feb. 1998.
- [96] C. C. Swanwick, N. R. Murthy, and J. Kapur, "Activity-dependent scaling of GABAergic synapse strength is regulated by brain-derived neurotrophic factor," *Mol. Cell. Neurosci.*, vol. 31, no. 3, pp. 481–492, Mar. 2006.
- [97] G. H. Diering, R. S. Nirujogi, R. H. Roth, P. F. Worley, A. Pandey, and R. L. Huganir, "Homer1a drives homeostatic scaling-down of excitatory synapses during sleep," *Science (80-. )*, vol. 355, no. 6324, pp. 511–515, Feb. 2017.
- [98] T. C. Thiagarajan, E. S. Piedras-Renteria, and R. W. Tsien, "alpha- and betaCaMKII.

- Inverse regulation by neuronal activity and opposing effects on synaptic strength.," *Neuron*, vol. 36, no. 6, pp. 1103–14, Dec. 2002.
- [99] G. Turrigiano, "Homeostatic synaptic plasticity: local and global mechanisms for stabilizing neuronal function.," *Cold Spring Harb. Perspect. Biol.*, vol. 4, no. 1, p. a005736, Jan. 2012.
- [100] L. A. Cingolani *et al.*, "Activity-Dependent Regulation of Synaptic AMPA Receptor Composition and Abundance by  $\beta$ 3 Integrins," *Neuron*, vol. 58, no. 5, pp. 749–762, Jun. 2008.
- [101] D. Stellwagen and R. C. Malenka, "Synaptic scaling mediated by glial TNF- $\alpha$ ," *Nature*, vol. 440, no. 7087, pp. 1054–1059, Mar. 2006.
- [102] A. Maffei and G. G. Turrigiano, "Multiple Modes of Network Homeostasis in Visual Cortical Layer 2/3," *J. Neurosci.*, vol. 28, no. 17, pp. 4377–4384, Apr. 2008.
- [103] A. Kleinschmidt, M. F. Bear, and W. Singer, "Blockade of &quot;NMDA&quot; receptors disrupts experience-dependent plasticity of kitten striate cortex.," *Science*, vol. 238, no. 4825, pp. 355–8, Oct. 1987.
- [104] M. F. Bear, A. Kleinschmidt, Q. A. Gu, and W. Singer, "Disruption of experience-dependent synaptic modifications in striate cortex by infusion of an NMDA receptor antagonist.," *J. Neurosci.*, vol. 10, no. 3, pp. 909–25, Mar. 1990.
- [105] E. B. Roberts, M. A. Meredith, and A. S. Ramoa, "Suppression of NMDA receptor function using antisense DNA block ocular dominance plasticity while preserving visual responses.," *J. Neurophysiol.*, vol. 80, no. 3, pp. 1021–32, Sep. 1998.
- [106] E. Tongiorgi, F. Ferrero, A. Cattaneo, and L. Domenici, "Dark-rearing decreases NR2A N-methyl-D-aspartate receptor subunit in all visual cortical layers.," *Neuroscience*, vol. 119, no. 4, pp. 1013–22, 2003.

- [107] B. D. Philpot and R. S. Zukin, "Synapse-specific metaplasticity: to be silenced is not to silence 2B.," *Neuron*, vol. 66, no. 6, pp. 814–6, Jun. 2010.
- [108] P. O. Kanold, Y. A. Kim, T. GrandPre, and C. J. Shatz, "Co-regulation of ocular dominance plasticity and NMDA receptor subunit expression in glutamic acid decarboxylase-65 knock-out mice," *J. Physiol.*, vol. 587, no. 12, pp. 2857–2867, Jun. 2009.
- [109] A. J. Heynen, B.-J. Yoon, C.-H. Liu, H. J. Chung, R. L. Huganir, and M. F. Bear, "Molecular mechanism for loss of visual cortical responsiveness following brief monocular deprivation," *Nat. Neurosci.*, vol. 6, no. 8, pp. 854–862, Aug. 2003.
- [110] Y. Rao and N. W. Daw, "Layer Variations of Long-Term Depression in Rat Visual Cortex," *J. Neurophysiol.*, vol. 92, no. 5, pp. 2652–2658, Jul. 2004.
- [111] G. Dölen and M. F. Bear, "Role for metabotropic glutamate receptor 5 (mGluR5) in the pathogenesis of fragile X syndrome," *J. Physiol.*, vol. 586, no. 6, pp. 1503–1508, Mar. 2008.
- [112] L. Domenici, N. Berardi, G. Carmignoto, G. Vantini, and L. Maffei, "Nerve growth factor prevents the amblyopic effects of monocular deprivation (visual plasticity/neurotrophic factors/rat)," *Neurobiology*, vol. 88, pp. 8811–8815, 1991.
- [113] L. Domenici, V. Parisi, and L. Maffei, "Exogenous supply of nerve growth factor prevents the effects of strabismus in the rat.," *Neuroscience*, vol. 51, no. 1, pp. 19–24, Nov. 1992.
- [114] C. Lodovichi, N. Berardi, T. Pizzorusso, and L. Maffei, "Effects of neurotrophins on cortical plasticity: same or different?," *J. Neurosci.*, vol. 20, no. 6, pp. 2155–65, Mar. 2000.
- [115] T. Pizzorusso *et al.*, "TrkA activation in the rat visual cortex by antirat trkA IgG

- prevents the effect of monocular deprivation.," *Eur. J. Neurosci.*, vol. 11, no. 1, pp. 204–12, Jan. 1999.
- [116] N. Berardi *et al.*, "Monoclonal antibodies to nerve growth factor affect the postnatal development of the visual system.," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 91, no. 2, pp. 684–8, Jan. 1994.
- [117] R. J. Cabelli, D. L. Shelton, R. A. Segal, and C. J. Shatz, "Blockade of endogenous ligands of trkB inhibits formation of ocular dominance columns.," *Neuron*, vol. 19, no. 1, pp. 63–76, Jul. 1997.
- [118] E. Castrén, F. Zafra, H. Thoenen, and D. Lindholm, "Light regulates expression of brain-derived neurotrophic factor mRNA in rat visual cortex.," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 89, no. 20, pp. 9444–8, Oct. 1992.
- [119] Y. Bozzi, T. Pizzorusso, F. Cremisi, F. M. Rossi, G. Barsacchi, and L. Maffei, "Monocular deprivation decreases the expression of messenger RNA for brain-derived neurotrophic factor in the rat visual cortex.," *Neuroscience*, vol. 69, no. 4, pp. 1133–44, Dec. 1995.
- [120] M. Poo, "Neurotrophins as synaptic modulators," *Nat. Rev. Neurosci.*, vol. 2, no. 1, pp. 24–32, Jan. 2001.
- [121] J. N. Jovanovic, A. J. Czernik, A. A. Fienberg, P. Greengard, and T. S. Sihra, "Synapsins as mediators of BDNF-enhanced neurotransmitter release.," *Nat. Neurosci.*, vol. 3, no. 4, pp. 323–329, Apr. 2000.
- [122] K. W. Kafitz, C. R. Rose, H. Thoenen, and A. Konnerth, "Neurotrophin-evoked rapid excitation through TrkB receptors.," *Nature*, vol. 401, no. 6756, pp. 918–21, Oct. 1999.
- [123] A. K. McAllister, L. C. Katz, and D. C. Lo, "NEUROTROPHINS AND SYNAPTIC PLASTICITY," *Annu. Rev. Neurosci.*, vol. 22, no. 1, pp. 295–318, Mar. 1999.

- [124] N. K. Isaev, E. V. Stelmashook, and E. E. Genrikhs, "Role of nerve growth factor in plasticity of forebrain cholinergic neurons," *Biochem.*, vol. 82, no. 3, pp. 291–300, Mar. 2017.
- [125] M. Hübener and T. Bonhoeffer, "Neuronal plasticity: Beyond the critical period," *Cell*, vol. 159, no. 4, pp. 727–737, 2014.
- [126] K. Kohara, A. Kitamura, M. Morishima, and T. Tsumoto, "Activity-Dependent Transfer of Brain-Derived Neurotrophic Factor to Postsynaptic Neurons," *Science (80-. )*, vol. 291, no. 5512, pp. 2419–2423, Mar. 2001.
- [127] C. S. von Bartheld, "Axonal transport and neuronal transcytosis of trophic factors, tracers, and pathogens," *J. Neurobiol.*, vol. 58, no. 2, pp. 295–314, Feb. 2004.
- [128] R. Sala *et al.*, "Nerve growth factor and brain-derived neurotrophic factor increase neurotransmitter release in the rat visual cortex.," *Eur. J. Neurosci.*, vol. 10, no. 6, pp. 2185–91, Jun. 1998.
- [129] L. Domenici, G. Fontanesi, A. Cattaneo, P. Bagnoli, and L. Maffei, "Nerve growth factor (NGF) uptake and transport following injection in the developing rat visual cortex," *Vis. Neurosci.*, vol. 11, no. 6, pp. 1093–1102, Nov. 1994.
- [130] R. A. Galuske, D. S. Kim, E. Castrén, and W. Singer, "Differential effects of neurotrophins on ocular dominance plasticity in developing and adult cat visual cortex.," *Eur. J. Neurosci.*, vol. 12, no. 9, pp. 3315–30, Sep. 2000.
- [131] D. C. Gillespie, M. C. Crair, and M. P. Stryker, "Neurotrophin-4/5 alters responses and blocks the effect of monocular deprivation in cat visual cortex during the critical period.," *J. Neurosci.*, vol. 20, no. 24, pp. 9174–86, Dec. 2000.
- [132] Z. J. Huang *et al.*, "BDNF regulates the maturation of inhibition and the critical period of plasticity in mouse visual cortex.," *Cell*, vol. 98, no. 6, pp. 739–55, Sep. 1999.

