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THE ANTIDEPRESSANT FLUOXETINE RESTORES PLASTICITY IN THE ADULT VISUAL CORTEX

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INTRODUCTION

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Depression and amine neurotransmitters

Mood disorders are among the most prevalent forms of mental illness. These are afflictions whereby the prevailing emotional mood is distorted or inappropriate to the circumstances, with a lifetime incidence of 10–25% in women and 5–12% in men (Blazer, 2000). Such pathologies are recurrent, life threatening due to the risk of suicide, and a major cause of morbidity worldwide. The most severe of these afflictions are major depression and manic depression; the latter also called "bipolar disorder" because such patients experience alternating episodes of depression and euphoria (Miklowitz & Johnson, 2006).

Depression has been described for several millennia, nevertheless, a distinction between a disturbance of cognitive faculties (a thought disorder) and a disturbance of emotions (a mood disorder), was recently introduced during early development of modern classification of mental illnesses in the 20th century. The term melancholia, which means "black bile" in greek, was first used by Hippocrates around 400 B.C. to refer to an alteration of mood, which was thought to depend on the balance of four humors, blood, phlegm, yellow and black bile. An excess of black bile was believed to cause depression (see Kandel et al., 2000). Most symptoms of this pathology were recognized in ancient times, as were the contributions of innate predispositions and external factors in causing the illness. Clinically, depression is defined by a set of standard criteria which include: depressed mood, low self esteem, feelings of hopelessness, worthlessness and guilt, decreased ability to concentrate and think, altered appetite, insomnia or hypersomnia, low energy, increased agitation, decreased interest in pleasurable stimuli, e.g., sex, food, social interactions, and recurrent thoughts of death and suicide. A diagnosis of depression is made when a certain number of the above symptoms are reported for longer than a 2 week period of time, and when symptoms disrupt normal social and occupational functioning (reviewed in Nestler et al., 2002).

There are four effective treatments for depression and bipolar disorders: electroconvulsive therapy (ECT), antidepressant drugs, lithium, and anticonvulsants. Of all the treatments, ECT has been used for the longest period of time, over 50 years. Although the therapeutic mechanism of ECT is not fully understood, it is thought to be related to alterations in the sensitivity of aminergic receptors (Nestler, 1998). Amine transmitters regulate many brain functions and are also active in the peripheral nervous system. Because biogenic amines are implicated in a wide range of behaviors, ranging from central homeostatic functions to cognitive phenomena such as attention, it is not surprising that defects in biogenic amines function are implicated in most psychiatric disorders. There are five well-established amine neurotransmitters, the three catecholamines: dopamine, norepinephrine (noradrenaline), and epinephrine (adrenaline), as well as histamine and serotonin (Figure 1).

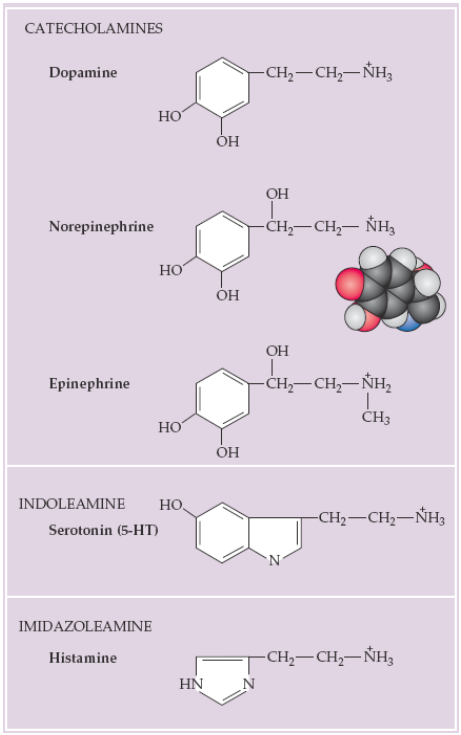


Figure 1. Amine neurotransmitters. The catecholamines, so named because they all share the catechol moiety (i.e., a hydroxylated benzene ring), make up a distinctive subgroup within the biogenic amines. Serotonin and histamine contain an indole ring and an imidazole ring, respectively. Insert, space-filling model for norepinephrine. Taken from Purves et al., 2004.

All catecholamines are derived from a common precursor, the amino acid tyrosine. Dopamine is synthesized in the brain stem and hypothalamus. Dopaminergic neurons in the substantia nigra and ventral tegmental area provide a major ascending pathway that terminates in the striatum, the prefrontal and temporal cortex as well as the limbic system. Hypothalamic dopaminergic neurons instead, provide descending pathways to autonomic areas of the brain stem and the spinal cord (see Kandel et al., 2000). Dopamine is believed to be involved in motivation, reward and reinforcement, and plays also a poorly understood role in some sympathetic ganglia (Feldman et al., 1997).

Norepinephrine is used as a neurotransmitter in the locus coeruleus, a brainstem nucleus that projects diffusely to a variety of forebrain targets and influences sleep and wakefulness, attention and feeding behavior (reviewed in Berridge & Waterhouse, 2003). The most prominent noradrenergic neurons are sympathetic ganglion cells, which employ norepinephrine as the major peripheral transmitter. Epinephrine-containing neurons in the central nervous system, instead, are primarily in the lateral tegmental system and in the medulla and project to the hypothalamus and thalamus (Feldman et al., 1997). Serotonin (5-HT), in contrast, is synthesized from the amino acid tryptophan and is found in groups of neurons in the raphe regions of the pons and upper brain stem, which have widespread projections to the forebrain with an important role in sleep, wakefulness and behavioral arousal (Abrams et al., 2004).

Antidepressant drugs (ADs). Historical perspective.

The functionality of amine synapses is critically important in psychopharmacology, with drugs affecting the synthesis, receptor binding, or catabolism of these neurotransmitters being among the most important agents of the modern pharmacology. Following their synthesis in the cytoplasm of presynaptic terminals, all the amine transmitters are packaged in synaptic vesicles and released into the synaptic cleft by means of exocytosis when the neurons fires an action potential. The neurotransmitter then interacts with pre- and post-synaptic receptors, and such activity is limited by active reuptake of the released molecule into presynaptic terminals as well as into glial cells (Feldman et al., 1997) (Figure 2). Inside the presynaptic terminals the

transmitters are packaged again into vesicles or catabolized primarily by the mitochondrial enzymes monoamine oxidase (MAO).

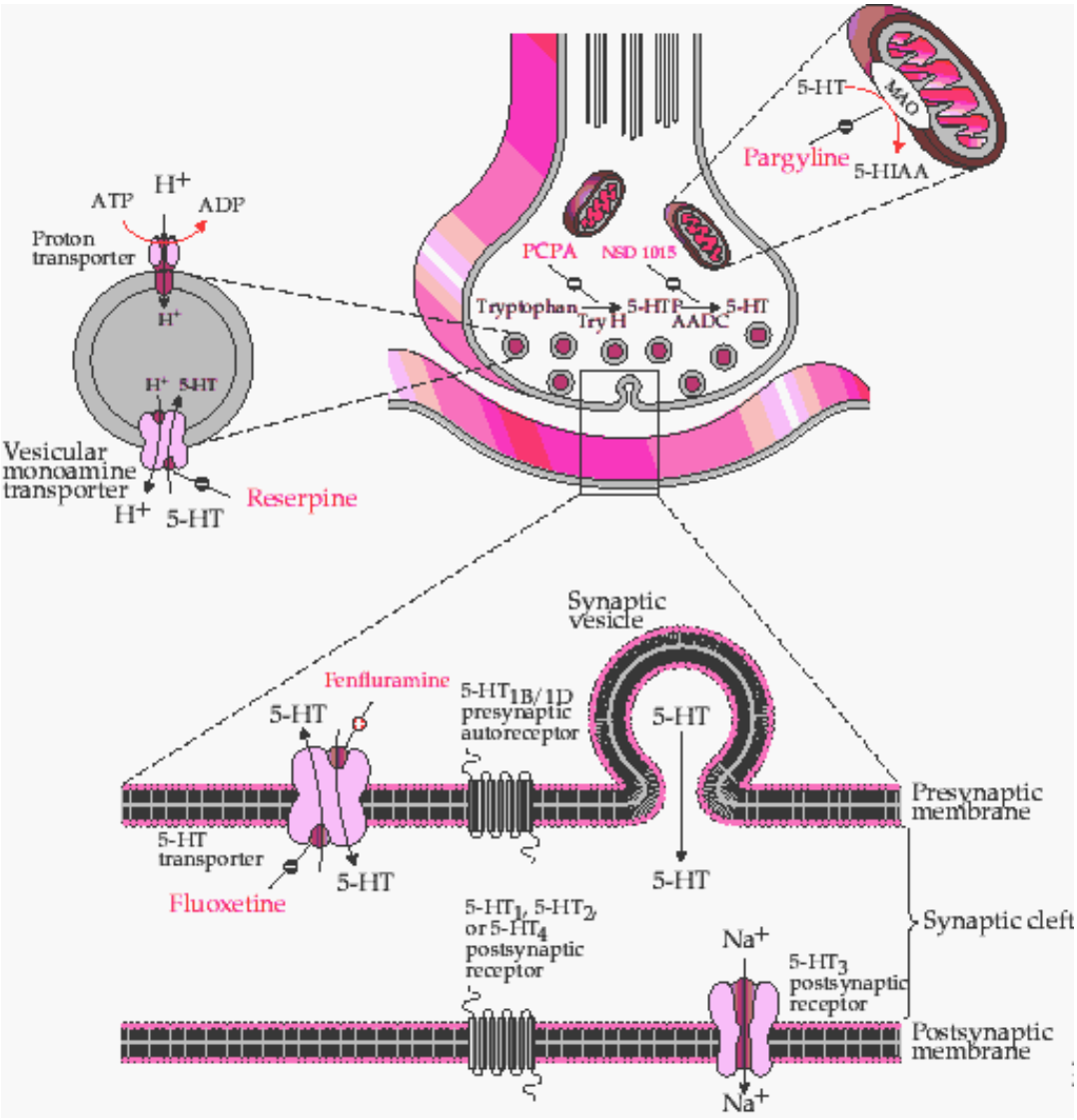


Figure 2. Serotonin synapse. Schematic diagram illustrating the synthesis, metabolism, neurotransmitter release and pre-synaptic reuptake. Pre and postsynaptic receptors and sites of action of some antidepressant drugs are shown. Note that fluoxetine selectively inhibits the presynaptic reuptake of 5-HT, which enhances the postsynaptic serotonergic transmission. Taken from Feldman et al., 1997.

Drugs effective in treating depression act primarily on the serotonergic and noradrenergic systems of the brain (reviewed in Wong et al., 1995). The first drug used to ameliorate mood disorders, in particular schizophrenia, was reserpine. It was developed in the 1950s and initially used as treatment for hypertension because it blocks the noradrenergic transmission causing a reduction in the ability of the sympathetic division of the visceral motor system to cause constriction of blood vessels (Purves et al., 2004). Soon after, it was clear that a major side effect in hypertensive patients treated with reserpine was depression. It was then found that reserpine decreases serotonin and noradrenaline levels by inhibiting the uptake of neurotransmitters into synaptic vesicles, in the presynaptic terminal (see Figure 2), thereby keeping the transmitter into the cytoplasm where it undergoes degradation by the mitochondrial enzymes monoamine oxidase (Pletscher et al., 1956). This finding was of importance because it suggested the possibility that alterations in monoaminergic transmission might be involved in disorders of mood.

The monoamine hypothesis of depression, as first described in the 1960s, stated that depression was caused by a deficiency in the serotonergic and noradrenergic systems at functionally important receptor sites in the brain (Bunney et al., 1965; Schildkraut, 1965; Coppen, 1967). Indeed, the first drugs identified as antidepressants were discovered by chance when medications developed for other illnesses were found to elevate mood of psychiatric patients. Particularly, isoniazid, a drug initially used as treatment of tuberculosis, was found to ameliorate mood of patients receiving it. Later studies performed in depressed patients, who did not have tuberculosis, demonstrated an antidepressant effect of the drug. Isoniazid increases the concentration of serotonin and noradrenaline by interfering with the degradation of these neurotransmitters by the enzyme MAO, thus increasing its total content in the axonic terminals of neurons (see Figure 2). Further support to this notion came with the discovery that imipramine, an experimental antihistamine with a tricyclic structure, induced antidepressant effects. Imipramine blocks the reuptake of both serotonin and noradrenaline to the presynaptic nerve endings, thereby prolonging the action of these transmitters in the synapse (reviewed in Castren, 2005). These findings revolutionized the recognition and treatment of mood disorders shedding light into some of the molecular mechanisms involved in

depression. Because it became clear that both isoniazid and imipramine increase the extracellular levels of two important neurotransmitters in the brain: serotonin and noradrenaline; an increase in the total concentration of these neurotransmitters after treatment with these two drugs was the starting point for the proposal of the monoamine hypothesis of depression.

Neuronal circuitries affected in depression

Abnormalities in many brain areas are thought to mediate the diverse symptoms of depression. This notion is supported by human brain imaging studies which have demonstrated changes in blood flow in several brain regions, including areas of prefrontal and cingulate cortex, hippocampus, striatum, amygdala and thalamus. Similarly, anatomic studies of brains of depressed patients obtained at autopsy report abnormalities in many of these same brain regions (for review see Nestler et al., 2002). Knowledge of the function of such brain areas under normal conditions suggest the aspects of depression to which they may contribute. Neocortex and hippocampus may mediate cognitive aspects of depression, such as memory impairments and feeling of worthlessness, hopelessness, guilt and recurrent thoughts of suicide. The ventral striatum or nucleus accumbens, and the amygdale, are important in emotional memory and could as a result mediate the decreased drive and reward for pleasurable activities, anxiety, and reduced motivation that predominate in many patients (reviewed in Nestler et al., 2006).