- [133] I. Abidin, U. T. Eysel, V. Lessmann, and T. Mittmann, "Impaired GABAergic inhibition in the visual cortex of brain-derived neurotrophic factor heterozygous knockout mice," *J. Physiol.*, vol. 586, no. 7, pp. 1885–901, Apr. 2008.
- [134] A. Bartoletti *et al.*, "Heterozygous knock-out mice for brain-derived neurotrophic factor show a pathway-specific impairment of long-term potentiation but normal critical period for monocular deprivation.," *J. Neurosci.*, vol. 22, no. 23, pp. 10072–7, Dec. 2002.
- [135] M. Kaneko, J. L. Hanover, P. M. England, and M. P. Stryker, "TrkB kinase is required for recovery, but not loss, of cortical responses following monocular deprivation," *Nat. Neurosci.*, vol. 11, no. 4, pp. 497–504, Apr. 2008.
- [136] L. Baroncelli *et al.*, "Experience-dependent reactivation of ocular dominance plasticity in the adult visual cortex.," *Exp. Neurol.*, vol. 226, no. 1, pp. 100–9, Nov. 2010.
- [137] J. A. Heimel, M. H. Saiepour, S. Chakravarthy, J. M. Hermans, and C. N. Levelt, "Contrast gain control and cortical TrkB signaling shape visual acuity," *Nat. Neurosci.*, vol. 13, no. 5, pp. 642–648, May 2010.
- [138] A. Sale *et al.*, "Environmental enrichment in adulthood promotes amblyopia recovery through a reduction of intracortical inhibition," *Nat. Neurosci.*, vol. 10, no. 6, pp. 679–681, 2007.
- [139] K. K. Cowansage, J. E. LeDoux, and M. H. Monfils, "Brain-derived neurotrophic factor: a dynamic gatekeeper of neural plasticity," *Curr Mol Pharmacol*, vol. 3, no. 1, pp. 12–29, Jan. 2010.
- [140] Q. Gu, "Neuromodulatory transmitter systems in the cortex and their role in cortical plasticity.," *Neuroscience*, vol. 111, no. 4, pp. 815–35, 2002.
- [141] N. Mechawar and L. Descarries, "The cholinergic innervation develops early and

- rapidly in the rat cerebral cortex: a quantitative immunocytochemical study.," *Neuroscience*, vol. 108, no. 4, pp. 555–67, 2001.
- [142] M. Latsari, I. Dori, J. Antonopoulos, M. Chiotelli, and A. Dinopoulos, "Noradrenergic innervation of the developing and mature visual and motor cortex of the rat brain: a light and electron microscopic immunocytochemical analysis.," *J. Comp. Neurol.*, vol. 445, no. 2, pp. 145–58, Apr. 2002.
- [143] T. Kasamatsu and J. D. Pettigrew, "Depletion of brain catecholamines: failure of ocular dominance shift after monocular occlusion in kittens.," *Science*, vol. 194, no. 4261, pp. 206–9, Oct. 1976.
- [144] T. Kasamatsu and J. D. Pettigrew, "Preservation of binocularity after monocular deprivation in the striate cortex of kittens treated with 6-Hydroxydopamine," *J. Comp. Neurol.*, vol. 185, no. 1, pp. 139–161, May 1979.
- [145] T. Kasamatsu, J. D. Pettigrew, and M. Ary, "Restoration of visual cortical plasticity by local microperfusion of norepinephrine," *J. Comp. Neurol.*, vol. 185, no. 1, pp. 163–181, May 1979.
- [146] T. Kasamatsu, J. D. Pettigrew, and M. Ary, "Cortical recovery from effects of monocular deprivation: acceleration with norepinephrine and suppression with 6-hydroxydopamine.," *J. Neurophysiol.*, vol. 45, no. 2, pp. 254–66, Feb. 1981.
- [147] K. Imamura and T. Kasamatsu, "Acutely induced shift in ocular dominance during brief monocular exposure: Effects of cortical noradrenaline infusion," *Neurosci. Lett.*, vol. 88, no. 1, pp. 57–62, May 1988.
- [148] K. Imamura and T. Kasamatsu, "Ocular dominance plasticity restored by NA infusion to aplastic visual cortex of anesthetized and paralyzed kittens.," *Exp. brain Res.*, vol. 87, no. 2, pp. 309–18, 1991.



- [149] T. Kasamatsu, K. Watabe, P. Heggelund, and E. Schöller, "Plasticity in cat visual cortex restored by electrical stimulation of the locus coeruleus.," *Neurosci. Res.*, vol. 2, no. 5, pp. 365–86, Jun. 1985.
- [150] N. Mataga, K. Imamura, and Y. Watanabe, "L-threo-3,4-dihydroxyphenylserine enhanced ocular dominance plasticity in adult cats.," *Neurosci. Lett.*, vol. 142, no. 2, pp. 115–8, Aug. 1992.
- [151] T. Kasamatsu and T. Shirokawa, "Involvement of  $\beta$ -adrenoreceptors in the shift of ocular dominance after monocular deprivation," *Exp. Brain Res.*, vol. 59, no. 3, pp. 507–514, Aug. 1985.
- [152] A. Kirkwood, C. Rozas, J. Kirkwood, F. Perez, and M. F. Bear, "Modulation of long-term synaptic depression in visual cortex by acetylcholine and norepinephrine.," *J. Neurosci.*, vol. 19, no. 5, pp. 1599–609, Mar. 1999.
- [153] R. C. Foehring, P. C. Schwindt, and W. E. Crill, "Norepinephrine selectively reduces slow  $\text{Ca}^{2+}$ - and  $\text{Na}^{+}$ -mediated  $\text{K}^{+}$  currents in cat neocortical neurons.," *J. Neurophysiol.*, vol. 61, no. 2, pp. 245–56, Feb. 1989.
- [154] K. Imamura, T. Kasamatsu, T. Shirokawa, and T. Ohashi, "Restoration of ocular dominance plasticity mediated by adenosine 3',5'-monophosphate in adult visual cortex.," *Proceedings. Biol. Sci.*, vol. 266, no. 1428, pp. 1507–16, Aug. 1999.
- [155] M. F. Bear and W. Singer, "Modulation of visual cortical plasticity by acetylcholine and noradrenaline," *Nature*, vol. 320, no. 6058, pp. 172–176, Mar. 1986.
- [156] Q. Gu and W. Singer, "The role of muscarinic acetylcholine receptors in ocular dominance plasticity.," *EXS*, vol. 57, pp. 305–14, 1989.
- [157] Q. Gu and W. Singer, "Effects of intracortical infusion of anticholinergic drugs on neuronal plasticity in kitten striate cortex.," *Eur. J. Neurosci.*, vol. 5, no. 5, pp. 475–85,

May 1993.

- [158] H. Markram and M. Segal, "The inositol 1,4,5-trisphosphate pathway mediates cholinergic potentiation of rat hippocampal neuronal responses to NMDA.," *J. Physiol.*, vol. 447, pp. 513–33, Feb. 1992.
- [159] Q. Gu and W. Singer, "Involvement of serotonin in developmental plasticity of kitten visual cortex.," *Eur. J. Neurosci.*, vol. 7, no. 6, pp. 1146–53, Jun. 1995.
- [160] Y. Wang, Q. Gu, and M. S. Cynader, "Blockade of serotonin-2C receptors by mesulergine reduces ocular dominance plasticity in kitten visual cortex.," *Exp. brain Res.*, vol. 114, no. 2, pp. 321–8, Apr. 1997.
- [161] M. M. Panicker, I. Parker, and R. Miledi, "Receptors of the serotonin 1C subtype expressed from cloned DNA mediate the closing of K<sup>+</sup> membrane channels encoded by brain mRNA.," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 88, no. 6, pp. 2560–2, Mar. 1991.
- [162] D. Hoyer and G. Martin, "5-HT receptor classification and nomenclature: towards a harmonization with the human genome.," *Neuropharmacology*, vol. 36, no. 4–5, pp. 419–28.
- [163] G. D. Cristo *et al.*, "Requirement of ERK Activation for Visual Cortical Plasticity," *Science (80-. )*, vol. 292, no. 5525, pp. 2337–2340, Jun. 2001.
- [164] S. Taha, J. L. Hanover, A. J. Silva, and M. P. Stryker, "Autophosphorylation of alphaCaMKII is required for ocular dominance plasticity.," *Neuron*, vol. 36, no. 3, pp. 483–91, Oct. 2002.
- [165] S. A. Taha and M. P. Stryker, "Molecular substrates of plasticity in the developing visual cortex," in *Progress in brain research*, vol. 147, 2005, pp. 101–114.
- [166] N. Berardi, T. Pizzorusso, G. M. Ratto, and L. Maffei, "Molecular basis of plasticity in the visual cortex," *Trends Neurosci.*, vol. 26, no. 7, pp. 369–378, Jul. 2003.

- [167] L. Cancedda, E. Putignano, S. Impey, L. Maffei, G. M. Ratto, and T. Pizzorusso, "Patterned vision causes CRE-mediated gene expression in the visual cortex through PKA and ERK.," *J. Neurosci.*, vol. 23, no. 18, pp. 7012–20, Aug. 2003.
- [168] S. Suzuki, S. Al-Noori, S. A. Butt, and T. A. Pham, "Regulation of the CREB signaling cascade in the visual cortex by visual experience and neuronal activity," *J. Comp. Neurol.*, vol. 479, no. 1, pp. 70–83, Nov. 2004.
- [169] T. A. Pham, S. Impey, D. R. Storm, and M. P. Stryker, "CRE-mediated gene transcription in neocortical neuronal plasticity during the developmental critical period.," *Neuron*, vol. 22, no. 1, pp. 63–72, Jan. 1999.
- [170] A. F. Mower, D. S. Liao, E. J. Nestler, R. L. Neve, and A. S. Ramoa, "cAMP/Ca<sup>2+</sup> response element-binding protein function is essential for ocular dominance plasticity.," *J. Neurosci.*, vol. 22, no. 6, pp. 2237–45, Mar. 2002.
- [171] E. Putignano *et al.*, "Developmental Downregulation of Histone Posttranslational Modifications Regulates Visual Cortical Plasticity," *Neuron*, vol. 53, no. 5, pp. 747–759, Mar. 2007.
- [172] A. W. Lyckman *et al.*, "Gene expression patterns in visual cortex during the critical period: Synaptic stabilization and reversal by visual deprivation," *Proc. Natl. Acad. Sci.*, vol. 105, no. 27, pp. 9409–9414, Jul. 2008.
- [173] Y. Yang, Q. S. Fischer, Y. Zhang, K. Baumgärtel, I. M. Mansuy, and N. W. Daw, "Reversible blockade of experience-dependent plasticity by calcineurin in mouse visual cortex," *Nat. Neurosci.*, vol. 8, no. 6, pp. 791–796, Jun. 2005.
- [174] T. Pizzorusso, P. Medini, N. Berardi, S. Chierzi, J. W. Fawcett, and L. Maffei, "Reactivation of Ocular Dominance Plasticity in the Adult Visual Cortex," *Science (80-. )*, vol. 298, no. 5596, pp. 1248–1251, Nov. 2002.

- [175] S. Hockfield, R. G. Kalb, S. Zaremba, and H. Fryer, "Expression of neural proteoglycans correlates with the acquisition of mature neuronal properties in the mammalian brain.," *Cold Spring Harb. Symp. Quant. Biol.*, vol. 55, pp. 505–14, 1990.
- [176] P. C. Kind *et al.*, "The development and activity-dependent expression of aggrecan in the cat visual cortex.," *Cereb. Cortex*, vol. 23, no. 2, pp. 349–60, Feb. 2013.
- [177] N. Mataga, K. Imamura, T. Shiomitsu, Y. Yoshimura, K. Fukamauchi, and Y. Watanabe, "Enhancement of mRNA expression of tissue-type plasminogen activator by L-threo-3,4-dihydroxyphenylserine in association with ocular dominance plasticity.," *Neurosci. Lett.*, vol. 218, no. 3, pp. 149–52, Nov. 1996.
- [178] C. M. Müller and C. B. Griesinger, "Tissue plasminogen activator mediates reverse occlusion plasticity in visual cortex," *Nat. Neurosci.*, vol. 1, no. 1, pp. 47–53, May 1998.
- [179] A. W. McGee, Y. Yang, Q. S. Fischer, N. W. Daw, and S. M. Strittmatter, "Experience-Driven Plasticity of Visual Cortex Limited by Myelin and Nogo Receptor," *Science (80-. )*, vol. 309, no. 5744, pp. 2222–2226, Sep. 2005.
- [180] S. J. Raiker, H. Lee, K. T. Baldwin, Y. Duan, P. Shrager, and R. J. Giger, "Oligodendrocyte-Myelin Glycoprotein and Nogo Negatively Regulate Activity-Dependent Synaptic Plasticity," *J. Neurosci.*, vol. 30, no. 37, pp. 12432–12445, Sep. 2010.
- [181] U. R. Karmarkar and Y. Dan, "Experience-Dependent Plasticity in Adult Visual Cortex," *Neuron*, vol. 52, no. 4, pp. 577–585, Nov. 2006.
- [182] C. D. Gilbert and W. Li, "Adult Visual Cortical Plasticity," *Neuron*, vol. 75, no. 2. Cell Press, pp. 250–264, 26-Jul-2012.
- [183] I. Fine and R. A. Jacobs, "Comparing perceptual learning across tasks: A review," *J. Vis.*,

vol. 2, no. 2, pp. 5–5, Apr. 2002.

- [184] D. M. Levi and R. W. Li, “Perceptual learning as a potential treatment for amblyopia: a mini-review,” *Vision Res.*, vol. 49, no. 21, pp. 2535–49, Oct. 2009.
- [185] J. Bonaccorsi, N. Berardi, and A. Sale, “Treatment of amblyopia in the adult: insights from a new rodent model of visual perceptual learning,” *Front. Neural Circuits*, vol. 8, no. July, p. 82, 2014.
- [186] D. M. Levi, D. C. Knill, and D. Bavelier, “Stereopsis and amblyopia: A mini-review,” *Vision Res.*, vol. 114, pp. 17–30, Sep. 2015.
- [187] J. H. Kaas, L. A. Krubitzer, Y. M. Chino, A. L. Langston, E. H. Polley, and N. Blair, “Reorganization of retinotopic cortical maps in adult mammals after lesions of the retina,” *Science*, vol. 248, no. 4952, pp. 229–31, Apr. 1990.
- [188] S. J. Heinen and A. A. Skavenski, “Recovery of visual responses in foveal V1 neurons following bilateral foveal lesions in adult monkey,” *Exp. brain Res.*, vol. 83, no. 3, pp. 670–4, 1991.
- [189] C. D. Gilbert and T. N. Wiesel, “Receptive field dynamics in adult primary visual cortex,” *Nature*, vol. 356, no. 6365, pp. 150–152, Mar. 1992.
- [190] C. Darian-Smith and C. D. Gilbert, “Axonal sprouting accompanies functional reorganization in adult cat striate cortex,” *Nature*, vol. 368, no. 6473, pp. 737–740, Apr. 1994.
- [191] C. Darian-Smith and C. D. Gilbert, “Topographic reorganization in the striate cortex of the adult cat and monkey is cortically mediated,” *J. Neurosci.*, vol. 15, no. 3 Pt 1, pp. 1631–47, Mar. 1995.
- [192] M. B. Calford, L. L. Wright, A. B. Metha, and V. Taglianetti, “Topographic plasticity in primary visual cortex is mediated by local corticocortical connections,” *J. Neurosci.*,

- vol. 23, no. 16, pp. 6434–42, Jul. 2003.
- [193] Y. Tagawa, P. O. Kanold, M. Majdan, and C. J. Shatz, “Multiple periods of functional ocular dominance plasticity in mouse visual cortex,” *Nat. Neurosci.*, vol. 8, no. 3, pp. 380–388, Mar. 2005.
- [194] S. B. Hofer, T. D. Mrsic-Flogel, T. Bonhoeffer, and M. Hübener, “Prior experience enhances plasticity in adult visual cortex,” *Nat. Neurosci.*, vol. 9, no. 1, pp. 127–132, Jan. 2006.
- [195] H.-Y. He, W. Hodos, and E. M. Quinlan, “Visual Deprivation Reactivates Rapid Ocular Dominance Plasticity in Adult Visual Cortex,” *J. Neurosci.*, vol. 26, no. 11, pp. 2951–2955, Mar. 2006.
- [196] H.-Y. He, B. Ray, K. Dennis, and E. M. Quinlan, “Experience-dependent recovery of vision following chronic deprivation amblyopia,” *Nat. Neurosci.*, vol. 10, no. 9, pp. 1134–1136, Sep. 2007.
- [197] M. R. Rosenzweig, E. L. Bennett, M. Hebert, and H. Morimoto, “Social grouping cannot account for cerebral effects of enriched environments.,” *Brain Res.*, vol. 153, no. 3, pp. 563–76, Sep. 1978.
- [198] N. Berardi, A. Sale, and L. Maffei, “Brain structural and functional development: genetics and experience,” *Dev. Med. Child Neurol.*, vol. 57, pp. 4–9, Apr. 2015.
- [199] M. R. Rosenzweig and E. L. Bennett, “Effects of differential environments on brain weights and enzyme activities in gerbils, rats, and mice,” *Dev. Psychobiol.*, vol. 2, no. 2, pp. 87–95, 1969.
- [200] P. Cornwell and W. Overman, “Behavioral effects of early rearing conditions and neonatal lesions of the visual cortex in kittens.,” *J. Comp. Physiol. Psychol.*, vol. 95, no. 6, pp. 848–62, Dec. 1981.