A prominent mechanism by which the brain reacts to acute and chronic stress is the activation of the hypothalamic-pituitary-adrenal (HPA) axis. Under such conditions, neurons in the paraventricular nucleus (PVN) of the hypothalamus secrete corticotrophin releasing factor (CRF), which stimulates the synthesis and release of adrenocorticotropin (ACTH) from the pituitary gland. ACTH then stimulates the synthesis and release of glucocorticoids (cortisol in humans, corticosterone in rodents) from the adrenal cortex. Glucocorticoids exert profound effects on general metabolism and dramatically affects behavior via direct actions on numerous brain regions (reviewed by Nestler et al., 2002). The activity of the HPA axis is controlled by different brain pathways, including an inhibitory action exerted by the hippocampus and an excitatory action mediated by the

amygdala. Levels of glucocorticoids that are seen under normal physiological conditions seem to enhance hippocampal inhibition of HPA activity. They may also enhance general hippocampal functions and promote certain cognitive abilities. However, sustained elevations of glucocorticoids seen under conditions of stress or depression may damage hippocampal neurons particularly CA3 pyramidal cells (Sapolsky, 2000). Stress and the resulting increase in cortisol also reduce neurogenesis in the adult hippocampal dentate gyrus (Fuchs & Gould, 2000), which in turn reduces the inhibitory hippocampal action on the HPA axis; further increasing circulating glucocorticoid levels and subsequent hippocampal damage. Such positive feedback process with pathological consequences has been implicated in the onset of depression. Abnormal, excessive activation of the HPA axis is observed in approximately 50% of depressed patients and these abnormalities are corrected by antidepressant treatment (Arborelius et al., 1999; Holsboer, 2001).

Hypothesis for the etiology of depression

The pathologic effects of stress on hippocampus have contributed to a hypothesis for depression that proposes a role for neurotrophic factors in the etiology of this pathology and its treatment (Duman et al., 1997). Neurotrophic factors were first characterized by regulating neuronal growth and differentiation during development, but it has become clear that they are also potent regulators of plasticity and survival of adult neurons. The neurotrophic hypothesis of depression states that a deficiency in neurotrophic support may contribute to hippocampal pathology during the development of the illness, and that reversal of this deficiency induced by antidepressant treatments ameliorate the symptoms. Work in this hypothesis has focused on the brain derived neurotrophic factor (BDNF) (Reviewed in D'Sa & Duman, 2002), one of the most prevalent neurotrophins in the adult brain. Acute and chronic stress decrease levels of BDNF expression in the dentate gyrus and pyramidal cell layer of hippocampus in rodents. This reduction appears to be mediated partly via stress-induced glucocorticoids and partly by stress-induced decrease in serotonergic transmission (Smith et al., 1995). Conversely, chronic administration of virtually all classes of antidepressant treatments

increases BDNF expression in these regions (Nibuya et al., 1995), and prevent the stress-induced decrease in the neurotrophin levels. These findings raise the possibility that antidepressant induced upregulation of BDNF could repair some stress-induced damage to the hippocampus and protect vulnerable neurons from further damage. Furthermore, an enhancement of long-term potentiation and other forms of synaptic plasticity induced by BDNF in the hippocampus has been reported (Korte et al., 1996; Kang et al., 1997), which suggests that BDNF induced by antidepressants may promote hippocampal function. Importantly, these findings could also explain why the clinical antidepressant response show a time delay of several weeks: it would require sufficient time for levels of BDNF to gradually raise and exert its neurotrophic effects.

The neurotrophic hypothesis predicts that agents that promote BDNF expression and signaling might be clinically effective antidepressants, so different approaches focusing on understanding BDNF gene expression following antidepressant treatment have been developed. There is evidence for the transcription factor cAMP-response-element binding protein (CREB) regulating BDNF expression. For instance, BDNF expression is induced *in vitro* and *in vivo* by CREB (Tao et al., 1998; Conti et al., 2002) and all major classes of antidepressants increase levels of CREB expression and function in several brain regions including the hippocampus (Nibuya et al., 1996; Thome et al., 2000). An increase in CREB activity by microinjections of a viral vector encoding CREB into the hippocampal dentate gyrus exerts antidepressant-like effects, as evidenced in the forced swim test and learned helplessness test (Chen et al., 2001). In contrast, levels of CREB are reduced in the temporal cortex of depressed patients (Dowlatshahi et al., 1998). While these effects could be mediated by numerous target genes under control of CREB, it does illustrate novel strategies by which influencing hippocampal function in the context of depression, and makes likely a role for BDNF on it. Indeed, it has been demonstrated that single bilateral infusions of BDNF into the dentate gyrus of hippocampus or the overexpression of its primary receptor TrkB produce an antidepressant effect in behavioral models of depression (Shirayama et al., 2002; Koponen et al., 2005).

Importantly, experimental evidence arguing against the possibility that an impairment of BDNF-TrkB signaling may underlie the pathophysiology of depression

has been recently found. Mice lacking the tyrosine kinase receptor TrkB in the forebrain and transgenic mice overexpressing the dominant-negative T1 form of TrkB, for instance, show no depressive behavior (Zorner et al., 2003; Saarelainen et al., 2003). In contrast, in both BDNF knockouts and TrkB.T1 transgenic mice antidepressant treatment no longer induces a reduction of immobility in behavioral models of depression (Saarelainen et al., 2003), indicating that BDNF-TrkB signaling is necessary to mediate the behavioral response induced by antidepressants but may not be involved in the etiology of depression (reviewed in Martinowich et al., 2007).

Most of the clinical studies have focused on the hippocampus as the site involved in the generation and treatment of depression (Reviewed by Campbell & Macqueen, 2004). However, while the hippocampus is undoubtedly involved in the illness, it is unlikely that it accounts completely for these phenomena. The pathology in the hippocampus explains symptoms like alterations in learning and memory seen in depression but it does not represent the wide spectrum of symptoms. Brain imaging and autopsy studies have provided evidence for abnormalities in several brain areas of depressed individuals well beyond the hippocampus. The role of subcortical structures like the hypothalamus and amygdala in the regulation of motivation, sleep, appetite, energy levels, circadian rhythms, and responses to pleasurable stimuli, prominently affected in depressed patients, has also been recognized (reviewed in Nestler et al., 2002). Given the pervasive symptoms of depression, it is likely that the pathophysiology of this disorder and the mechanisms by which currently available treatments reverse symptoms, involve diverse neural circuits in numerous brain regions.

Recent experimental findings support an alternative hypothesis for depression which proposes a role for neurogenesis in its etiology. Magnetic resonance imaging studies have shown a reduction of hippocampal volume in depressed patients, event that has been correlated to a reduction of the number of hippocampal neurons (Videbech & Ravnkilde, 2004; Campbell et al., 2004). In addition, all clinically effective antidepressant drugs increase neurogenesis in the adult hippocampus (Malberg et al., 2000). Even though these findings indicate adult hippocampal neurogenesis as a candidate mechanism for the etiology of depression and as a substrate of antidepressant action, there is evidence arguing against it. For instance, at structural level it is highly

unlikely that changes in adult hippocampal neurogenesis account for the reduction of hippocampal volume observed in depressed patients. Stereological analysis of hippocampal volume in irradiated mice indeed, a strategy that impairs adult hippocampal neurogenesis, revealed no a significant reduction of volume in the hippocampus (Santarelli et al., 2003). In addition the fact that ablation of neurogenesis does not elicit a depression-like behavioral phenotype undermines a role for neurogenesis in the etiology of depression (Airan et al., 2007; Santarelli et al., 2003).

In contrast to the neurotrophic or neurogenic view of depression recent observations support an alternative hypothesis for such neurological disorder, which suggests that an impaired activity-dependent neuronal signaling may underlie this pathology and that antidepressant drugs might exert a therapeutic effect by enhancing information processing in the affected neuronal networks through activity-dependent mechanisms of synaptic plasticity (reviewed by Castren, 2005). This alternative idea, known as the network hypothesis, predicts that depression arise from an impaired synaptic plasticity in the CNS rather than from an alteration in the balance of signaling molecules. Evidence supporting this notion comes from the finding that disruption of adult hippocampal neurogenesis impairs the antidepressant-like behavioral response induced by antidepressants drugs (Santarelli et al., 2003). According to this idea, antidepressants may promote mechanisms of neuronal plasticity through an initial effect on monoamine metabolism, leading to an improved processing of information in neuronal networks involved in mood regulation (reviewed by Castren, 2005). Interestingly, differentiation of newly generated neurons takes several weeks (van Praag et al., 2002) which is a time course that correlates with the delayed onset of the therapeutic effects induced by antidepressant drugs. These findings suggest a role for neuronal plasticity in the action of ADs.

ADs and neuronal plasticity

Despite years of study, the biological basis of depression and the precise mechanisms of antidepressant efficacy remains unclear. Early research on depression focused on changes in neurotransmitter concentrations and its receptors. The results of

these studies, however, were in disagreement with clinical observations of the therapeutic action of ADs. Although the effects of antidepressants on monoamine metabolism occur soon after administration, it typically takes several weeks of continued treatment for the clinical antidepressant response to appear (Nestler et al, 1998). Further research made clear that long-term antidepressant treatment produces adaptive changes in monoamine receptors and intracellular signal transduction pathways associated (Sulser et al., 1978), which centered the attention on the effects of long-term antidepressant treatments on neurotrophic factors and coupled receptors as well as on intracellular signaling molecules (Duman et al., 1997; Manji et al., 2001; Coyle & Duman, 2003).

Increased BDNF expression induced by ADs

Research on the therapeutic effects induced by chronic administration of antidepressants has been developed focusing on the regulation of key signaling pathways involved in cellular survival, neurogenesis and neuronal plasticity in the adult brain. The fact that chronic antidepressant treatment increases the expression of BDNF and its primary receptor TrkB, in the frontal cortex and hippocampus of adult rats, was early demonstrated by Nibuya et al. in 1995 using in situ hybridization and northern blot analysis. Because neurotrophins were known to promote growth and development of immature neurons as well as to enhance the survival and function in neural cells in the adult (for review see Lindvall et al, 1994), a possible role for BDNF in mediating the therapeutic effects induced by antidepressants was suggested. That neurogenesis in the adult rat hippocampus is increased by chronic antidepressant treatment was elegantly shown by Malberg et al. (2000). In this study, different types of ADs were systemically given to adult animals and the acute and chronic effects on hippocampal neurogenesis were assessed by immunohistochemistry using the thymidine analogue bromodeoxyuridine (BrdU) as a marker for dividing cells. The authors found an increased number of BrdU labelled cells in the dentate gyrus of the adult rat hippocampus after chronic antidepressant administration but not after acute treatment. Moreover, an enhanced proliferation of hippocampal cells and differentiation into mature neurons was shown by combining BrdU labelling with specific markers for neuronal and glial cells.

These findings not only suggested a common molecular event induced by different ADs but highlighted a mechanism that is consistent with the time course for the therapeutic actions of antidepressants.

It has also been observed that adult neurogenesis and BDNF-induced TrkB signaling are critical molecular events in mediating the therapeutic effects of ADs. In a very elegant set of experiments Santarelli et al. (2003) showed that the antidepressant-like behavioral response to ADs is blocked if hippocampal neurogenesis is disrupted through a restricted x-irradiation in the mouse brain. These data provided a correlation between behavioral responses induced by antidepressants and the induction of hippocampal neurogenesis and suggest that generation of neural cells in the adult hippocampus may be necessary for the therapeutic action induced by ADs. On the other hand, single bilateral infusion of BDNF into the dentate gyrus of hippocampus was shown to induce an antidepressant effect in two behavioral models of depression: the learn helplessness and forced swim test (Shirayama et al., 2002). Furthermore, the authors evaluated whether a decreased phosphorylation of the primary BDNF receptor TrkB, induced by co-administration of BDNF with a broad spectrum tyrosine kinase inhibitor (K252a) into the hippocampus, impaired the antidepressant-like behavioral response induced by the neurotrophin in the learn helplessness test. K252a cortical administration completely blocked the behavioral effects induced by cortical BDNF administration. Taken together, these data suggest a correlation between BDNF-TrkB signaling and adult hippocampal neurogenesis, which may account for the behavioral effects induced by antidepressants. Experimental evidence in support to this notion comes from the finding that cortical administration of exogenous BDNF in adult animals not only increases CREB phosphorylation, but enhances long-term potentiation (LTP) of neural transmission in the adult hippocampus (Ying et al., 2002).

One important mechanism at the basis of the therapeutic action of ADs is the phosphorylation of the transcription factor cAMP-response element binding protein (CREB), which is known to promote BDNF expression. Direct evidence for the regulation of gene transcription via the cAMP-mediated second messenger pathway implicated in the actions induced by antidepressants was obtained *in vivo* using transgenic mice with a CRE-LacZ reporter gene construct (Thome et al., 2000). In these

mice, stimulation of the CRE site leads to an increase expression of the LacZ gene product, β-galactosidase, making it possible to evaluate the influence of antidepressant administration on CRE-mediated gene transcription. Levels of β-galactosidase assessed by fluorescence immunohistochemistry revealed an increased CRE-mediated gene transcription in limbic structures and cerebral cortex, induced by chronic treatment with three distinct classes of antidepressants. Furthermore, immunohistochemical analysis for p-CREB revealed that chronic antidepressant treatment increased phosphorylation of CREB, result that was consistent with the enhanced CRE-mediated gene expression. These findings, together with those of Nakagawa et al. (2002) in which the phosphorylation of CREB was shown to be required for hippocampal neurogenesis induced by the antidepressant rolipram, indicate a critical role for CREB phosphorylation in mediating the therapeutic effects of antidepressant drugs.