- [201] Hansen and Berthelsen, "The effect of environmental enrichment on the behaviour of caged rabbits (*Oryctolagus cuniculus*).," *Appl. Anim. Behav. Sci.*, vol. 68, no. 2, pp. 163–178, May 2000.
- [202] Y. Kozorovitskiy *et al.*, "Experience induces structural and biochemical changes in the adult primate brain," *Proc. Natl. Acad. Sci.*, vol. 102, no. 48, pp. 17478–17482, Nov. 2005.
- [203] M. Lazic, S. M. Schneider, and R. Lickliter, "Enriched rearing facilitates spatial exploration in northern bobwhite (*Colinus virginianus*) neonates," *Dev. Psychobiol.*, vol. 49, no. 5, pp. 548–551, Jul. 2007.
- [204] J. Jansen, J. E. Bolhuis, W. G. P. Schouten, B. M. Spruijt, and V. M. Wiegant, "Spatial learning in pigs: effects of environmental enrichment and individual characteristics on behaviour and performance," *Anim. Cogn.*, vol. 12, no. 2, pp. 303–315, Mar. 2009.
- [205] M. R. ROSENZWEIG, D. KRECH, E. L. BENNETT, and J. F. ZOLMAN, "Variation in environmental complexity and brain measures.," *J. Comp. Physiol. Psychol.*, vol. 55, pp. 1092–5, Dec. 1962.
- [206] M. R. ROSENZWEIG, D. KRECH, E. L. BENNETT, and M. C. DIAMOND, "Effects of environmental complexity and training on brain chemistry and anatomy: a replication and extension.," *J. Comp. Physiol. Psychol.*, vol. 55, pp. 429–37, Aug. 1962.
- [207] E. L. BENNETT, D. KRECH, and M. R. ROSENZWEIG, "RELIABILITY AND REGIONAL SPECIFICITY OF CEREBRAL EFFECTS OF ENVIRONMENTAL COMPLEXITY AND TRAINING.," *J. Comp. Physiol. Psychol.*, vol. 57, pp. 440–1, Jun. 1964.
- [208] E. L. BENNETT, M. C. DIAMOND, D. KRECH, and M. R. ROSENZWEIG, "CHEMICAL AND ANATOMICAL PLASTICITY BRAIN.," *Science*, vol. 146, no. 3644, pp. 610–9, Oct. 1964.
- [209] M. C. DIAMOND, D. KRECH, and M. R. ROSENZWEIG, "THE EFFECTS OF AN ENRICHED

- ENVIRONMENT ON THE HISTOLOGY OF THE RAT CEREBRAL CORTEX.," *J. Comp. Neurol.*, vol. 123, pp. 111–20, Aug. 1964.
- [210] M. C. Diamond *et al.*, "Increases in cortical depth and glia numbers in rats subjected to enriched environment," *J. Comp. Neurol.*, vol. 128, no. 1, pp. 117–125, Sep. 1966.
- [211] M. C. Diamond, M. R. Rosenzweig, E. L. Bennett, B. Lindner, and L. Lyon, "Effects of environmental enrichment and impoverishment on rat cerebral cortex," *J. Neurobiol.*, vol. 3, no. 1, pp. 47–64, 1972.
- [212] J. ALTMAN and G. D. DAS, "Autoradiographic Examination of the Effects of Enriched Environment on the Rate of Glial Multiplication in the Adult Rat Brain," *Nature*, vol. 204, no. 4964, pp. 1161–1163, Dec. 1964.
- [213] F. R. Volkmar, W. T. Greenough, and T. DeVoogd, "Rearing complexity affects branching of dendrites in the visual cortex of the rat.," *Science*, vol. 176, no. 4042, pp. 1445–7, Jun. 1972.
- [214] W. T. Greenough and F. R. Volkmar, "Pattern of dendritic branching in occipital cortex of rats reared in complex environments.," *Exp. Neurol.*, vol. 40, no. 2, pp. 491–504, Aug. 1973.
- [215] E. J. Green, W. T. Greenough, and B. E. Schlumpf, "Effects of complex or isolated environments on cortical dendrites of middle-aged rats.," *Brain Res.*, vol. 264, no. 2, pp. 233–40, Apr. 1983.
- [216] A. Globus, M. R. Rosenzweig, E. L. Bennett, and M. C. Diamond, "Effects of differential experience on dendritic spine counts in rat cerebral cortex.," *J. Comp. Physiol. Psychol.*, vol. 82, no. 2, pp. 175–81, Feb. 1973.
- [217] K. Mollgaard, M. C. Diamond, E. L. Bennett, M. R. Rosenzweig, and B. Lindner, "Quantitative synaptic changes with differential experience in rat brain.," *Int. J.*



- Neurosci.*, vol. 2, no. 3, pp. 113–27, Sep. 1971.
- [218] M. C. Diamond, B. Lindner, R. Johnson, E. L. Bennett, and M. R. Rosenzweig, “Difference in occipital cortical synapses from environmentally enriched, impoverished, and standard colony rats,” *J. Neurosci. Res.*, vol. 1, no. 2, pp. 109–119, 1975.
- [219] A. M. Turner and W. T. Greenough, “Differential rearing effects on rat visual cortex synapses. I. Synaptic and neuronal density and synapses per neuron,” *Brain Res.*, vol. 329, no. 1–2, pp. 195–203, Mar. 1985.
- [220] C. Beaulieu and M. Colonnier, “Effect of the richness of the environment on the cat visual cortex,” *J. Comp. Neurol.*, vol. 266, no. 4, pp. 478–494, Dec. 1987.
- [221] J. Ekstrand, J. Hellsten, and A. Tingström, “Environmental enrichment, exercise and corticosterone affect endothelial cell proliferation in adult rat hippocampus and prefrontal cortex,” *Neurosci. Lett.*, vol. 442, no. 3, pp. 203–207, Sep. 2008.
- [222] P. A. Ferchmin and V. A. Eterovic, “Forty minutes of experience increase the weight and RNA content of cerebral cortex in periadolescent rats,” *Dev. Psychobiol.*, vol. 19, no. 6, pp. 511–519, Nov. 1986.
- [223] M. C. Diamond, C. A. Ingham, R. E. Johnson, E. L. Bennett, and M. R. Rosenzweig, “Effects of environment on morphology of rat cerebral cortex and hippocampus,” *J. Neurobiol.*, vol. 7, no. 1, pp. 75–85, Jan. 1976.
- [224] M. R. Rosenzweig and E. L. Bennett, “Psychobiology of plasticity: effects of training and experience on brain and behavior,” *Behav. Brain Res.*, vol. 78, no. 1, pp. 57–65, Jun. 1996.
- [225] C. Rampon and J. Z. Tsien, “Genetic analysis of learning behavior-induced structural plasticity,” *Hippocampus*, vol. 10, no. 5, pp. 605–609, 2000.

- [226] T. A. Comery, R. Shah, and W. T. Greenough, "Differential Rearing Alters Spine Density on Medium-Sized Spiny Neurons in the Rat Corpus Striatum: Evidence for Association of Morphological Plasticity with Early Response Gene Expression," *Neurobiol. Learn. Mem.*, vol. 63, no. 3, pp. 217–219, May 1995.
- [227] M. Ichikawa, M. Matsuoka, and Y. Mori, "Effect of differential rearing on synapses and soma size in rat medial amygdaloid nucleus," *Synapse*, vol. 13, no. 1, pp. 50–56, Jan. 1993.
- [228] M. K. Floeter and W. T. Greenough, "Cerebellar plasticity: modification of Purkinje cell structure by differential rearing in monkeys," *Science*, vol. 206, no. 4415, pp. 227–9, Oct. 1979.
- [229] J. A. Kleim, K. Vij, D. H. Ballard, and W. T. Greenough, "Learning-dependent synaptic modifications in the cerebellar cortex of the adult rat persist for at least four weeks," *J. Neurosci.*, vol. 17, no. 2, pp. 717–21, Jan. 1997.
- [230] G. Kempermann, H. G. Kuhn, and F. H. Gage, "More hippocampal neurons in adult mice living in an enriched environment," *Nature*, vol. 386, no. 6624, pp. 493–495, Apr. 1997.
- [231] M. Nilsson, E. Perfilieva, U. Johansson, O. Orwar, and P. S. Eriksson, "Enriched environment increases neurogenesis in the adult rat dentate gyrus and improves spatial memory," *J. Neurobiol.*, vol. 39, no. 4, pp. 569–78, Jun. 1999.
- [232] H. van Praag, G. Kempermann, and F. H. Gage, "Neural consequences of environmental enrichment," *Nat. Rev. Neurosci.*, vol. 1, no. December, pp. 191–198, 2000.
- [233] E. Bruel-Jungerman, S. Davis, C. Rampon, and S. Laroche, "Long-term potentiation enhances neurogenesis in the adult dentate gyrus," *J. Neurosci.*, vol. 26, no. 22, pp.

5888–93, May 2006.

- [234] G. Kempermann, E. P. Brandon, and F. H. Gage, “Environmental stimulation of 129/SvJ mice causes increased cell proliferation and neurogenesis in the adult dentate gyrus,” *Curr. Biol.*, vol. 8, no. 16, pp. 939–42, 1998.
- [235] D. Young, P. A. Lawlor, P. Leone, M. Dragunow, and M. J. During, “Environmental enrichment inhibits spontaneous apoptosis, prevents seizures and is neuroprotective,” *Nat. Med.*, vol. 5, no. 4, pp. 448–53, Apr. 1999.
- [236] S. M. G. Braun and S. Jessberger, “Adult neurogenesis: mechanisms and functional significance,” *Development*, vol. 141, no. 10, pp. 1983–6, May 2014.
- [237] Gould, Tanapat, Hastings, and Shors, “Neurogenesis in adulthood: a possible role in learning,” *Trends Cogn. Sci.*, vol. 3, no. 5, pp. 186–192, May 1999.
- [238] T. J. Shors, G. Miesegaes, A. Beylin, M. Zhao, T. Rydel, and E. Gould, “Neurogenesis in the adult is involved in the formation of trace memories,” *Nature*, vol. 410, no. 6826, pp. 372–376, Mar. 2001.
- [239] E. Bruel-Jungerman, S. Laroche, and C. Rampon, “New neurons in the dentate gyrus are involved in the expression of enhanced long-term memory following environmental enrichment,” *Eur. J. Neurosci.*, vol. 21, no. 2, pp. 513–521, Jan. 2005.
- [240] D. Meshi *et al.*, “Hippocampal neurogenesis is not required for behavioral effects of environmental enrichment,” *Nat. Neurosci.*, vol. 9, no. 6, pp. 729–731, Jun. 2006.
- [241] T. Kobilko, C. Yuan, and H. van Praag, “Endurance factors improve hippocampal neurogenesis and spatial memory in mice,” *Learn. Mem.*, vol. 18, no. 2, pp. 103–107, Jan. 2011.
- [242] M. W. Voss, C. Vivar, A. F. Kramer, and H. van Praag, “Bridging animal and human models of exercise-induced brain plasticity,” *Trends Cogn. Sci.*, vol. 17, no. 10, pp.

525–544, 2013.

- [243] E. J. Green and W. T. Greenough, “Altered synaptic transmission in dentate gyrus of rats reared in complex environments: evidence from hippocampal slices maintained in vitro.,” *J. Neurophysiol.*, vol. 55, no. 4, pp. 739–50, Apr. 1986.
- [244] T. C. Foster, J. Gagne, and G. Massicotte, “Mechanism of altered synaptic strength due to experience: relation to long-term potentiation.,” *Brain Res.*, vol. 736, no. 1–2, pp. 243–50, Oct. 1996.
- [245] T. C. Foster and T. C. Dumas, “Mechanism for increased hippocampal synaptic strength following differential experience.,” *J. Neurophysiol.*, vol. 85, no. 4, pp. 1377–83, Apr. 2001.
- [246] G. I. Irvine and W. C. Abraham, “Enriched environment exposure alters the input-output dynamics of synaptic transmission in area CA1 of freely moving rats,” *Neurosci. Lett.*, vol. 391, no. 1–2, pp. 32–37, Dec. 2005.
- [247] J. A. Nichols, V. P. Jakkamsetti, H. Salgado, L. Dinh, M. P. Kilgard, and M. Atzori, “Environmental enrichment selectively increases glutamatergic responses in layer II/III of the auditory cortex of the rat,” *Neuroscience*, vol. 145, no. 3, pp. 832–840, Mar. 2007.
- [248] S. N. Duffy, K. J. Craddock, T. Abel, and P. V. Nguyen, “Environmental enrichment modifies the PKA-dependence of hippocampal LTP and improves hippocampus-dependent memory.,” *Learn. Mem.*, vol. 8, no. 1, pp. 26–34, 2001.
- [249] A. Artola *et al.*, “Long-lasting modulation of the induction of LTD and LTP in rat hippocampal CA1 by behavioural stress and environmental enrichment,” *Eur. J. Neurosci.*, vol. 23, no. 1, pp. 261–272, Jan. 2006.
- [250] F. W. F. Shum *et al.*, “Alteration of cingulate long-term plasticity and behavioral

- sensitization to inflammation by environmental enrichment.," *Learn. Mem.*, vol. 14, no. 4, pp. 304–12, Apr. 2007.
- [251] H. P. Edwards, W. F. Barry, and J. O. Wyspianski, "Effect of differential rearing on photic evoked potentials and brightness discrimination in the albino rat," *Dev. Psychobiol.*, vol. 2, no. 3, pp. 133–138, 1969.
- [252] M. L. Seo, "Effect of environmental complexity on the latency of cortical vibrissa potentials," *Dev. Psychobiol.*, vol. 25, no. 1, pp. 67–76, Jan. 1992.
- [253] J. O. Coq and C. Xerri, "Environmental enrichment alters organizational features of the forepaw representation in the primary somatosensory cortex of adult rats.," *Exp. brain Res.*, vol. 121, no. 2, pp. 191–204, Jul. 1998.
- [254] D. B. Polley, E. Kvašňák, and R. D. Frostig, "Naturalistic experience transforms sensory maps in the adult cortex of caged animals," *Nature*, vol. 429, no. 6987, pp. 67–71, May 2004.
- [255] C. Beaulieu and M. Cynader, "Effect of the richness of the environment on neurons in cat visual cortex. II. Spatial and temporal frequency characteristics.," *Brain Res. Dev. Brain Res.*, vol. 53, no. 1, pp. 82–8, Apr. 1990.
- [256] N. D. Engineer, C. R. Percaccio, P. K. Pandya, R. Moucha, D. L. Rathbun, and M. P. Kilgard, "Environmental Enrichment Improves Response Strength, Threshold, Selectivity, and Latency of Auditory Cortex Neurons," *J. Neurophysiol.*, vol. 92, no. 1, pp. 73–82, Mar. 2004.
- [257] C. R. Percaccio *et al.*, "Environmental Enrichment Increases Paired-Pulse Depression in Rat Auditory Cortex," *J. Neurophysiol.*, vol. 94, no. 5, pp. 3590–3600, Nov. 2005.
- [258] C. R. Percaccio, A. L. Pruette, S. T. Mistry, Y. H. Chen, and M. P. Kilgard, "Sensory experience determines enrichment-induced plasticity in rat auditory cortex," *Brain*

*Res.*, vol. 1174, pp. 76–91, Oct. 2007.

- [259] R. Cai, F. Guo, J. Zhang, J. Xu, Y. Cui, and X. Sun, “Environmental enrichment improves behavioral performance and auditory spatial representation of primary auditory cortical neurons in rat,” *Neurobiol. Learn. Mem.*, vol. 91, no. 4, pp. 366–376, May 2009.
- [260] P. A. Ferchmin, V. A. Eterovic, and R. Caputto, “Studies of brain weight and RNA content after short periods of exposure to environmental complexity,” *Brain Res.*, vol. 20, no. 1, pp. 49–57, May 1970.
- [261] L. D. Grouse, B. K. Schrier, E. L. Bennett, M. R. Rosenzweig, and P. G. Nelson, “Sequence diversity studies of rat brain RNA: effects of environmental complexity on rat brain RNA diversity,” *J. Neurochem.*, vol. 30, no. 1, pp. 191–203, Jan. 1978.
- [262] C. Rampon *et al.*, “Effects of environmental enrichment on gene expression in the brain,” *Proc. Natl. Acad. Sci. U. S. A.*, vol. 97, no. 23, pp. 12880–4, Nov. 2000.
- [263] K. Keyvani, N. Sachser, O. W. Witte, and W. Paulus, “Gene expression profiling in the intact and injured brain following environmental enrichment,” *J. Neuropathol. Exp. Neurol.*, vol. 63, no. 6, pp. 598–609, Jun. 2004.
- [264] R. Pinaud, M. R. Penner, H. A. Robertson, and R. W. Currie, “Upregulation of the immediate early gene arc in the brains of rats exposed to environmental enrichment: implications for molecular plasticity,” *Brain Res. Mol. Brain Res.*, vol. 91, no. 1–2, pp. 50–6, Jul. 2001.
- [265] K. McNair, J. Broad, G. Riedel, C. H. Davies, and S. R. Cobb, “Global changes in the hippocampal proteome following exposure to an enriched environment,” *Neuroscience*, vol. 145, no. 2, pp. 413–422, Mar. 2007.
- [266] N. Thiriet *et al.*, “Environmental enrichment during adolescence regulates gene

- expression in the striatum of mice.," *Brain Res.*, vol. 1222, pp. 31–41, Jul. 2008.
- [267] M. R. Rosenzweig, E. L. Bennett, and M. C. Diamond, "Effects of differential environments on brain anatomy and brain chemistry.," *Proc. Annu. Meet. Am. Psychopathol. Assoc.*, vol. 56, pp. 45–56, 1967.
- [268] S. Rasmuson *et al.*, "Environmental enrichment selectively increases 5-HT<sub>1A</sub> receptor mRNA expression and binding in the rat hippocampus.," *Brain Res. Mol. Brain Res.*, vol. 53, no. 1–2, pp. 285–90, Jan. 1998.
- [269] R. Galani, L. E. Jarrard, B. E. Will, and C. Kelche, "Effects of Postoperative Housing Conditions on Functional Recovery in Rats with Lesions of the Hippocampus, Subiculum, or Entorhinal Cortex," *Neurobiol. Learn. Mem.*, vol. 67, no. 1, pp. 43–56, Jan. 1997.
- [270] J. C. Brenes, O. Rodríguez, and J. Fornaguera, "Differential effect of environment enrichment and social isolation on depressive-like behavior, spontaneous activity and serotonin and norepinephrine concentration in prefrontal cortex and ventral striatum," *Pharmacol. Biochem. Behav.*, vol. 89, no. 1, pp. 85–93, Mar. 2008.
- [271] R. M. Escorihuela *et al.*, "Early environmental stimulation produces long-lasting changes on beta-adrenoceptor transduction system.," *Neurobiol. Learn. Mem.*, vol. 64, no. 1, pp. 49–57, Jul. 1995.
- [272] F. Naka, T. Shiga, M. Yaguchi, and N. Okado, "An enriched environment increases noradrenaline concentration in the mouse brain.," *Brain Res.*, vol. 924, no. 1, pp. 124–6, Jan. 2002.
- [273] F. Cordoba, B. Yusta, and J. Muñoz-Blanco, "Changes in neurotransmitter amino acids and protein in CNS areas of mice subjected to differential housing conditions.," *Pharmacol. Biochem. Behav.*, vol. 21, no. 3, pp. 349–52, Sep. 1984.