ADs and synaptic plasticity

Recent studies in animal models suggest that antidepressant treatments enhance synaptic connectivity in the brain. Particularly, an increased dendritic spine synapse formation in the adult rat hippocampus and frontal cortex was shown to be induced by chronic administration of the selective serotonin reuptake inhibitor (SSRI) fluoxetine (Hajszan et al., 2005). In depressed patients, brain imaging and neuropathological studies point toward a reduced neuronal activity and synaptic connectivity in the brain as evidenced by a decreased hippocampal and prefrontal cortex volume (reviewed by Castren, 2004) and antidepressant treatment seems to prevent or restore the structural and functional deficits observed in depression (Czeh et al., 2001).

The role of ADs in the remodeling and strengthening of specific neural synapses in the adult brain has also been addressed in rodents. Sairanen et al. (2007) evaluated the effect of chronic antidepressant treatment on the expression of three plasticity associated marker proteins: the polysialylated form of the nerve cell adhesion molecule (PSA-NCAM), the phosphorylated form of CREB (p-CREB) and the growth-associated protein 43 (GAP-43). PSA-NCAM is a cell surface protein involved in the regulation of axon growth (Cremer et al., 1997) and has been associated with the differentiation of newly

generated neurons in the rodent hippocampus (Seki & Arai, 1993), cell migration (Yoshida et al., 1999), synaptogenesis (Dityatev et al., 2004) and long-term potentiation of neural transmission (Muller et al., 1996; Cremer et al., 1998), while the phosphorylated form of CREB is known to be a permissive factor for neuronal plasticity. The GAP-43 protein is critically involved in synaptogenesis, axonal sprouting and the regulation of the cytoskeletal organization in the nerve ending (Benowitz & Routtenberg, 1997). Thus, Sairanen et al. (2007) assessed the expression of these proteins using immunohistochemistry to study mechanisms of synaptic plasticity induced by chronic treatment with the antidepressant imipramine on different areas in the forebrain of adult rats. Increased levels of PSA-NCAM, p-CREB and GAP-43 were found to be induced by chronic but not acute imipramine treatment in the hippocampus, medial prefrontal cortex and piriform cortex of adult animals, indicating an effect of antidepressants on the remodeling of neuronal networks.

Recent experimental evidence also suggests that a disturbance of brain plasticity is involved in animal models of depression and that chronic antidepressant treatment may counteract these alterations. In a set of elegant experiments adult rats were exposed to chronic mild stress conditions for three weeks to examine later long-term synaptic plasticity in the hippocampal CA1 region by whole-cell patch clamp (Holderbach et al., 2007). The authors found that chronic mild stress facilitated long-term depression (LTD) and had no effect on long-term potentiation (LTP), while chronic treatment of the SSRI fluvoxamine during the stress paradigm prevented the induction of LTD and increased the extent of LTP induction. Moreover, LTP was shown to be enhanced in non-stressed animals treated with fluvoxamine compared to stressed and non-stressed rats, which suggested an effect of antidepressants on synaptic plasticity of normal subjects.

More recently, Normann et al. (2007) assessed this notion by using visual evoked potentials (VEPs) as a model to study cortical responses and its plasticity in the human visual system. Since repeated presentation of visual stimuli is known to produce a form of learning which subsequently improves the perception of these stimuli, the authors were able to assessed synaptic transmission in depressed patients and healthy individuals after chronic treatment with the SSRI sertraline. The neuronal correlates of perceptual learning in the visual system have been studied in awake mice (Frenkel et al., 2006), in which the

enhancement of evoked responses after repetitive presentation of visual stimuli is thought to reflect an increased long-term synaptic plasticity, as similarly suggested for the human visual system (Teyler et al., 2005). Normann et al. (2007) showed that chronic antidepressant administration in healthy human subjects increases the amplitude of early components (P1 and N1) of visual evoked potentials (VEPs) in response to repeated presentation of visual stimuli, whereas the polarity of such modulation was inverted in depressed patients. Taken together, these data suggest a role for neuronal plasticity in the action of ADs. How changes in plasticity are translated into the therapeutic effects induced by ADs is, however, currently unknown.

Critical periods for experience-dependent plasticity

The rich diversity of human personalities, abilities, and behavior is undoubtedly generated by the uniqueness of individual human brains. These fascinating neurobiological differences among human beings derive from both genetic and environmental influences. The first steps in the construction of neural circuitries, i.e., the establishment of distinct brain regions, the generation of neurons, the formation of major axon tracts, the guidance of growing axons to appropriate targets, and the initiation of synaptogenesis, rely largely on the tight interaction between genes and environment. Once the basic patterns of neural connections in the brain are established, however, patterns of neuronal activity (including those that are elicited by experience) modify the synaptic circuitry of the developing brain. Neuronal activity generated by interactions with the external world in postnatal life thus provides a mechanism by which the environment can influence brain structure and function.

The neural mechanisms that result from genetic and environmental interactions and their developmental consequences are sufficient to create some remarkably sophisticated innate behaviors. For most animals, the behavioral repertoire, including foraging, fighting and mating strategies, largely relies on patterns of connectivity established by intrinsic developmental mechanisms. However, the nervous system clearly adapts to and is influenced by the particular circumstances of an individual's environment. These environmental factors are especially influential in early life, during

temporal windows called “critical periods”. Psychologists and ethologists have long recognized this notion, that early postnatal life is a period of special sensitivity to environmental influences on animal’s behavior. It is thought to be a critical period during which a given behavior is especially susceptible to, an indeed requires, specific environmental influences to develop normally (Purves et al., 2004). Once this period ends, such behavior is largely unaffected by subsequent experience and the failure to be exposed to appropriate stimuli during the critical period causes alterations that are irreversible on late developmental stages.

Avian song learning

The existence of critical periods has been demonstrated in several species (reviewed by Berardi et al., 2000) which include, for instance, song in birds and language in humans. Particularly well characterized is the sensitive period for learning courtship songs by oscine songbirds such as canaries and finches (reviewed by Doupe & Kuhl, 1999). In these species, the quality of early sensory exposure is the major determinant of subsequent perceptual and behavioral capabilities. The developmental periods for learning these and other behaviors are restricted during postnatal life, suggesting that the nervous system changes in some manner to become refractory to further experience.

Thus, avian song learning illustrates the interactions between intrinsic and environmental factors in this developmental process. Many birds sing to attract mates, but oscine songbirds are special in that their courtship songs are dependent on auditory and vocal experience. The sensitive period for song learning comprises an initial stage of sensory acquisition, when the juvenile bird listens to and memorizes the song of a nearby adult male tutor (usually of its own species), and a subsequent stage of vocal learning, when the young bird matches its own song to the now memorized tutor model via auditory feedback. This sensory motor learning stage ends with the onset of sexual maturity, when songs become acoustically stable. Even constant exposure to other songs after sensory acquisition at the end of the sensitive period does not affect this memory: the songs heard during sensory acquisition, but not later, are those that the bird vocally mimics (Purves et al., 2004).

On the other hand, additional features of song learning indicate an intrinsic predisposition for this specialized form of vocal learning. First, juveniles often need to hear the tutor song only 10 or 20 times to then vocally mimic it many months later. Second, when presented with a variety of songs played from tape recordings that include their own and other species' songs, juvenile birds preferentially copy the song of their own species, even with no external reinforcement (reviewed in Doupe & Kuhl, 1999). These observations show that juveniles are not really "naive," but are innately biased to learn the songs of their own species over those of others. Therefore, intrinsic factors make the nervous system of oscine birds especially sensitive to songs that are species typical. It is likely that similar biases influence human language learning.

Language in human beings

In contrast to canaries and finches, human beings require large postnatal experience to produce and decode speech sounds that are the basis of language. The various forms of early language exposure, including the "baby talk" that parents and other adults often use to communicate with children as they begin to acquire language may actually serve to emphasize important perceptual distinctions that facilitate proper language production and comprehension (Purves et al., 2004). Importantly, for this linguistic experience to be effective it must occur in early life. The requirement for perceiving and practicing language during a critical period is apparent in studies of language acquisition in congenitally deaf children. Whereas most babies begin producing speech like sounds at about 7 months (babbling), congenitally deaf infants show obvious deficits in their early vocalizations, and such individuals fail to develop language if not provided with an alternative form of symbolic expression. If, however, these deaf children are exposed to sign language at an early age, they begin to "babble" with their hands just as a hearing infant babbles audibly (Petitto & Marenhette, 1991). This suggests that, regardless of the modality, early experience shapes language behavior.

Examples of pathological situations in which normal children were never exposed to a significant amount of language point toward the same notion. One well-documented case is that of a girl who was raised by deranged parents until the age of 13 under

conditions of almost total language deprivation. Despite intense subsequent training, she never learned more than a rudimentary level of communication (see Kandel et al., 2002). This and other examples of so-called "feral children" starkly define the importance of early experience for language development as well as other aspects of social communication and personality. In contrast to the devastating effects of deprivation on children, adults retain their ability to speak and comprehend language even after long periods without exposure to human communication. The normal acquisition of human speech is subject to a critical period: the process is sensitive to experience or deprivation during a restricted period early in life and is relatively refractory to similar experience or deprivations in adulthood.

Critical period for visual system development

Although critical periods for language and other distinctively human behaviors are in some ways the most compelling examples of this phenomenon, it is difficult to study the underlying changes in the human brain. A much clearer understanding of how changes in connectivity might contribute to critical periods has come from studies of the developing visual system in experimental animals with highly developed visual abilities, particularly cats and monkeys. In an extraordinarily influential series of experiments in the 1960s, David Hubel and Torsten Wiesel found that depriving animals of normal visual experience during a restricted period of early postnatal life irreversibly alters neuronal connections and functions in the visual cortex. These observations provided the first evidence that the brain translates the effects of early experience, i.e., patterns of neural activity, into more or less permanently altered wiring of neural circuitries.

Electrophysiological recordings from the primary visual cortex of adult cats evidenced that the two eyes differentially activated cortical neurons and cells with similar preference for one eye were grouped together into ocular dominance columns (Hubel & Wiesel, 1963). It became clear that sensory inputs from the two eyes are first integrated in the primary visual cortex, where most afferents from the lateral geniculate nucleus of the thalamus terminate. In most mammals the afferent terminals form an alternating series of eye-specific domains in cortical layer IV called ocular dominance columns,

which are represented as stripes of cortical neurons that are driven only by stimulation of one eye or the other. All these units, together with orientation columns, are functionally organized into hypercolumns which process visual stimuli from discrete regions of the visual field. Thus, using electrophysiological recordings Hubel & Wiesel (1963) found that ocular dominance distribution across cortical layers of the visual cortex is roughly Gaussian in adult normal cats (Figure 3).

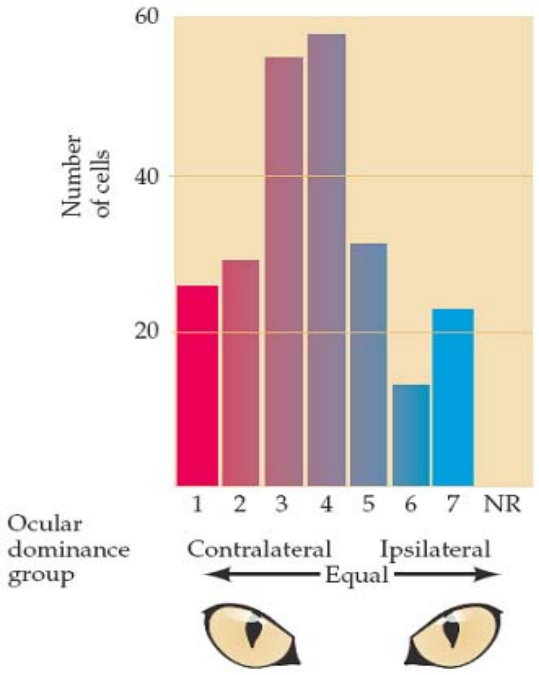


Figure 3. Ocular dominance distribution of single cell recordings in the visual cortex of adult cats. Cells in group 1 and 7 are activated only by the contralateral or by the ipsilateral eye, respectively. Group 4 cells are driven by both eyes equally while 2/3 and 5/6 driven mainly by contra and ipsi eye, respectively. Taken from Purves et al., 2004.

The researchers then asked whether this normal distribution of ocular dominance could be altered by visual experience. Using single cell recordings, they observed that occluding one eye early in development (a treatment referred to as monocular deprivation) led to the reduction in the number of cortical cells responding to that eye, in parallel with a robust increment in the number of neurons activated by the open eye (Wiesel & Hubel, 1963; Hubel & Wiesel, 1970) (Figure 4A). In contrast, monocular deprivation has no effect on ocular dominance distribution of adult animals (Figure 4B).