- [274] C. Rampon, Y. P. Tang, J. Goodhouse, E. Shimizu, M. Kyin, and J. Z. Tsien, "Enrichment induces structural changes and recovery from nonspatial memory deficits in CA1 NMDAR1-knockout mice.," *Nat. Neurosci.*, vol. 3, no. 3, pp. 238–244, Mar. 2000.
- [275] Y. P. Tang, H. Wang, R. Feng, M. Kyin, and J. Z. Tsien, "Differential effects of enrichment on learning and memory function in NR2B transgenic mice.," *Neuropharmacology*, vol. 41, no. 6, pp. 779–90, Nov. 2001.
- [276] F. Naka, N. Narita, N. Okado, and M. Narita, "Modification of AMPA receptor properties following environmental enrichment," *Brain Dev.*, vol. 27, no. 4, pp. 275–278, Jun. 2005.
- [277] J. Andin, M. Hallbeck, A. H. Mohammed, and J. Marcusson, "Influence of environmental enrichment on steady-state mRNA levels for EAAC1, AMPA1 and NMDA2A receptor subunits in rat hippocampus," *Brain Res.*, vol. 1174, pp. 18–27, Oct. 2007.
- [278] X. Q. Zhang *et al.*, "Increased protein expression levels of pCREB, BDNF and SDF-1/CXCR4 in the hippocampus may be associated with enhanced neurogenesis induced by environmental enrichment," *Mol. Med. Rep.*, vol. 14, no. 3, pp. 2231–2237, Sep. 2016.
- [279] R. Pinaud, L. A. Tremere, M. R. Penner, F. F. Hess, H. A. Robertson, and R. W. Currie, "Complexity of sensory environment drives the expression of candidate-plasticity gene, nerve growth factor induced-A.," *Neuroscience*, vol. 112, no. 3, pp. 573–82, 2002.
- [280] T. M. Pham, B. Winblad, A.-C. Granholm, and A. H. Mohammed, "Environmental influences on brain neurotrophins in rats.," *Pharmacol. Biochem. Behav.*, vol. 73, no. 1, pp. 167–75, Aug. 2002.



- [281] S. W. Zhu, B. K. Yee, M. Nyffeler, B. Winblad, J. Feldon, and A. H. Mohammed, "Influence of differential housing on emotional behaviour and neurotrophin levels in mice," *Behav. Brain Res.*, vol. 169, no. 1, pp. 10–20, Apr. 2006.
- [282] T. M. Pham, B. Ickes, D. Albeck, S. Söderström, A. C. Granholm, and A. H. Mohammed, "Changes in brain nerve growth factor levels and nerve growth factor receptors in rats exposed to environmental enrichment for one year," *Neuroscience*, vol. 94, no. 1, pp. 279–286, Sep. 1999.
- [283] T. B. Franklin, J. A. Murphy, T. L. Myers, D. B. Clarke, and R. W. Currie, "Enriched environment during adolescence changes brain-derived neurotrophic factor and TrkB levels in the rat visual system but does not offer neuroprotection to retinal ganglion cells following axotomy," *Brain Res.*, vol. 1095, no. 1, pp. 1–11, Jun. 2006.
- [284] B. M. Williams *et al.*, "Environmental enrichment: effects on spatial memory and hippocampal CREB immunoreactivity.," *Physiol. Behav.*, vol. 73, no. 4, pp. 649–58, Jul. 2001.
- [285] A. Fischer, F. Sananbenesi, X. Wang, M. Dobbin, and L.-H. Tsai, "Recovery of learning and memory is associated with chromatin remodelling," *Nature*, vol. 447, no. 7141, pp. 178–182, May 2007.
- [286] T. Pizzorusso, N. Berardi, and L. Maffei, "A Richness that Cures," *Neuron*, vol. 54, no. 4. Cell Press, pp. 508–510, 24-May-2007.
- [287] C. T. Ramey and S. L. Ramey, "Prevention of Intellectual Disabilities: Early Interventions to Improve Cognitive Development," *Prev. Med. (Baltim).*, vol. 27, no. 2, pp. 224–232, Mar. 1998.
- [288] A. Raine, K. Mellinger, J. Liu, P. Venables, and S. A. Mednick, "Effects of Environmental Enrichment at Ages 3–5 Years on Schizotypal Personality and Antisocial Behavior at

- Ages 17 and 23 Years," *Am. J. Psychiatry*, vol. 160, no. 9, pp. 1627–1635, Sep. 2003.
- [289] G. Sansevero and A. Sale, "Environment as therapy : neuroscience for intellectual disability and dementia," pp. 1–2, 2017.
- [290] F. Fernandez and C. C. Garner, "Over-inhibition: a model for developmental intellectual disability," *Trends Neurosci.*, vol. 30, no. 10, pp. 497–503, Oct. 2007.
- [291] V. S. Dani, Q. Chang, A. Maffei, G. G. Turrigiano, R. Jaenisch, and S. B. Nelson, "Reduced cortical activity due to a shift in the balance between excitation and inhibition in a mouse model of Rett syndrome.," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 102, no. 35, pp. 12560–5, Aug. 2005.
- [292] Y. Asaka, D. G. M. Jugloff, L. Zhang, J. H. Eubanks, and R. M. Fitzsimonds, "Hippocampal synaptic plasticity is impaired in the Mecp2-null mouse model of Rett syndrome," *Neurobiol. Dis.*, vol. 21, no. 1, pp. 217–227, Jan. 2006.
- [293] P. Moretti *et al.*, "Learning and Memory and Synaptic Plasticity Are Impaired in a Mouse Model of Rett Syndrome," *J. Neurosci.*, vol. 26, no. 1, pp. 319–327, Jan. 2006.
- [294] S. Durand *et al.*, "NMDA receptor regulation prevents regression of visual cortical function in the absence of Mecp2.," *Neuron*, vol. 76, no. 6, pp. 1078–90, Dec. 2012.
- [295] M. T. Davison, C. Schmidt, and E. C. Akeson, "Segmental trisomy of murine chromosome 16: a new model system for studying Down syndrome.," *Prog. Clin. Biol. Res.*, vol. 360, pp. 263–80, 1990.
- [296] P. V. Belichenko *et al.*, "Excitatory-inhibitory relationship in the fascia dentata in the Ts65Dn mouse model of down syndrome," *J. Comp. Neurol.*, vol. 512, no. 4, pp. 453–466, Feb. 2009.
- [297] A. M. Kleschevnikov, P. V Belichenko, A. J. Villar, C. J. Epstein, R. C. Malenka, and W. C. Mobley, "Hippocampal Long-Term Potentiation Suppressed by Increased Inhibition in

- the Ts65Dn Mouse, a Genetic Model of Down Syndrome," *J. Neurosci.*, vol. 24, no. 37, pp. 8153–8160, Sep. 2004.
- [298] D. Pérez-Cremades, S. Hernández, J. M. Blasco-Ibáñez, C. Crespo, J. Nacher, and E. Varea, "Alteration of inhibitory circuits in the somatosensory cortex of Ts65Dn mice, a model for Down's syndrome," *J. Neural Transm.*, vol. 117, no. 4, pp. 445–455, Apr. 2010.
- [299] R. J. Siarey, E. J. Carlson, C. J. Epstein, A. Balbo, S. I. Rapoport, and Z. Galdzicki, "Increased synaptic depression in the Ts65Dn mouse, a model for mental retardation in Down syndrome.," *Neuropharmacology*, vol. 38, no. 12, pp. 1917–20, Dec. 1999.
- [300] A. M. Kleschevnikov *et al.*, "Deficits in Cognition and Synaptic Plasticity in a Mouse Model of Down Syndrome Ameliorated by GABAB Receptor Antagonists," *J. Neurosci.*, vol. 32, no. 27, pp. 9217–9227, Jul. 2012.
- [301] F. Fernandez *et al.*, "Pharmacotherapy for cognitive impairment in a mouse model of Down syndrome," *Nat. Neurosci.*, vol. 10, no. 4, pp. 411–3, Feb. 2007.
- [302] M. E. Blue, S. Naidu, and M. V. Johnston, "Altered Development of Glutamate and GABA Receptors in the Basal Ganglia of Girls with Rett Syndrome," *Exp. Neurol.*, vol. 156, no. 2, pp. 345–352, Apr. 1999.
- [303] R. A. Rissman and W. C. Mobley, "Implications for treatment: GABAA receptors in aging, Down syndrome and Alzheimer's disease," *J. Neurochem.*, vol. 117, no. 4, p. no-no, Mar. 2011.
- [304] G. Lonetti, A. Angelucci, L. Morando, E. M. Boggio, M. Giustetto, and T. Pizzorusso, "Early Environmental Enrichment Moderates the Behavioral and Synaptic Phenotype of MeCP2 Null Mice," *Biol. Psychiatry*, vol. 67, no. 7, pp. 657–665, Apr. 2010.
- [305] T. Begenisic, G. Sansevero, L. Baroncelli, G. Cioni, and A. Sale, "Early environmental

- therapy rescues brain development in a mouse model of Down syndrome," *Neurobiol. Dis.*, vol. 82, pp. 409–419, Oct. 2015.
- [306] J. Nithianantharajah and A. J. Hannan, "Enriched environments, experience-dependent plasticity and disorders of the nervous system," *Nat. Rev. Neurosci.*, vol. 7, no. 9, pp. 697–709, Sep. 2006.
- [307] N. Berardi, C. Braschi, S. Capsoni, A. Cattaneo, and L. Maffei, "Environmental enrichment delays the onset of memory deficits and reduces neuropathological hallmarks in a mouse model of Alzheimer-like neurodegeneration.," *J. Alzheimers. Dis.*, vol. 11, no. 3, pp. 359–70, Jun. 2007.
- [308] W. Comeau, R. Gibb, E. Hastings, J. Cioe, and B. Kolb, "Therapeutic effects of complex rearing or bFGF after perinatal frontal lesions," *Dev. Psychobiol.*, vol. 50, no. 2, pp. 134–146, Mar. 2008.
- [309] G. C. Koopmans *et al.*, "Circulating insulin-like growth factor I and functional recovery from spinal cord injury under enriched housing conditions," *Eur. J. Neurosci.*, vol. 23, no. 4, pp. 1035–1046, Feb. 2006.
- [310] B. B. Johansson, "Functional outcome in rats transferred to an enriched environment 15 days after focal brain ischemia.," *Stroke*, vol. 27, no. 2, pp. 324–6, Feb. 1996.
- [311] L. Cancedda, E. Putignano, A. Sale, A. Viegi, N. Berardi, and L. Maffei, "Acceleration of Visual System Development by Environmental Enrichment," *J. Neurosci.*, vol. 24, no. 20, pp. 4840–4848, May 2004.
- [312] S. Landi, A. Sale, N. Berardi, A. Viegi, L. Maffei, and M. C. Cenni, "Retinal functional development is sensitive to environmental enrichment: a role for BDNF.," *FASEB J.*, vol. 21, no. 1, pp. 130–9, Jan. 2007.
- [313] A. Sale *et al.*, "Enriched environment and acceleration of visual system

- development.," *Neuropharmacology*, vol. 47, no. 5, pp. 649–60, Oct. 2004.
- [314] F. Ciucci, E. Putignano, L. Baroncelli, S. Landi, N. Berardi, and L. Maffei, "Insulin-Like Growth Factor 1 (IGF-1) Mediates the Effects of Enriched Environment (EE) on Visual Cortical Development," *PLoS One*, vol. 2, no. 5, p. e475, May 2007.
- [315] A. Pignataro, A. Borreca, M. Ammassari-Teule, and S. Middei, "CREB Regulates Experience-Dependent Spine Formation and Enlargement in Mouse Barrel Cortex.," *Neural Plast.*, vol. 2015, p. 651469, 2015.
- [316] K. Fox, "Experience-dependent plasticity mechanisms for neural rehabilitation in somatosensory cortex.," *Philos. Trans. R. Soc. Lond. B. Biol. Sci.*, vol. 364, no. 1515, pp. 369–81, Feb. 2009.
- [317] A. Bartoletti, P. Medini, N. Berardi, and L. Maffei, "Environmental enrichment prevents effects of dark-rearing in the rat visual cortex," *Nat. Neurosci.*, vol. 7, no. 3, pp. 215–216, Mar. 2004.
- [318] S. Landi, M. C. Cenni, L. Maffei, and N. Berardi, "Environmental Enrichment Effects on Development of Retinal Ganglion Cell Dendritic Stratification Require Retinal BDNF," *PLoS One*, vol. 2, no. 4, p. e346, Apr. 2007.
- [319] A. Guzzetta *et al.*, "Massage Accelerates Brain Development and the Maturation of Visual Function," *J. Neurosci.*, vol. 29, no. 18, pp. 6042–6051, May 2009.
- [320] M. J. Meaney and M. Szyf, "Maternal care as a model for experience-dependent chromatin plasticity?," *Trends Neurosci.*, vol. 28, no. 9, pp. 456–463, Sep. 2005.
- [321] I. C. G. Weaver, "Epigenetic programming by maternal behavior and pharmacological intervention - Nature versus nurture: Let's call the whole thing off," *Epigenetics*, vol. 2, no. 1, pp. 22–28, 2007.
- [322] D. Silingardi, M. Scali, G. Belluomini, and T. Pizzorusso, "Epigenetic treatments of adult

- rats promote recovery from visual acuity deficits induced by long-term monocular deprivation," *Eur. J. Neurosci.*, vol. 31, no. 12, pp. 2185–2192, Jun. 2010.
- [323] P. Tognini, E. Putignano, A. Coatti, and T. Pizzorusso, "Experience-dependent expression of miR-132 regulates ocular dominance plasticity," *Nat. Neurosci.*, vol. 14, no. 10, pp. 1237–1239, 2011.
- [324] L. Baroncelli, C. Braschi, and L. Maffei, "Visual depth perception in normal and deprived rats: Effects of environmental enrichment," *Neuroscience*, vol. 236, pp. 313–319, Apr. 2013.
- [325] L. Baroncelli, C. Braschi, M. Spolidoro, T. Begenisic, L. Maffei, and A. Sale, "Brain Plasticity and Disease: A Matter of Inhibition," *Neural Plast.*, vol. 2011, pp. 1–11, 2011.
- [326] P. Tognini, I. Manno, J. Bonaccorsi, M. C. Cenni, A. Sale, and L. Maffei, "Environmental Enrichment Promotes Plasticity and Visual Acuity Recovery in Adult Monocular Amblyopic Rats," *PLoS One*, vol. 7, no. 4, p. e34815, Apr. 2012.
- [327] E. P. Vereecken and P. Brabant, "Prognosis for vision in amblyopia after the loss of the good eye.," *Arch. Ophthalmol. (Chicago, Ill. 1960)*, vol. 102, no. 2, pp. 220–4, Feb. 1984.
- [328] M. E. Wilson, "Adult amblyopia reversed by contralateral cataract formation.," *J. Pediatr. Ophthalmol. Strabismus*, vol. 29, no. 2, pp. 100–2.
- [329] L. Baroncelli *et al.*, "Enriched experience and recovery from amblyopia in adult rats: impact of motor, social and sensory components.," *Neuropharmacology*, vol. 62, no. 7, pp. 2388–97, Jun. 2012.
- [330] A. T. Astle, B. S. Webb, and P. V McGraw, "Can perceptual learning be used to treat amblyopia beyond the critical period of visual development?," *Ophthalmic Physiol. Opt.*, vol. 31, no. 6, pp. 564–573, Nov. 2011.

- [331] C. S. Green and D. Bavelier, "Learning, attentional control, and action video games.," *Curr. Biol.*, vol. 22, no. 6, pp. R197-206, Mar. 2012.
- [332] D. M. Levi and R. W. Li, "Improving the performance of the amblyopic visual system.," *Philos. Trans. R. Soc. Lond. B. Biol. Sci.*, vol. 364, no. 1515, pp. 399–407, Feb. 2009.
- [333] R. W. Li, C. Ngo, J. Nguyen, and D. M. Levi, "Video-Game Play Induces Plasticity in the Visual System of Adults with Amblyopia," *PLoS Biol.*, vol. 9, no. 8, p. e1001135, Aug. 2011.
- [334] A. Sale *et al.*, "Visual perceptual learning induces long-term potentiation in the visual cortex," *Neuroscience*, vol. 172, pp. 219–225, Jan. 2011.
- [335] M. S. Rioult-Pedotti, D. Friedman, and J. P. Donoghue, "Learning-induced LTP in neocortex.," *Science*, vol. 290, no. 5491, pp. 533–6, Oct. 2000.
- [336] M. T. Rogan, U. V. Stäubli, and J. E. LeDoux, "Fear conditioning induces associative long-term potentiation in the amygdala.," *Nature*, vol. 390, no. 6660, pp. 604–607, Dec. 1997.
- [337] J. R. Whitlock, A. J. Heynen, M. G. Shuler, and M. F. Bear, "Learning Induces Long-Term Potentiation in the Hippocampus," *Science (80-. )*, vol. 313, no. 5790, pp. 1093–1097, Aug. 2006.
- [338] S. F. Cooke and M. F. Bear, "Visual Experience Induces Long-Term Potentiation in the Primary Visual Cortex," *J. Neurosci.*, vol. 30, no. 48, pp. 16304–16313, Dec. 2010.
- [339] U. Polat, Y. Bonnef, T. Ma-Naim, M. Belkin, and D. Sagi, "Spatial interactions in amblyopia: Effects of stimulus parameters and amblyopia type," *Vision Res.*, vol. 45, no. 11, pp. 1471–1479, May 2005.
- [340] D. M. Levi, S. Hariharan, and S. A. Klein, "Suppressive and facilitatory spatial interactions in amblyopic vision.," *Vision Res.*, vol. 42, no. 11, pp. 1379–94, May 2002.

- [341] B. Thompson, B. Mansouri, L. Koski, and R. F. Hess, "Brain Plasticity in the Adult: Modulation of Function in Amblyopia with rTMS," *Curr. Biol.*, vol. 18, no. 14, pp. 1067–1071, Jul. 2008.
- [342] A. H. Nagahara and M. H. Tuszynski, "Potential therapeutic uses of BDNF in neurological and psychiatric disorders," *Nat. Rev. Drug Discov.*, vol. 10, no. 3, pp. 209–219, Mar. 2011.
- [343] G. T. Prusky, P. W. . West, and R. M. Douglas, "Behavioral assessment of visual acuity in mice and rats," *Vision Res.*, vol. 40, no. 16, pp. 2201–2209, 2000.
- [344] B.-S. Wang, L. Feng, M. Liu, X. Liu, and J. Cang, "Environmental enrichment rescues binocular matching of orientation preference in mice that have a precocious critical period.," *Neuron*, vol. 80, no. 1, pp. 198–209, Oct. 2013.
- [345] L. Cancedda, E. Putignano, A. Sale, A. Viegi, N. Berardi, and L. Maffei, "Acceleration of visual system development by environmental enrichment.," *J. Neurosci.*, vol. 24, no. 20, pp. 4840–8, May 2004.
- [346] M. Metsis, T. Timmusk, E. Arenas, and H. Persson, "Differential usage of multiple brain-derived neurotrophic factor promoters in the rat brain following neuronal activation.," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 90, no. 19, pp. 8802–6, Oct. 1993.
- [347] J.-S. Guan *et al.*, "HDAC2 negatively regulates memory formation and synaptic plasticity.," *Nature*, vol. 459, no. 7243, pp. 55–60, May 2009.
- [348] M. Kilgore *et al.*, "Inhibitors of class 1 histone deacetylases reverse contextual memory deficits in a mouse model of Alzheimer's disease.," *Neuropsychopharmacology*, vol. 35, no. 4, pp. 870–80, Mar. 2010.
- [349] H. Scharfman, "Does BDNF Contribute to Temporal Lobe Epilepsy?," *Epilepsy Curr.*, vol. 2, no. 3, pp. 92–94, May 2002.