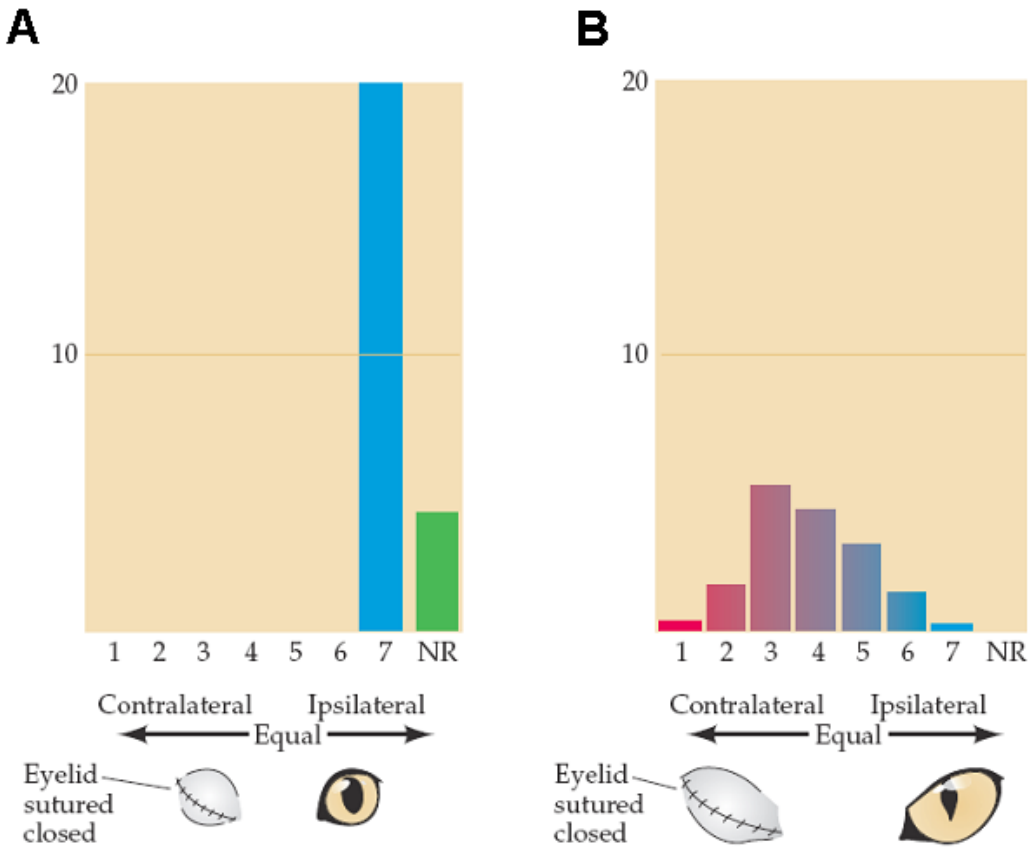


Figure 4. Effect of early closure of one eye on the ocular dominance distribution in the visual cortex. **(A)** Following closure of one eye from 1 week after birth until 2.5 months, no cells could be activated by the deprived contralateral eye. Some cells could not be activated by either eye (NR). **(B)** A much longer period of monocular deprivation in an adult cat had little effect on ocular dominance distribution, though the overall cortical activity was diminished. Taken from Purves et al., 2004.

Recordings from the retina and lateral geniculate layers related to the deprived eye indicated that these more peripheral stations in the visual pathway worked quite normally. Thus, the absence of cortical cells that responded to stimulation of the closed eye was not a result of retinal degeneration or a loss of retinal connections to the thalamus. Rather, the deprived eye had been functionally disconnected from the visual cortex. Consequently, such animals are behaviorally blind in the deprived eye.

Because the same treatment is completely ineffective in the adult animal (Figure 4B), this early temporal window characterized by enhanced plasticity in response to experience is a typical example of a critical period. The shift in the ocular dominance distribution in response to monocular deprivation has been reported to occur in all mammals tested (reviewed by Berardi et al., 2000), accompanied by other dramatic effects such as poor development of visual acuity for the deprived eye, a condition known as amblyopia (reviewed in Odom, 1983) which is a permanent pathology in the adult. Even if the formerly deprived eye is subsequently left open indefinitely, little or no recovery of visual acuity occurs. Similar experiments in the monkey have shown that the same phenomenon occurs in primates, although the critical period is longer than in cats, up to six months (Horton & Hocking, 1999).

Determinant factors of the critical period for visual cortical plasticity

A further question for understanding how experience modulates neural circuits during critical periods is how patterns of activity are transduced to modify neural connections and to make these changes permanent. The ability of the central nervous system to process external stimuli into long term changes rely on activity dependent neural mechanisms that induce structural and functional modifications which underlie, for instance, processes of learning and memory. Extensive research during the last years shed light on determinant aspects of critical periods for experience-dependent plasticity. The mechanisms that modulate neural circuits functioning and connectivity rely on signals generated by the synaptic activity associated with sensory experience or motor performance, the basic neural processes by which experience is represented. A number of different signaling molecules, including NMDA receptors, neurotrophic factors, the

activity of the CRE-CREB system, extracellular matrix molecules and the GABAergic inhibitory transmission have been recognized as important regulators of visual cortical plasticity through changes that occur with correlated neural activity (reviewed by Berardi et al., 2003).

Glutamatergic NMDA receptors

Experimental evidence for the role of NMDA receptors in the regulation of visual cortical plasticity first came from the finding that blockade of such receptors inhibits the effects of monocular deprivation (Bear et al., 1990). The use of NMDA receptors antagonists and antisense oligonucleotides to reduce the expression of the NR1 subunit, without affecting visually driven activity, confirmed this notion (Roberts et al., 1998; Daw et al., 1999), demonstrating the NMDA receptor involvement in visual cortical plasticity. Additionally, the expression of NMDA receptors is developmentally regulated and modified by electrical activity. Their subunit composition varies in the visual cortex, from an increased expression of NMDA receptors containing the 2B subunit to a high presence of receptors containing the subunit 2A, with a time course that nearly parallels that of the functional development of the visual cortex. The expression of the NR2A subunit also correlates with the progressive diminishment of NMDA mediated currents (Flint et al., 1997). Dark rearing, which delays the critical period closure and impairs functional properties of the visual cortex, delays both the developmental shortening of NMDA receptor currents and expression of the NR2A subunit, suggesting a possible role for the 2B-to-2A switch in the closure of the critical period (Philpot et al., 2001). Nevertheless, experimental evidence against a causal role of NMDA receptors in determining the closure of the critical period comes from the finding that transgenic mice engineered to maintain prolonged NMDA responses by deletion of the NR2A subunit, show an unaltered sensitivity to MD which was restricted to the typical critical period and normally delayed by dark rearing from birth (Fagiolini et al., 2003). Moreover, western blot analysis confirmed a late postnatal onset of NR2A protein expression in wild type animals (P18) well in advance of the critical period (P28). Taken together, these observations indicate that the late onset and experience-dependent profile of NR2A

subunits, known to determine LTP occurrence in the hippocampus, is not relevant for critical period expression in the visual cortex (reviewed by Hensch, 2005).

Neurotrophic factors

Experimental evidence that link neurotrophins and neuronal plasticity, particularly in the neocortex and hippocampus, came out in the 1990s. One of the first evidences regarding neurotrophic factors and visual cortical plasticity came from the work of Maffei et al., in 1992, who demonstrated that the physiological shift in ocular dominance distribution could be prevented by infusion of the nerve growth factor (NGF) into the cortex during the critical period. Later, Cabelli et al., in 1995, evidenced that infusion of BDNF and the neurotrophin 4/5 (NT4/5) during the critical period, but not NGF, prevented the formation of ocular dominance columns in the cat visual cortex; finding of importance not only because it showed that neurotrophins modulate the patterning of projections within the visual cortex, but also because it suggested that thalamic axons compete for limiting amounts of neurotrophic factors.

Although plausible, such experiments, did not address the role of neurotrophins in activity dependent plasticity, since the effects observed still could be explain by a growth promoting action of BDNF and NT4/5 on thalamic axons within layer IV of the cortex, independent of neuronal activity. The requirement of neuronal activity for the occurrence of BDNF effects was evidenced by McAllister et al., in 1996, who demonstrated that inhibition of spontaneous electrical activity, glutamatergic synaptic transmission or inhibition of activation of L-type Ca^{2+} channels, prevented the BDNF induced dendritic growth in the visual cortex.

In addition, the requirement of electrical activity for the occurrence of the effects induced by NGF on ocular dominance plasticity has also been demonstrated. It was tested in animals subjected to complete monocular blockade of retinal discharges through intravitreal tetrodoxin (TTX) injections, at the peak of the critical period, while NGF was concurrently delivered into the visual cortex by osmotic minipumps. Analysis of single cell recordings revealed that while infusion of NGF is effective in preventing the ocular

dominance shift in young monocularly deprived rats, no rescue can be observed in TTX injected animals intracortically infused with NGF (Caleo et al., 1999).

Striking evidence for the role of BDNF in regulating the critical period for visual cortical plasticity came from studies performed in transgenic mice overexpressing BDNF in the visual cortex, with no alterations in the normal cellular pattern of expression or release and an effectively restricted expression of the neurotrophin in excitatory neocortical neurons (Huang et al., 1999). In these animals BDNF overexpression accelerates both the development of visual acuity and the time course of ocular dominance, thus supporting a crucial role for neurotrophins in visual cortical development and plasticity. The reciprocal regulation between neurotrophins and electrical activity seems to provide a mechanism by which active neuronal connections are selectively strengthened. As mentioned before, neurotrophins seem to require the presence of electrical activity to exert their actions (Sala et al., 1998; Caleo et al., 1999). Indeed, it has been demonstrated that both weak synaptic plasticity and localized BDNF application, which by themselves do not alter synaptic efficacy, induces long-term potentiation of synaptic transmission (Kovalchuk et al., 2002), finding that suggest a synergistic effect of neurotrophins and electrical activity in promoting synaptic plasticity. Accordingly, although BDNF promotes the phosphorylation of the transcription factor cAMP-response-element binding protein (CREB), it evokes only weak CREB mediated gene transcription unless it is coupled with electrical activity (Hu et al., 1999).

Activity of the CRE-CREB system

The initial molecular events of neural plasticity, which are changes in synaptic efficacy that do not require protein synthesis, are followed by long-lasting changes in neural circuitries that depend on gene expression and subsequent synthesis of proteins (reviewed in Berardi et al., 2003). The intracellular signaling pathways underlying the integration of electrical activity and neurotrophin transmission, for instance, involve the activation of three different protein kinases: cAMP-dependent protein kinase (PKA), extracellular-signal-regulated kinase (ERK1/2) and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), whose activity is necessary for the shift of ocular dominance after

monocular deprivation in young animals (Taha et al., 2002; Di Cristo et al., 2001; Beaver et al., 2001). Each kinase is activated by specific patterns of extracellular signals and is tightly woven within a network of mutual interactions. Activation of such intracellular pathways leads to changes in gene expression mediated by an up-regulation of transcription factors like egr1/zif68 and CREB, and subsequent protein synthesis, as demonstrated for ocular dominance plasticity in the visual cortex (Mower et al., 2002). Activated kinases must translocate to the nucleus where they phophorylate CREB, which then initiates the expression of genes under control of the cAMP-response element (CRE) promoter. Then, the consequent production of transcripts essential for establishment and maintenance of plasticity, does take place (reviewed by Silva et al., 1998).

A critical role for the CRE-CREB system in regulating the expression of genes involve in the physiological plasticity events during postnatal neocortical development, using transgenic mice carrying a CRE-lacZ reporter, has been demonstrated as well (Pham et al., 1999). It was found that calcium- and cAMP-regulated signaling pathways in visual cortical neurons are activated by monocular deprivation in young animals, events that precede the plastic modification typically observed after manipulations of visual stimuli early in development. Indeed, immunofluorescence analysis of visual cortical sections of CRE-lacZ transgenic mice monocularly deprived during the critical period, demonstrated an increased number of lacZ-positive cells in the visual cortex contralateral to the deprived eye. Moreover, the induction of these molecular events was dramatically down-regulated following the end of the critical period for visual cortical plasticity (Pham et al., 1999). These results show that CREB mediated gene expression is involved in the occurrence of visual cortical plasticity during development and suggest that a reduction in the activity of the CRE-CREB system may be implicated in the decline of plasticity observed during late postnatal development.

Extracellular matrix molecules

A correlation between the extracellular environment and adult visual cortical plasticity has also been demonstrated. There is evidence indicating that removal of important components present in the extracellular environment of the central nervous

system is necessary for experience-dependent plasticity to take place. The extracellular protease tissue plasminogen activator (tPA), for instance, is induced by electrical activity as an immediate early gene (Qian et al., 1993) and its proteolytic activity in the visual cortex is increased during monocular deprivation (Mataga et al., 2002). Initial evidence for the role of tPA in visual cortical plasticity came from the work of Mataga et al., in 1996, with the finding that its pharmacological inhibition attenuates the ocular dominance shift induced by monocular deprivation in young animals. This finding was then confirmed through pharmacological studies of the effects of monocular deprivation on tPA-knockout mice. Ocular dominance plasticity in these mice is impaired and can be rescued by exogenous tPA administration (Mataga et al., 2002). tPA has a wide spectrum of possible molecular targets, including extracellular matrix proteins (Wu et al., 2000), growth factors (Yuan et al., 2002), membrane receptors and cell adhesion molecules (Endo et al., 1999) which suggest an important role for this protease in visual cortical plasticity.

Additional data confirming the inhibitory action of the extracellular environment on visual cortical plasticity came from the work of Pizzorusso et al., in 2002, which focused on a class of glycoproteins that are major components of the extracellular matrix, the chondroitin-sulphate proteoglycans (CSPGs). Such molecules are composed of a core protein and CSPG glycosaminoglycan chains, which show a wide expression in the central nervous system, where they give a structural support to the extracellular environment acting mainly as physical barriers (Faissner & Steindler, 1995). CSPGs typically condense in lattice-like structures designated as perineuronal nets (PNNs) during postnatal development (Hockfield et al., 1990; Bruckner et al., 2000), and completely ensheath inhibitory interneurons assuming a perisynaptic localization at sites of synaptic contacts (Zaremba et al., 1989; reviewed by Celio et al., 1998). In a set of very elegant experiments, Pizzorusso et al. (2002) showed that the developmental maturation of the extracellular matrix was inhibitory for experience-dependent plasticity in the visual cortex. The authors used immunohistochemical analysis for Wisteria Floribunda Agglutinin (WFA), which binds to CSPG glycosaminoglycan chains, to demonstrate that the formation of adult-like PNNs around inhibitory interneurons coincided with the end of the critical period. Then, they showed that rearing animals in

complete darkness from birth, a strategy that is known to prolong the critical period for ocular dominance plasticity (see Berardi et al., 2000), inhibits the developmental maturation of PNNs. Soon after, the authors analyzed the effects of CSPGs degradation *in vivo* with the enzyme chondroitin ABC (chABC) on visual cortical plasticity. This treatment caused a degradation of PNNs in the adult visual cortex and reactivated plasticity for ocular dominance in monocularly deprived adult rats, demonstrating that developmental maturation of PNNs contribute to the progressive reduction of plasticity that occurs in the visual cortex at the end of the critical period.

That degradation of extracellular matrix components is a useful strategy to promote structural and functional recovery from visual deficits in the adult visual cortex, was recently demonstrated by Pizzorusso et al. (2006). In this case, long-term monocularly deprived animals were subjected to reverse suture in parallel to intracortical injections of the enzyme chABC which digest glycosaminoglycan chains of CSPGs, and single-unit recordings were then performed. The electrophysiological analysis showed a complete recovery of ocular dominance distribution in the visual cortex contralateral to the long-term deprived eye in chABC treated animals. Moreover, full recovery of both visual acuity and receptive field size of the long-term deprived eye was shown to be induced by chABC treatment. Thus, the authors demonstrated that intracortical infusion of chABC, coupled to reverse lid-suturing, favored the recovery of vision in adult rats with normal visual functions permanently impaired after long-term monocular deprivation.

A critical role for myelin-associated proteins in adult visual cortical plasticity, which include ligands for the Nogo receptor (NgR) has also been recognized. In particular, some proteins that are components of the myelin sheath including Nogo and the myelin basic protein (MBP) have been found to be chemo-repulsive for growing axons. Indeed, such molecules display an important function during development of the central nervous system in mediating axon growth and guidance (Purves et al., 2004). In addition, axon growth inhibition after injury of the adult nervous system appears to reflect the activity of inhibitory signals produced by glia and other cells at the lesion site through NgR signaling.

As the vast majority of visual cortical neurons express the NgR, McGee et al. (2005) evaluated whether the NgR-mediated myelin inhibition of neurite outgrowth contributes to the closure of the critical period for ocular dominance plasticity in rodents. Initially, the authors demonstrated that maturation of intracortical myelination correlates with the end of the critical period. Afterwards, they used transgenic mice lacking the NgR to investigate the involvement of NgR-signaling in restricting OD plasticity in the adulthood. Analysis of adult NgR transgenic mice, well after the end of the critical period (P120), showed that OD plasticity persisted into adulthood since an OD shift of visual cortical neurons was elicited by MD. Moreover, it was demonstrated that transgenic mice lacking main ligand of the NgR: Nogo, showed a similar susceptibility to MD in the adulthood thus confirming that NgR-dependent mechanisms participate directly in restricting visual cortex experience-dependent plasticity (McGee et al., 2005).

Intracortical GABAergic inhibition

It has become clear that inhibition has an important role in sculpting patterns of electrical activity. This action contributes to the detection of imbalance of activity between the afferents to single cortical neurons. A failure in the timing of arrival of synaptic inputs on a post-synaptic neuron has been correlated with a failure in plasticity. The fact that manifestation of visual cortical plasticity requires inhibitory transmission was first shown by Hensch et al. (1998), using transgenic mice lacking the 65-Kda isoform of the GABA-synthesizing enzyme GAD (GAD65). Experience dependent plasticity in young animals in response to monocular deprivation, is impaired in these transgenic mice. Normal plasticity in these animals is rescued by enhancement of GABAergic transmission, in the visual cortex, by means of benzodiazepines administration. Thus, if the intracortical inhibition is reduced in young animals, the critical period onset is delayed, suggesting that there is an inhibitory threshold to be surpassed before the critical period can start. In contrast, if the intracortical inhibition is precociously enhanced by diazepam administration (Fagiolini & Hensch, 2000) the critical period starts earlier.

It has also been demonstrated that the time course and closure of the critical period for visual cortical plasticity is mediated by the maturation of intracortical inhibition, which involves BDNF expression. The development of intracortical inhibition, indeed, is accelerated in BDNF overexpressing mice (Huang et al., 1999) which suggest that BDNF controls the time course of the critical period by accelerating the maturation of the GABA-mediated inhibition. It is known that intracortical inhibition provides strong control over activity-dependent synaptic plasticity (Artola & Singer, 1987; Kirkwood & Bear, 1994) and matures slowly in comparison to excitation. It has been suggested that such a developmental mismatch between inhibition and excitation provides a temporal window for the critical period, when the organization of neuronal circuitries can be strongly influenced by sensory experience. Thus, the maturation of intracortical inhibitory circuitries sets the threshold for both the start and the end of the critical period. Consistent with this notion, dark rearing which prolongs the closure of the critical period also delay the development of intracortical inhibition (Fagiolini et al., 1994; Benevento et al., 1992).

The use of *in vitro* models of neuronal plasticity, long-term potentiation and long-term depression (LTP/LTD) of neural transmission, has also shed light on the molecular mechanisms underlying activity-dependent modifications of synaptic plasticity that depend on intracortical inhibition. In particular, reliable LTP of synaptic responses in layer II/III can be elicited by theta burst stimulation of layer IV in visual cortical slices of both young and adult animals. In contrast, LTP in layer II/III after electrical stimulation from the white matter (WM-LTP) can be obtained only in slices from the visual cortex of young rats but not in those of adult animals. Kirkwood & Bear (1994) first suggested that the maturation of inhibitory circuitries in layer IV is one of the mechanisms responsible for the closure of the critical period for the occurrence of WM-LTP. Indeed, theta burst stimulation from the white matter, in the adult rat, failed to induce LTP unless a GABAA receptor antagonist was applied to visual cortical slices. Notably, susceptibility to WM-LTP roughly coincides with the critical period for ocular dominance plasticity and, as the critical period, can be prolonged by dark rearing (Kirkwood et al., 1995). In support to this hypothesis, BDNF overexpressing mice, which show an accelerated maturation of intracortical inhibition, displayed an accelerated developmental decline of WM-LTP in

the visual cortex. The magnitude of LTP in wild-type mice undergoes a sharp decline between the fourth and fifth postnatal weeks, whereas in transgenic mice such decline in LTP occurred one week earlier (Huang et al., 1999). Moreover, it has also been demonstrated that WM-LTP can be rescued by blocking intracortical inhibition in slices derived from BDNF transgenic mice.

As to the diversity of intracortical inhibitory networks involved in the occurrence of plasticity in the visual cortex, the use of transgenic knockin animals with mutations in particular α-subunits of GABAA receptors, has given the opportunity to analyze whether specific GABA circuitries underlie plasticity in the visual system (Fagiolini et al., 2004). Because specific GABAA receptor-mediated currents can be enhanced by certain benzodizepines, whose sensitivity is determined by particular α-subunit composition, the triggering of ocular dominance plasticity through cortical administration of the benzodiazepine diazepam in parallel to monocular deprivation in young animals with point mutations in the α-1 α-2 and α-3 subunits that contribute to the benzodiazepine binding site, showed that only α1-containing inhibitory GABAergic circuitries regulate the expression of the critical period for visual cortical plasticity. In particular, point mutations of a histidine (H) to an arginin (R) in different subunits: α-1(H101R), α-2(H101R) and α-3(H101R), which renders individual GABAA receptors insensitive to diazepam, were used. The authors initially showed that wild-type mice (in which normally no plasticity occurs after brief monocular deprivation soon after eye opening) that were cortically treated with the benzodiazepine agonist zoldipem, showed a marked ocular dominance shift in favor of the non deprived eye. Afterwards, they went on to examined ocular dominance plasticity in α-1(H101R) animals and found that no shift of ocular dominance occurred after cortical infusion of diazepam in parallel to brief monocular deprivation. In contrast, α-2(H101R) animals displayed an ocular dominance shift of visual cortical neurons similar to that of control animals after benzodiazepine administration (Fagiolini et al., 2004).

Visual Evoked Potentials (VEPs) and visual acuity

The visual evoked potentials (VEPs) in response to sinusoidal gratings alternated in phase several times per sec have proved to be very useful for the study of visual functions in man and animals. The extensive application of this technique is based on the fact that it does allow to predict either the psychophysical contrast sensitivity or limit of spatial resolution. Indeed, extrapolation to zero amplitude of the regression line obtained by plotting VEP amplitude against log contrast or log spatial frequency of the stimulus grating, gives contrast sensitivity or visual acuity values which are very similar to the psychophysical estimations (Campbell & Maffei, 1970). This has been evidenced not only in man but also in other animal species in which the subjective contrast thresholds or acuity estimations were behaviorally evaluated (Campbell et al., 1973; Nakayama & Mackeben, 1982). Because of the coincidence between contrast threshold estimated from psychophysical and from electrophysiological data, VEPs are assumed to be a reliable technique used to predict the subjective contrast sensitivity in all those cases in which other techniques are difficult to apply (Maffei & Fiorentini, 1990).

The pattern-reversal VEPs have also been useful to study the postnatal development of visual contrast sensitivity in infants (Pirchio et al., 1978) and to investigate the physiological mechanisms underlying the processing of spatial information in man as well. In particular, evidence has been found supporting the existence in the human visual cortex of orientation channels (Maffei & Campbell, 1970) and of spatial frequency channels (Fiorentini et al., 1983). In addition, methods based on the recording of pattern VEPs have been successfully applied in infants to study the postnatal development of orientation and spatial frequency selectivity (Fiorentini et al., 1983). Moreover, VEP techniques have become an important tool in clinical practice both in adults and young children with neurological diseases.

Initial studies aimed at identifying the site of origin of the pattern reversal VEP suggested that pre- and post-synaptic mechanisms very likely contributed to VEPs (Maffei & Fiorentini, 1990). The contribution of post-synaptic components in the generation of VEPs is highlighted by the fact that VEPs share some of the properties of visual cortical cells such as, for instance, orientation selectivity (Hubel & Wiesel, 1962)

and susceptibility to contrast adaptation (Maffei et al., 1973). These are properties that are not found in the lateral geniculate nucleus or the retina.

As to the dependence upon stimulus orientation, the amplitudes of VEPs recorded in man has been found to be larger for horizontal and vertical gratings than for oblique ones (Maffei & Campbell, 1970). In the cat, orientational interactions between grating stimuli have been observed in VEP responses. Other properties of visual cortical neurons that have been shown to be shared by VEPs, are the adaptation after impairments of contrast sensitivity following the observation of high-contrast gratings, which has been shown to occur both in man and animals as well as cross orientation inhibition, i.e., inhibition of the response to a phase-reversed grating by a superimposed orthogonal grating (Morrone & Burr, 1986). As to the nature of the post-synaptic neuronal events that contribute to the pattern VEP, these are likely to consist mainly of source-sink pairs related to excitatory post-synaptic potentials. The spike activity of individual neurons seems not to contribute to the occurrence of VEPs (Petsche et al., 1984).

Another characteristic property of steady-state pattern reversal VEPs is that their waveform has a temporal frequency that corresponds to the second harmonic of contrast orientation, at least for temporal frequencies of 10-20 reversal per sec. In the visual cortex this is a property of complex cells, which are neurons that present orientation selectivity. Simple cells, in contrast, respond to phase-reversal gratings with a modulation of their discharge mainly at the fundamental temporal frequency of the stimulus. This, however, does not rule out the possibility that simple cells contribute to the generation of steady-state VEPs. Indeed, the response of a simple cell is not a pure fundamental; it also contains the second and higher harmonics although with a much smaller amplitude. Thus, when the response of a large population of cells adds up, the first and the higher odds harmonics cancel out but the second harmonics responses do not. Therefore, the steady state VEPs in response to alternating gratings could reflect the activity of both complex and simple cells (Maffei & Fiorentini, 1990).

VEPs have also been used to characterize the physiology of the visual system in rodents (Porciatti et al., 1999). Through electrophysiological recordings from the primary visual cortex of the wild-type C57BL/6J mouse, the authors evaluated spatial and temporal aspects of VEPs. In particular, the mouse VEP limit of spatial frequency (visual

acuity) was found to be 0.6 c deg^{-1} an estimated value that was comparable to the behavioral visual acuity. VEPs recordings at different depths of the binocular portion of the visual cortex contralateral to the stimulated eye also revealed that the VEP major component is positive in the superficial layers, whereas in deeper layers it is negative. Local VEPs had their maximal amplitude about 400 μm of depth in the cortex, which correspond to the termination of geniculate afferents at the level of cortical layer IV (Porciatti et al, 2002). Importantly, this pattern of VEPs depth profile is very consistent among animals. That the intracortical VEP profile shows a clear polarity inversion at a point between 150 and 200 μm electrode advancement, suggest a major dipole source of VEPs located at this cortical level. Indeed, anatomical reconstruction of electrode tracks indicates that the major dipole source is generated at the level of pyramidal cells in supragranular layers II-III of the visual cortex (Porciatti et al., 1999). Electrophysiological recordings of VEPs also provide the advantage that different aspects of vision can be screened readily and simultaneously in the same animals, including those with poor visual behavior due to motor or learning deficits.