- [350] D. K. Binder, S. D. Croll, C. M. Gall, and H. E. Scharfman, "BDNF and epilepsy: too much of a good thing?," *Trends Neurosci.*, vol. 24, no. 1, pp. 47–53, Jan. 2001.
- [351] Y. Bozzi, D. Vallone, and E. Borrelli, "Neuroprotective role of dopamine against hippocampal cell death.," *J. Neurosci.*, vol. 20, no. 22, pp. 8643–9, Nov. 2000.
- [352] T. Pizzorusso, P. Medini, S. Landi, S. Baldini, N. Berardi, and L. Maffei, "Structural and functional recovery from early monocular deprivation in adult rats," *Proc. Natl. Acad. Sci.*, vol. 103, no. 22, pp. 8517–8522, May 2006.
- [353] W. A. Gottschalk, H. Jiang, N. Tartaglia, L. Feng, A. Figurov, and B. Lu, "Signaling mechanisms mediating BDNF modulation of synaptic plasticity in the hippocampus.," *Learn. Mem.*, vol. 6, no. 3, pp. 243–56, 1999.
- [354] B.-S. Wang, L. Feng, M. Liu, X. Liu, and J. Cang, "Environmental Enrichment Rescues Binocular Matching of Orientation Preference in Mice that Have a Precocious Critical Period," *Neuron*, vol. 80, no. 1, pp. 198–209, Oct. 2013.
- [355] A. Sale, N. Berardi, and L. Maffei, "Enrich the environment to empower the brain," no. March, pp. 233–239, 2009.
- [356] M. Fuchikami, S. Yamamoto, S. Morinobu, S. Takei, and S. Yamawaki, "Epigenetic Regulation of *BDNF* Gene in Response to Stress," *Psychiatry Investig.*, vol. 7, no. 4, p. 251, Dec. 2010.
- [357] K. A. Intlekofer and C. W. Cotman, "Exercise counteracts declining hippocampal function in aging and Alzheimer's disease," *Neurobiol. Dis.*, vol. 57, pp. 47–55, Sep. 2013.
- [358] I. C. G. Weaver *et al.*, "Epigenetic programming by maternal behavior," *Nat. Neurosci.*, vol. 7, no. 8, pp. 847–854, Aug. 2004.
- [359] L. Baroncelli *et al.*, "Enriched experience and recovery from amblyopia in adult rats:

- Impact of motor, social and sensory components," *Neuropharmacology*, vol. 62, no. 7, pp. 2387–2396, 2012.
- [360] M. Mainardi *et al.*, "A sensitive period for environmental regulation of eating behavior and leptin sensitivity," *Proc. Natl. Acad. Sci.*, vol. 107, no. 38, pp. 16673–16678, Sep. 2010.
- [361] F. Greifzu *et al.*, "Environmental enrichment extends ocular dominance plasticity into adulthood and protects from stroke-induced impairments of plasticity.," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 111, no. 3, pp. 1150–5, Jan. 2014.
- [362] Z. J. Huang *et al.*, "BDNF regulates the maturation of inhibition and the critical period of plasticity in mouse visual cortex.," *Cell*, vol. 98, no. 6, pp. 739–55, Sep. 1999.
- [363] T. Tanaka, H. Saito, and N. Matsuki, "Inhibition of GABA<sub>A</sub> synaptic responses by brain-derived neurotrophic factor (BDNF) in rat hippocampus.," *J. Neurosci.*, vol. 17, no. 9, pp. 2959–66, May 1997.
- [364] M. Frerking, R. C. Malenka, and R. A. Nicoll, "Brain-derived neurotrophic factor (BDNF) modulates inhibitory, but not excitatory, transmission in the CA1 region of the hippocampus.," *J. Neurophysiol.*, vol. 80, no. 6, pp. 3383–6, Dec. 1998.
- [365] C. Henneberger, R. Jüttner, T. Rothe, and R. Grantyn, "Postsynaptic action of BDNF on GABAergic synaptic transmission in the superficial layers of the mouse superior colliculus.," *J. Neurophysiol.*, vol. 88, no. 2, pp. 595–603, Aug. 2002.
- [366] I. Brünig, S. Penschuck, B. Berninger, J. Benson, and J. M. Fritschy, "BDNF reduces miniature inhibitory postsynaptic currents by rapid downregulation of GABA(A) receptor surface expression.," *Eur. J. Neurosci.*, vol. 13, no. 7, pp. 1320–8, Apr. 2001.
- [367] Y. Mizoguchi, H. Ishibashi, and J. Nabekura, "The action of BDNF on GABA(A) currents changes from potentiating to suppressing during maturation of rat hippocampal CA1

- pyramidal neurons.," *J. Physiol.*, vol. 548, no. Pt 3, pp. 703–9, May 2003.
- [368] J. N. Jovanovic, P. Thomas, J. T. Kittler, T. G. Smart, and S. J. Moss, "Brain-Derived Neurotrophic Factor Modulates Fast Synaptic Inhibition by Regulating GABAA Receptor Phosphorylation, Activity, and Cell-Surface Stability," *J. Neurosci.*, vol. 24, no. 2, pp. 522–530, Jan. 2004.
- [369] C. M. Niell and M. P. Stryker, "Modulation of Visual Responses by Behavioral State in Mouse Visual Cortex," *Neuron*, vol. 65, no. 4, pp. 472–479, 2010.
- [370] J. F. Maya-Vetencourt *et al.*, "IGF-1 Restores Visual Cortex Plasticity in Adult Life by Reducing Local GABA Levels," *Neural Plast.*, vol. 2012, pp. 1–10, 2012.
- [371] A. Sale, N. Berardi, and L. Maffei, "Environment and Brain Plasticity: Towards an Endogenous Pharmacotherapy," *Physiol. Rev.*, vol. 94, no. 1, pp. 189–234, Jan. 2014.
- [372] J. F. M. Vetencourt *et al.*, "The Antidepressant Fluoxetine Restores Plasticity in the Adult Visual Cortex," *Science (80-. )*, vol. 320, no. 5874, pp. 385–388, Apr. 2008.
- [373] M. Spolidoro, L. Baroncelli, E. Putignano, J. F. Maya-Vetencourt, A. Viegi, and L. Maffei, "Food restriction enhances visual cortex plasticity in adulthood," *Nat. Commun.*, vol. 2, p. 320, May 2011.
- [374] R. F. Hess and B. Thompson, "Amblyopia and the binocular approach to its therapy," *Vision Res.*, vol. 114, pp. 4–16, Sep. 2015.
- [375] A. L. Webber and J. Wood, "Amblyopia: prevalence, natural history, functional effects and treatment.," *Clin. Exp. Optom.*, vol. 88, no. 6, pp. 365–75, Nov. 2005.
- [376] D. E. Mitchell, K. MacNeill, N. A. Crowder, K. Holman, and K. R. Duffy, "Recovery of visual functions in amblyopic animals following brief exposure to total darkness.," *J. Physiol.*, vol. 594, no. 1, pp. 149–67, Jan. 2016.
- [377] J. P. Gavornik and M. F. Bear, "Learned spatiotemporal sequence recognition and

- prediction in primary visual cortex," *Nat. Neurosci.*, vol. 17, no. 5, pp. 732–737, Mar. 2014.
- [378] P. Sengupta, "The Laboratory Rat: Relating Its Age With Human's," *Int. J. Prev. Med.*, vol. 4, no. 6, pp. 624–30, Jun. 2013.
- [379] F. Malerba, F. Paoletti, S. Capsoni, and A. Cattaneo, "Intranasal delivery of therapeutic proteins for neurological diseases," *Expert Opin. Drug Deliv.*, vol. 8, no. 10, pp. 1277–1296, Oct. 2011.
- [380] M. V. Chao, "Neurotrophins and their receptors: A convergence point for many signalling pathways," *Nat. Rev. Neurosci.*, vol. 4, no. 4, pp. 299–309, Apr. 2003.
- [381] G. M. Ratto and T. Pizzorusso, "A Kinase with a Vision," in *Brain Repair*, vol. 557, Boston, MA: Springer US, 2006, pp. 122–132.
- [382] L. F. Reichardt, "Neurotrophin-regulated signalling pathways," *Philos. Trans. R. Soc. B Biol. Sci.*, vol. 361, no. 1473, pp. 1545–1564, Sep. 2006.
- [383] J.-M. Revest *et al.*, "BDNF-TrkB signaling through Erk1/2MAPK phosphorylation mediates the enhancement of fear memory induced by glucocorticoids," *Mol. Psychiatry*, vol. 19, no. 9, pp. 1001–1009, Sep. 2014.
- [384] D. Tropea *et al.*, "Gene expression changes and molecular pathways mediating activity-dependent plasticity in visual cortex," *Nat. Neurosci.*, vol. 9, no. 5, pp. 660–668, May 2006.
- [385] M. L. Rietman, J.-P. Sommeijer, N.-B. M. P. Neuro-Bsik Mouse Phenomics Consortium, C. N. Levelt, and J. A. Heimel, "Candidate genes in ocular dominance plasticity," *Front. Neurosci.*, vol. 6, p. 11, 2012.
- [386] G. D. Cristo *et al.*, "Requirement of ERK Activation for Visual Cortical Plasticity," *Science (80-. )*, vol. 292, no. 5525, pp. 2337–2340, Jun. 2001.