Functional development of the rat primary visual cortex

Early electrophysiological studies in the adult visual system of the rat found that cortical neurons have well defined functional properties (Parnavelas et al., 1981; Maffei et al., 1992) and are distributed in distinct classes of ocular dominance, with high proportion of binocular cells, comparable to that in cats and monkeys (Maffei et al., 1992; Berardi et al., 1993). The functional postnatal development of the rat primary visual cortex was initially investigated by Fagiolini et al., in 1994. The authors demonstrated that physiological properties of visual cortical neurons are immature soon after eye opening, and gradually develop during the first month of postnatal life. Visual cortical responses are slow and variable at P17, particularly, they present habituation which is the tendency of cell response to diminish after continues stimulation. Neuron responsiveness, evaluated as amplitude of modulation of cell discharge in response to an optimal visual stimulus, increases progressively with age over the third postnatal week, while the sluggishness and tendency to habituation disappears by P23. Ocular dominance

distribution does not change significantly through development; indeed the vast majority of visual cortical neurons are binocular and preferentially driven by the contralateral eye. The major component of age dependent changes in ocular dominance distribution is the increase of monocular, contralateraly driven cells. Receptive fields in adult rats are small and well defined, but this is not the case in younger animals, in which receptive fields at P17 are very large, extending through almost the whole binocular hemifield. At P19-21 receptive field size is around 34 degrees (deg), and it reaches the value of 10 deg or less in the adult. Similarly, visual acuity, which is the highest spatial frequency that can be perceived, increases from 0,5 c deg^{-1} to 0,9 c deg^{-1} within the first month of life in the rat and then reaches the value of about 1 c deg^{-1} in the adult at P45 (Fagiolini at al., 1994).

Importantly, a precocious development of visual acuity has been shown to occur in mice overexpressing the neurotrophin BDNF (Huang et al., 1999). In these transgenic mice adult levels of visual acuity were reached precociously, at about P25, one week earlier than that of wild-type littermates. On the other hand, there is evidence that the progressive decrease in receptive field size is correlated with the time course of visual acuity development (Fagiolini at al., 1994). Therefore, one plausible mechanism by which increased levels of BDNF would accelerate such property of visual cortical neurons is the acceleration of inhibitory circuitries maturation, which by affecting receptive field development, could promote the faster maturation of visual acuity.

In support to this notion, an accelerated development of visual acuity has also been evidenced in animals under environmental enrichment conditions. This is a condition characterized by an increased exploratory behavior, somatosensory and social stimulation which is known to increase the expression of BDNF and promote a precocious maturation of intracortical inhibition in young animals (Cancedda et al., 2004; Sale et al., 2004). As in the case of BDNF overexpressing mice, environmental enriched pups also show a precocious developmental decline of WM-LTP, which is an *in vitro* model of visual function development, as well as an accelerated development of visual acuity.

Visual deprivation and amblyopia in humans

During development, the cortical representation of both eyes starts out equal and in a normal animal this balance is retained if both eyes experience roughly comparable levels of visual stimulation. When, however, an imbalance in visual experience is induced by monocular deprivation, the active eye gains a competitive advantage and replaces many of the synaptic inputs from the closed eye, such that few if any neurons can be driven by the deprived eye. Thus, an acquired defect in vision due to an abnormal visual experience during a sensitive period of visual development that is characterized by reductions in visual acuity and contrast sensitivity, does take place (Grigg et al., 1996). These observations in experimental animals have important implications for children with birth defects or ocular injuries that cause an imbalance of inputs from the two eyes. Unless the imbalance is corrected during the critical period, the child may ultimately have visual functions permanently impaired, as evidenced by poor binocularity, diminished depth perception, and reduced visual acuity (see Levi et al., 1997).

The developmental phenomena in the visual system of experimental animals are in agreement with clinical problems in children who have experienced similar deprivation. The loss of visual acuity and diminished stereoscopic vision that arise from early deficiencies of visual experience is called “amblyopia”. In humans, amblyopia is most often the result of strabismus, a misalignment of the two eyes due to improper control of the direction of gaze by the eye muscles (reviewed in Grigg et al., 1996). Since such misalignments produce double vision, the response of the visual system in some of these individuals is to suppress input from one eye by mechanisms that are not completely understood, but are thought to reflect competitive interactions during the critical period. Functionally, however, the suppressed eye eventually comes to have very low visual acuity and may render the affected individual effectively blind in that eye. Thus, early surgical correction of ocular misalignment, by adjusting lengths of extra-ocular muscles, has become an essential treatment for children with strabismus (Purves et al., 2004). Another cause of visual deprivation in humans is cataracts which can be caused by several congenital conditions that render the lenses opaque. A cataract in one eye is functionally equivalent to monocular deprivation in experimental animals; left

untreated in children, this defect also results in an irreversible effect on the visual acuity of the deprived eye. If either the cataract or corneal opacity is removed at about 4 months of age, however, the consequences of monocular deprivation are largely avoided (reviewed by Holmes & Clark, 2006).

In animal models, the role of correlated activity in driving the competitive postnatal rearrangement of cortical connections can be assessed inducing a situation in which activity levels in each eye remain the same but the correlations between the two eyes are altered. This circumstance can be obtained by cutting one of the extra-ocular muscles in one eye, condition known as strabismus in which the two eyes can no longer be aligned (Purves et al., 2004). The major consequence of strabismus is that corresponding points on the two retinas are no longer stimulated by objects in the same location in visual space at the same time. As a result, differences in the visually evoked patterns of activity between the two eyes are far greater than normal. Unlike monocular deprivation, however, the overall amount of activity in each eye remains roughly the same; only the correlation of activity arising from corresponding retinal points is changed. Misalignment of the two eyes can lead to suppression of the input from one eye and eventual loss of the related cortical connections. The anatomical pattern of ocular dominance columns in layer IV of cats in which input from both eyes remains (but is asynchronous) is sharper than normal, implying that the uncoordinated patterns of activity have accentuated the normal separation of cortical inputs from the two eyes. In addition, the ocular asynchrony prevents the binocular convergence that normally occurs in cortical neurons: ocular dominance histograms from such animals show that most cells in all layers are driven exclusively by one eye or the other (Hubel & Wiesel, 1965).

Even before visual experience exerts these effects, innate mechanisms have ensured that the basic outlines of a functional system are present. These intrinsic mechanisms establish the general circuitry required for vision, but allow modifications to accommodate the individual requirements that occur with age (Crawley & Katz, 2002). Normal visual experience validates the initial wiring, preserving and adjusting the normal arrangement. In the case of abnormal experience, the mechanisms that allow these adjustments result in more dramatic anatomical and ultimately behavioral changes, such as those that occur in amblyopia. The eventual decline of this capacity to remodel cortical

and subcortical neuronal connections is presumably the cellular basis of critical periods in a variety of neural systems, including the development of language and other higher brain functions.

Aim of the thesis and experimental design

Recent experimental evidence suggests a critical role for neuronal plasticity in mediating the therapeutic effects induced by ADs. It has been observed that chronic antidepressant administration promotes neurogenesis and synaptogenesis in the adult hippocampus (Malberg et al., 2000; Hajszan et al., 2005) as well as an increased expression of the neurotrophin BDNF and its primary receptor TrkB (Nibuya et al., 1995; Castren et al., 2004). These cellular and molecular events seem to be necessary in mediating the therapeutic effects of ADs. The antidepressant-like behavioral response to ADs, indeed, is blocked if the induced neurogenesis is disrupted (Santarelli et al., 2003) whereas direct infusion of BDNF into the hippocampus, or the overexpression of its receptor in transgenic mice, induce an antidepressant effect (Shirayama et al., 2002; Koponen et al., 2005). While neurogenesis, synaptogenesis and BDNF signaling are events that correlate with neuronal plasticity, whether ADs promote a functional modification of neuronal circuitries, is currently unknown.

In the present study we investigated whether chronic treatment with fluoxetine, a selective serotonin reuptake inhibitor (SSRI), restores plasticity in the adult visual system of the rat. We used two well characterized models of plasticity, the ocular dominance (OD) shift of visual cortical neurons following monocular deprivation (MD) and the recovery of visual functions in the adult after long-term MD (amblyopia). These two plastic phenomena are restricted to a critical period during postnatal development and are absent in the adult because of a decline of plasticity that has been largely attributed to the maturation of intracortical inhibition (Huang et al., 1999; Fagiolini & Hensch, 2000). A shift in the composition of NMDA receptor subunits (Erisir & Harris, 2003), the activity of the CRE-CREB system (Pham et al., 1999), and the condensation of extracellular matrix molecules in perineuronal nets mainly around inhibitory interneurons (Pizzorusso

et al., 2002), are cellular events which have been correlated to the closure of the critical period for visual cortical plasticity as well.

In particular, we investigated the effects of monocular deprivation (MD) on the ocular dominance (OD) plasticity of adult animals chronically treated with fluoxetine, by recording VEPs in the binocular region of the primary visual cortex contralateral to the deprived eye. We next used VEPs to evaluate the recovery of visual acuity and binocularity in adult rats that were rendered amblyopic by long-term MD and then reverse-sutured (RS) during the last two weeks of antidepressant treatment. Recovery of visual acuity was also evaluated behaviorally in the same animals.

Afterwards, we used *in vivo* brain microdialysis to investigate whether chronic fluoxetine administration affected the ratio of intracortical inhibition relative to excitation in the adult visual cortex. In addition, long-term potentiation of layer II-III field potentials after theta-burst stimulation from the white matter (WM-LTP), an in vitro model of synaptic plasticity, was also examined. BDNF protein expression in the visual cortex and hippocampus of adult rats chronically treated with fluoxetine was assessed using the ELISA method. Western blot analysis of GAD65/67 expression following antidepressant treatment was performed as well.

We finally evaluated the effects of fluoxetine on visual cortical plasticity in adult animals monocularly deprived in parallel to intracortical infusion of the benzodiazepine agonist diazepam. The antidepressant-like behavioral response produced by fluoxetine treatment was also evaluated using the forced swim test (FST).

EXPERIMENTAL PROCEDURES

Animal treatment and fluoxetine administration

A total of 76 adult Long-Evans hooded rats were used in this study, which was approved by the Italian Ministry of Public Health. Animals were group-housed under standard conditions with food and water *ad libitum* in plexiglas cages (40x30x20 cm) and kept in a 12:12 light/dark cycle. Adult rats at the postnatal day 70 (P70) were systemically treated with fluoxetine (0.2 mg ml^{-1} drinking water) (Fluoxetine-hydrochloride, Galeno, Prato-Italy) during 04 weeks (28 Days). Control animals were housed under the same standard conditions except for fluoxetine administration. This method of administration yields fluoxetine plasma levels of 356 ± 99 ng ml^{-1} in rats, which is within the recommended plasma concentration for the treatment of depression in humans (50-450 ng ml^{-1}) (Castren, personal communication).

Surgical procedures

To assess ocular dominance (OD) plasticity, one week of monocular deprivation (MD) was performed through eyelid suturing at the beginning of the last week of chronic fluoxetine or vehicle administration (Day 21 of treatment). Adult animals (P90), under treatment, were anesthetized with avertin (1 ml hg^{-1}) and mounted on a stereotaxic apparatus to be monocularly deprived. Eyelid closure was inspected daily until complete cicatrization; subjects with even minimal spontaneous re-opening were excluded. Great care was taken during the first days after MD to prevent inflammation or infection of the deprived eye through topical application of antibiotic and cortisone.

To perform analysis of long-term MD, rats were anesthetized with avertin (1 ml hg^{-1}) and monocularly deprived through eyelid suturing at P21. Eyelid closure was inspected daily until complete cicatrization; subjects with even minimal spontaneous re-opening were excluded. Adult amblyopic rats were then subjected to reverse suture (RS), under anesthesia, at the beginning of the third week (P85) of chronic fluoxetine or vehicle administration (Day 14 of treatment). Reverse suture (RS), i.e., reopening of the deprived

eye while suturing shut the previously open eye, was performed using thin scissors. Great care was taken during the first days after RS to prevent inflammation or infection in the previously deprived eye through topical application of antibiotic and cortisone.

***In vivo* electrophysiology**

At the end of chronic fluoxetine or vehicle administration, adult animals monocularly deprived for one week and long-term MD animals subjected to RS, were anesthetized with urethane (0.7 ml hg^{-1}; 20% solution in saline; Sigma) by i.p. injection and placed in a stereotaxic frame. Additional doses of urethane were used to keep the anesthesia level stable throughout the experiment. Body temperature was continuously monitored and maintained at ~37°C by a thermostated electric blanket during the experiment. An electrocardiogram was continuously monitored. A hole was drilled in the skull, corresponding to the binocular area of the primary visual cortex (Oc1B) contralateral to the 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 λ (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. We measured visual acuity through both eyes recording visual evoked potentials (VEPs) (Figure 5). 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, as described previously (Huang et al., 1999). Briefly, at least 128 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^{-2}) positioned 20 cm from the rat's eyes and centred on the previously determined receptive fields. Visual acuity was obtained by extrapolation to

zero amplitude of the linear regression through the last four to five data points in a curve where VEP amplitude is plotted against log spatial frequency. Binocularity (ocular dominance) was assessed calculating the contralateral to ipsilateral (C/I) VEP ratio, i.e. the ratio of VEP amplitudes recorded by stimulating the eye respectively contralateral and ipsilateral to the visual cortex where recording is performed.

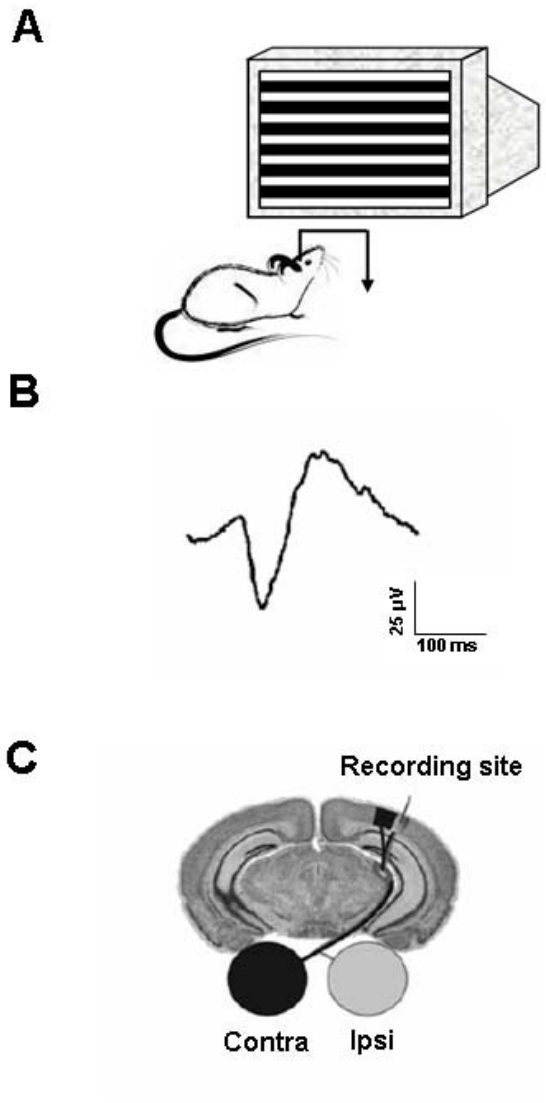


Figure 5. Schematic diagram of the Visual Evoked Potentials (VEPs). **A,** Visual stimuli are horizontal sinusoidal gratings of different spatial frequencies and contrast. **B,** Typical VEP waveform recorded from an electrode positioned 400 μm below the cortical surface. The response consists of a negative wave of 90-100 ms latency followed by a late positive wave. **C,** The recording site for VEPs is the binocular region (Oc1B) of the primary visual cortex contralateral to the deprived eye.

Behavioral assessment of visual acuity

We measured visual acuity of the fellow eye (not deprived) in long-term monocularly deprived rats before performing RS, thus behavioral assessment of visual acuity for the normal eye started at the beginning of chronic treatment (P70). Next, we started measuring visual acuity of the formerly deprived eye (long-term deprived), after RS, at the beginning of the third week (P90) of chronic fluoxetine or vehicle administration (Day 14 of treatment). Therefore, visual acuity measurement of the formerly deprived eye was completed when animals were about P100, at the end of chronic fluoxetine or vehicle treatment. To measure visual acuity, we used the visual water task (Prusky et al., 2000) which trains animals to first distinguish a low (0.1 cycles per degree (c deg^{-1}) spatial frequency vertical grating from grey, and then tests the limit of this ability at higher spatial frequencies. The apparatus consists of a trapezoidal-shaped pool with two panels placed side by side at one end (Figure 6). A midline divider is extended from the wide end of the pool into the middle, creating a maze with a stem and two arms. The length of the divider sets the choice point and effective spatial frequency. An escape platform is placed below the grating. Animals are released from the centre at the end of the pool opposite the panels. The position of the grating and the platform is alternated in a pseudorandom sequence over training trials while the rats are shaped to swim towards the grating in one of the maze arms. A trial is recorded as incorrect if an animal enters the arm without the platform. Animals are removed from the pool when they find the platform. Once 80% accuracy is achieved, the limit of the discrimination is estimated by increasing the spatial frequency of the grating. 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.

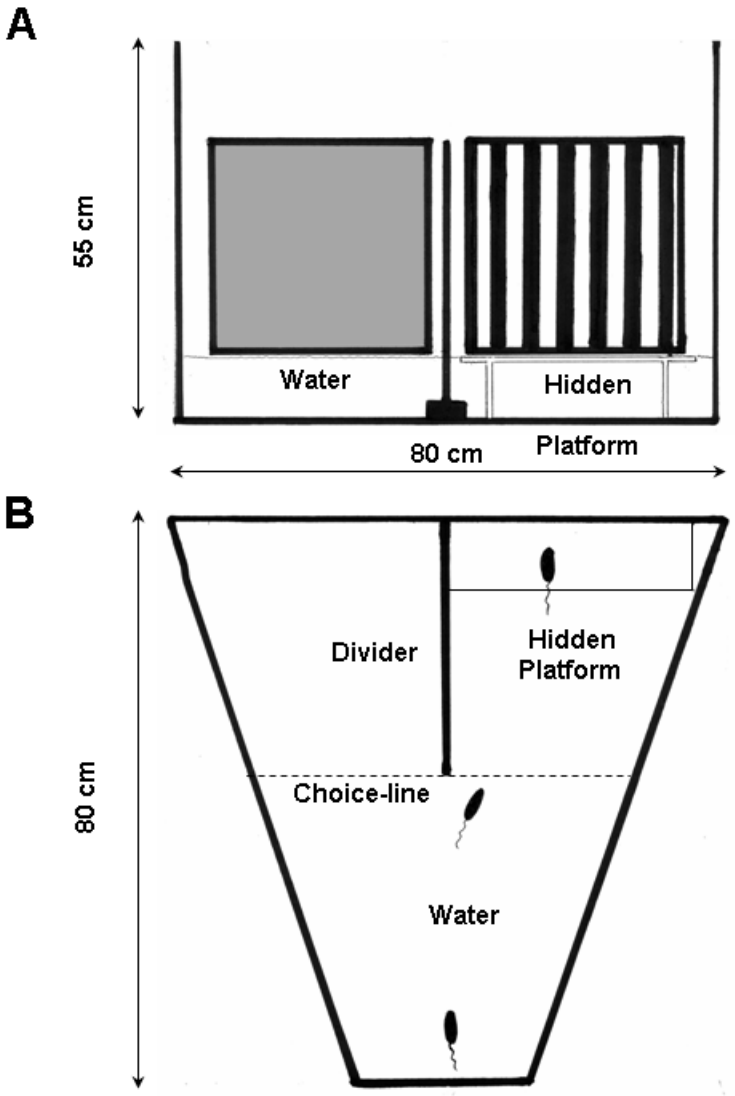


Figure 6. Schematic diagram of the visual water box task. **A,** Front view of the apparatus which is a trapezoidal-shaped pool with two panels placed side by side at one end. A midline divider is extended from the wide end of the pool into the middle, creating a maze with a stem and two arms. An escape platform is placed below the grating. **B,** view from above showing the major components of the devise.

***In vivo* brain microdialysis**

One week before the initiation of the chronic fluoxetine or vehicle administration, adult rats (P60) were anesthetized and stereotaxically implanted with a guide shaft above the binocular visual cortex (binocular area Oc1B), at coordinates: 7.3 mm posterior to bregma, 4.4 mm lateral to the midsagittal suture and 1 mm ventral to the skull. After the end of chronic treatment with fluoxetine, the *in vivo* sampling of dialysates was performed inserting a microdialysis probe into the guide shaft previously implanted. A detailed description of the procedure is reported in (Hernandez et al., 1986) (Figure 7).

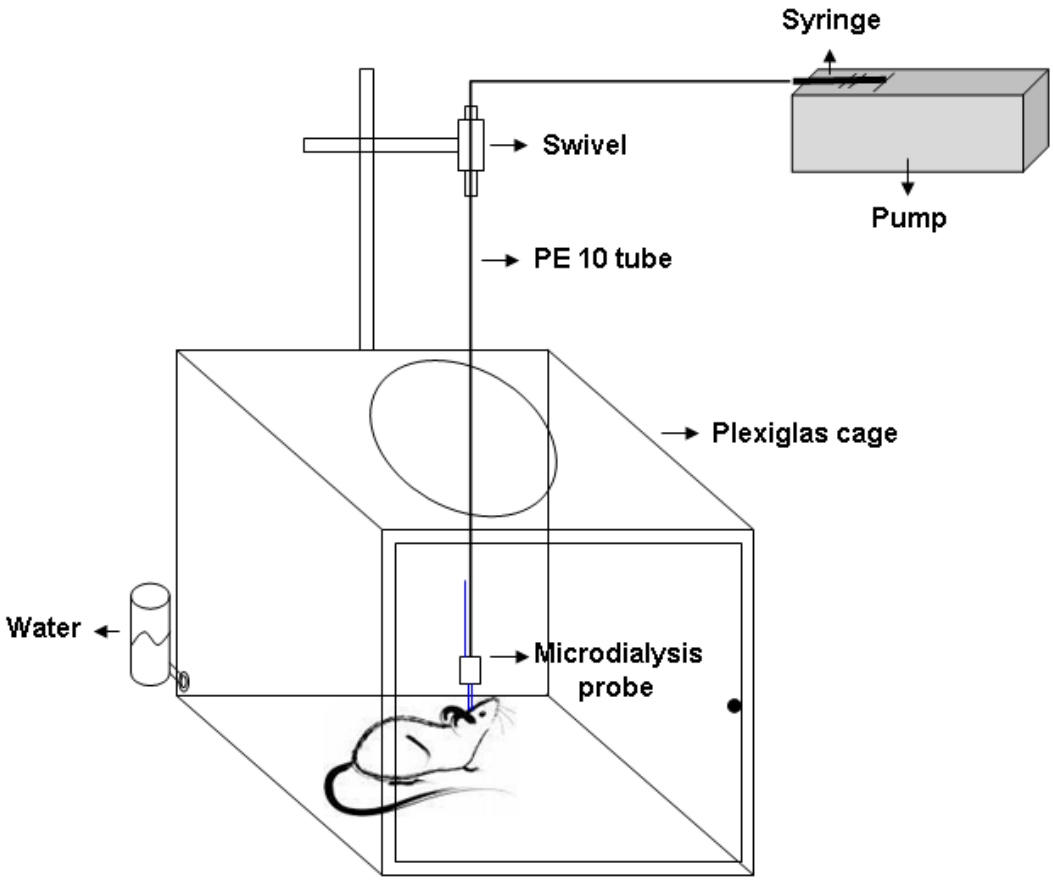


Figure 7. Schematic diagram of *in vivo* brain microdialysis. Sampling of basal extracellular levels of neurotransmitters from the binocular area of the visual cortex in fluoxetine treated adult rats. The microdialysis probe is connected to a dialysis system pumping artificial CSF. After insertion of the probe into the guide shaft *in vivo* sampling of dialysates is performed in each freely moving animal.

The microdialysis probe (Custom BR 1mm probe, BASi Instruments LTD, UK) with a 1 mm long tip of exposed cellulose membrane (6000 MW cut-off) was connected to a dialysis system pumping an artificial CSF (142 mM NaCl, 3.9 mM KCl, 1.2 mM $CaCl_2$, 1 mM $MgCl_2$, 1.35 mM Na_2HPO_4 , pH 7.4) at a flow rate of 1 µl min^{-1}. The probe protruded 1 mm from the tip of the guide shaft (Figure 8). Six hours after insertion of the probe (stabilization period), sampling was carried out. Six samples (20 µl/each) were collected every 20 min along 2 hours for each freely moving fluoxetine treated and control animal.

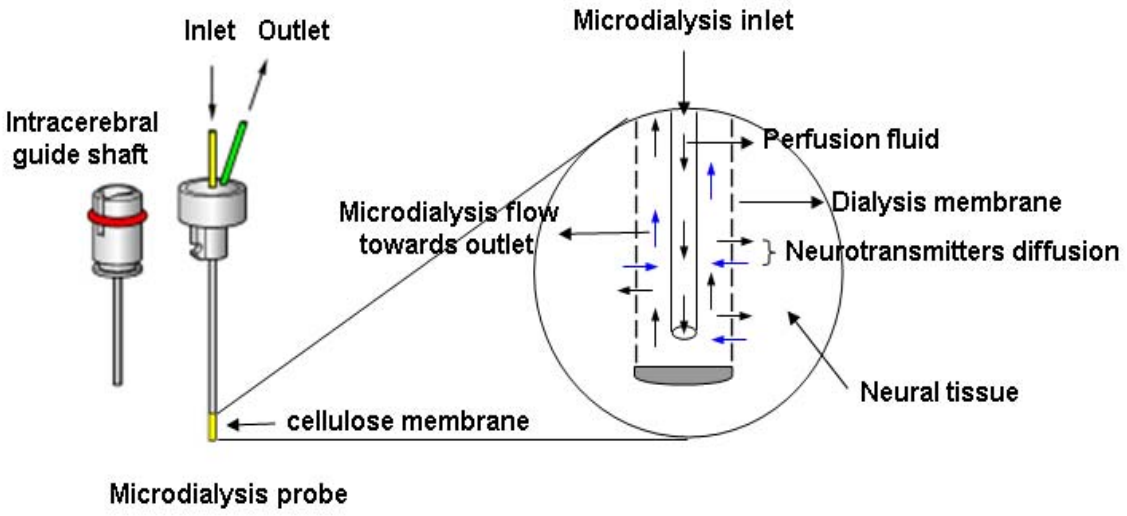


Figure 8. Scheme of the microdialysis probe and diffusion of neurotransmitters (blue arrows) through the dialysis membrane. The probe permits the delivery of CSF directly into the visual cortex to sample levels of neurotransmitters, which diffuse from the neural tissue to the perfusion fluid into the probe, through the cellulose membrane with a 6000 Da cut-off.

Histology

After brain microdialysis, rats were sacrificed with an overdose of chloral hydrate and perfused intracardially. Brains were post-fixed for two hours before being immersed in 30% sucrose in PBS. Forty (40) μm coronal sections from the occipital cortex were cut on a sledge microtome and collected in PBS. Brain sections were then stained for cresyl violet to verify probes' location in Oc1B**.** Only those animals with a correct location of the probe were taken into account for further analysis.

High Performance Liquid Chromatography (HPLC)

Analysis of γ-aminobutyric acid (GABA) and glutamate (GLU) basal levels from microdialysates obtained from fluoxetine treated and control animals, was performed using High Performance Liquid Chromatography (HPLC) coupled to a fluorimetric detection system. A sample automatic derivatization (Waters 2690 Alliance) with o-phtalaldehyde was followed (Calabresi et al., 1995). Resolution was obtained through a C18 reverse phase chromatographic column coupled to the fluorimetric detection (Waters 474; excitation wavelength 350nm, emission wavelength recorder 450nm). Buffer and gradient program was as follows: by definition, solvent A: 0.1M Sodium Acetate pH 5.8/methanol 20/80; solvent B: 0.1M Sodium Acetate pH 5.8/methanol 80/20; solvent C: 0.1M Sodium Acetate pH 6.0/methanol 80/20. Concerning the gradient program, initial isocratic step 5%A, 95%C from 0 to 5 min; 15%A, 85%B from 4 to 5 min and then isocratic until 9 min; 22%A, 66%B until 14.5 min and then 34%A, 66% B until 17 min; 5%A, 95%C until 19 min and then isocratic until 23 min. Flow rate was 0.9 ml min^{-1}. Homoserine was used as internal standard and aminoacid concentrations were calculated from a linear standard curve built upon known concentrations of injected aminoacids. Area of the peaks was used to make comparisons (Waters Millenium 32).

LTP recordings

Brains from fluoxetine treated and control adult rats (P100) were removed and immersed in ice-cold cutting solution containing (in mM): 220 sucrose, 3.1 KCl, 1.0 K_2HPO_4, 4.0 $NaHCO_3$, 2.0 $MgCl_2$, 1.0 $CaCl_2$, 10 HEPES, 1.0 ascorbic acid, 0.5 myo-Inositol, 2.0 pyruvic acid, and 1.0 kynurenate, pH 7.3. Slices (0.35mm thick) of visual cortex were obtained using a Leica (Nussloch, Germany) vibratome. Slices (n = 12 slices for fluoxetine treated animals and n = 14 slices for controls) were then perfused at a rate of 2 ml min^{-1} with 35°C oxygenated recording solution. The recording solution was composed of as the cutting solution with the following differences (in mM): 130 NaCl, 5.0 dextrose, 1.0 $MgCl_2$, 2.0 $CaCl_2$, 0.01 glycine, no kynurenate, no sucrose. Electrical stimulation (100 μsec duration) was delivered with a bipolar concentric stimulating electrode (FHC, St. Bowdoinham, ME) placed at the border of the white matter and layer VI. Field potentials in layers II-III were recorded by a micropipette (1–3MΩ) filled with NaCl (3 M). Baseline responses were obtained every 30 sec with a stimulation intensity that yielded a half-maximal response. After achievement of a 15 min stable baseline (field potential amplitude within 15% of change and with no evident increasing or decreasing trends), θ burst stimulation (TBS) was delivered. Postsynaptic field potentials after TBS were recorded every 30 sec during 30 min.

Western Blot

At the end of the chronic fluoxetine or vehicle administration the expression of the GABA synthesizing enzymes (GAD65/67) was assessed using Western Blot. Proteins from fresh visual cortex were extracted using lysis buffer (1% Triton X-100, 10% Glycerol, 20 mM TrisHCl pH 7.5, 150 mM NaCl, 10 mM EDTA, 0.1 mM Na_3VO_4, 1 ¼ g/ml Leupeptin, 1 ¼ g/ml Aprotinin, 1 mM PMSF) and the total concentration of samples was quantified with the protein assay kit (Bio-Rad, Hercules, CA) using a BSA based standard curve. Then, 10 μg of proteins were run on 12% SDS-PAGE gels to ultimately be electroblotted on nitrocellulose membranes. Blots were blocked with 4% dry milk powder (Bio-Rad Laboratories Inc., Hercules, CA), 0.2% Tween-20 in TBS for 2h and

incubated overnight at 4 °C with the polyclonal anti-GAD65/67 antibody (Chemicon, Temecula, CA) diluted at 1 μg ml^{-1} in TBS, 2% milk, 0.1% Tween-20. After washing, blots were incubated 1h at 37 °C with HRP-conjugated secondary antibody (Goat Anti Rabbit HRP; Bio-Rad, Hercules, CA), developed by ECL chemiluminescence's system (Amersham, UK) and captured on autoradiographic films.

To account for loading errors blots were then stripped with Re-Blot (Chemicon, Temecula, CA), blocked with 4% dry milk powder, 0.2% tween-20 in TBS for 2h and re-probed overnight at 4 °C with the monoclonal anti-tubulin antibody (Tubulin, Sigma, St Louis, MO; diluted 1 μg/ml in TBS, 2% milk, 0.1% Tween-20). Soon after, blots were incubated with HRP-conjugated secondary antibody (Goat Anti Rabbit HRP; Bio-Rad, Hercules, CA) as above described. Films were digitalized and band optical densities (OD) relative to the proteins of interest, as well as, to the corresponding tubulin internal standards, were measured with the Image-J software. The ratio of protein/tubulin OD mean (± S.E.M.) values, corresponding to the visual cortex of a single animal, was calculated for each sample.

Enzyme Linked ImmunoSorbent Assay (ELISA)

The expression of the protein BDNF was assessed at the end of chronic fluoxetine or vehicle administration using the ELISA method. Proteins from fresh visual cortex and hippocampus were initially extracted, respectively, using lysis buffer (1% Triton X-100, 10% Glycerol, 20 mM TrisHCl pH 7.5, 150 mM NaCl, 10 mM EDTA, 0.1 mM Na_3VO_4, 1 ¼ g/ml Leupeptin, 1 ¼ g/ml Aprotinin, 1 mM PMSF). The total concentration of samples was quantified with the protein assay kit (Bio-Rad, Hercules, CA) using a BSA based standard curve. Afterwards, BDNF expression was assessed loading 100 μg of total proteins in triplicate in a standard ELISA plate together with a standard curve and processed as indicated in the manufacturer protocol (BDNF, Promega). Results are reported in ng BDNF/μg proteins.

Cortical infusion of the benzodiazepine diazepam (Dz)

At the beginning of the last week of chronic treatment with fluoxetine (Day 21 of treatment), a different group of adult rats was subjected to MD. In parallel, under anesthesia, 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 deprived eye. Osmotic minipumps (flow rate, 0.5 µl hr^{-1}) were filled up with the benzodiazepine agonist diazepam (Dz; 2mg ml^{-1}; n = 4) or vehicle solution (50% propylene glycol; n = 4). Soon after surgery, rats were transferred to standard condition cages and kept under fluoxetine treatment for one more week. Electrophysiological recordings of the C/I ratio by VEPs was performed at the end of antidepressant treatment as previously described.

Cortical infusion of mercaptopropionic acid (MPA)

A different group of adult (not-deprived) rats was cortically infused with MPA via an osmotic minipump (model 2002; Alzet, Palo Alto, CA) connected via PE tubing to a stainless steel cannula (30 gauge), which was implanted in the binocular region of the primary visual cortex. Osmotic minipumps (flow rate, 0.5 µl hr^{-1}) were filled up with a non-epileptic dose of MPA (100 µM; n = 4) or vehicle solution (Saline; n = 4). Soon after surgery, rats were transferred to the cages and kept under standard conditions for one more week. Brains were then removed and proteins from fresh visual cortex were extracted to perform GAD65/67 analysis by western blot as previously described.

Rat Forced Swim Test

Behavioural response to fluoxetine treatment was studied in adult rats (P100) chronically treated with fluoxetine and control animals, using the forced swim test which is sensitive to the antidepressant activity of SSRIs (Cryan and Lucki, 2000). Briefly, rats were individually placed in a glass container (30 x 40 cm) that was filled with water at 22 °C to a depth of 30 cm, in the first day session (Figure 9). Each animal was removed after 10 min, dried and placed back in the standard condition cage. Twenty-four hours after the first exposure, animals were replaced in the water filled apparatus for 10 min and the session was recorded using a video camera placed above the bucket for subsequent analysis of behavioural response of fluoxetine treated and control rats. Swimming behaviour was defined as horizontal movements throughout the swim chamber. Immobility was considered as passive floating with only slight movements of tail or one hind limb. Climbing behaviour consisted of upward directed movements of the forepaws along the sides of the swim chamber.

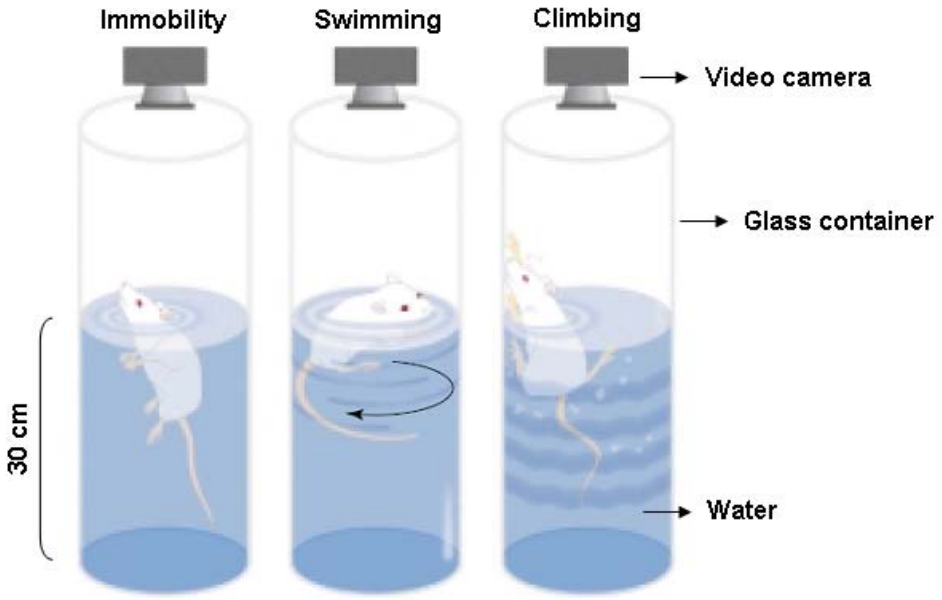


Figure 9. Scheme of the forced swim test. Animals are placed in the apparatus depicted, which is filled up with water (30 cm). During the FST rats exhibit three well recognized forms of behavior: immobility, swimming and climbing. Each session lasts 10 min. and is recorded using a video camera. Taken from Cryan et al., 2002.

Locomotor activity

To control for the spontaneous locomotor activity in both fluoxetine treated and control animals, we performed a mobility test at the end of antidepressant treatment using "Opto M3-2 cage system" with horizontal and vertical laser beam sensors. Spontaneous movement measurements for each animal were monitored at the end of chronic antidepressant treatment, from 00:00 to 06:00 am, by quantification of laser beam breaks during each session.

RESULTS

Chronic fluoxetine administration restores OD plasticity in the adult visual cortex

We initially investigated the effects of one week of monocular deprivation (MD) on the ocular dominance (OD) plasticity of adult animals chronically treated with fluoxetine, by recording visual evoked potentials (VEPs) in the binocular region of the primary visual cortex contralateral to the deprived eye. VEPs represent the integrated response of a population of neurons to patterned visual stimuli and are routinely used to evaluate visual acuity (VA) and binocularity alterations (Huang et al., 1999; Porciatti et al., 1999; He et al., 2006). We assessed OD (binocularity) calculating the contralateral to ipsilateral (C/I) VEP ratio, i.e. the ratio of VEP amplitudes recorded by stimulating the eye contralateral and ipsilateral, respectively, to the visual cortex where recording is performed. The C/I VEP ratio is around 2.5 in adult animals, reflecting the predominance of crossed fibres in the rat retinal projections. MD in control adult animals did not change binocularity in the visual cortex contralateral to the deprived eye (C/I VEP ratio 2.73 ± 0.2, $n = 5$) (Figure 10). In contrast, fluoxetine treated adult rats showed a marked OD shift in favor of the non deprived eye after MD (C/I VEP ratio 1.0 ± 0.08 *t-test* $P < 0.001$, $n = 5$), thus displaying a plastic modification normally restricted to early stages of brain development.

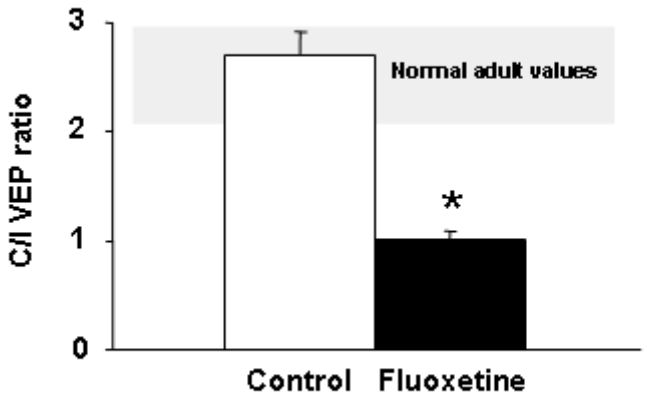


Figure 10. Reactivation of visual cortical plasticity in adulthood after chronic treatment with fluoxetine. MD in fluoxetine treated adult rats induced an OD shift of visual cortical neurons in favour of the non deprived eye (C/I VEP ratio 1.0 ± 0.08 *t-test* $P < 0.001$, $n = 5$) but not in control rats (C/I VEP ratio 2.73 ± 0.2 $n = 5$). Error bars represent S.E.M. * indicates statistical significance.

Chronic fluoxetine administration promotes the recovery of normal visual functions in adult amblyopic rats

To further assess the effects induced by chronic treatment with fluoxetine on visual cortical plasticity we next evaluated the recovery of visual functions in adult rats that were rendered amblyopic by long-term MD and then reverse-sutured (RS) during the last two weeks of antidepressant treatment. We measured VA by recording VEPs from the VC contralateral to the long-term deprived eye. In control animals, VA of the formerly deprived eye did not show any sign of recovery (0.62 ± 0.06 c deg^{-1}) compared to the fellow eye (1.06 ± 0.01 c deg^{-1}) (Figure 11A). In contrast, fluoxetine treated adult rats showed complete rescue of VA (0.97 ± 0.04 c deg^{-1}) in the formerly deprived eye. Behavioral measure of VA (using the visual water task), performed in the same animals before recording of VEPs, confirmed the electrophysiological data: complete recovery of VA (0.88 ± 0.02 c deg^{-1}) was evident in fluoxetine treated long-term deprived rats but not in control animals (Fig. 11B).

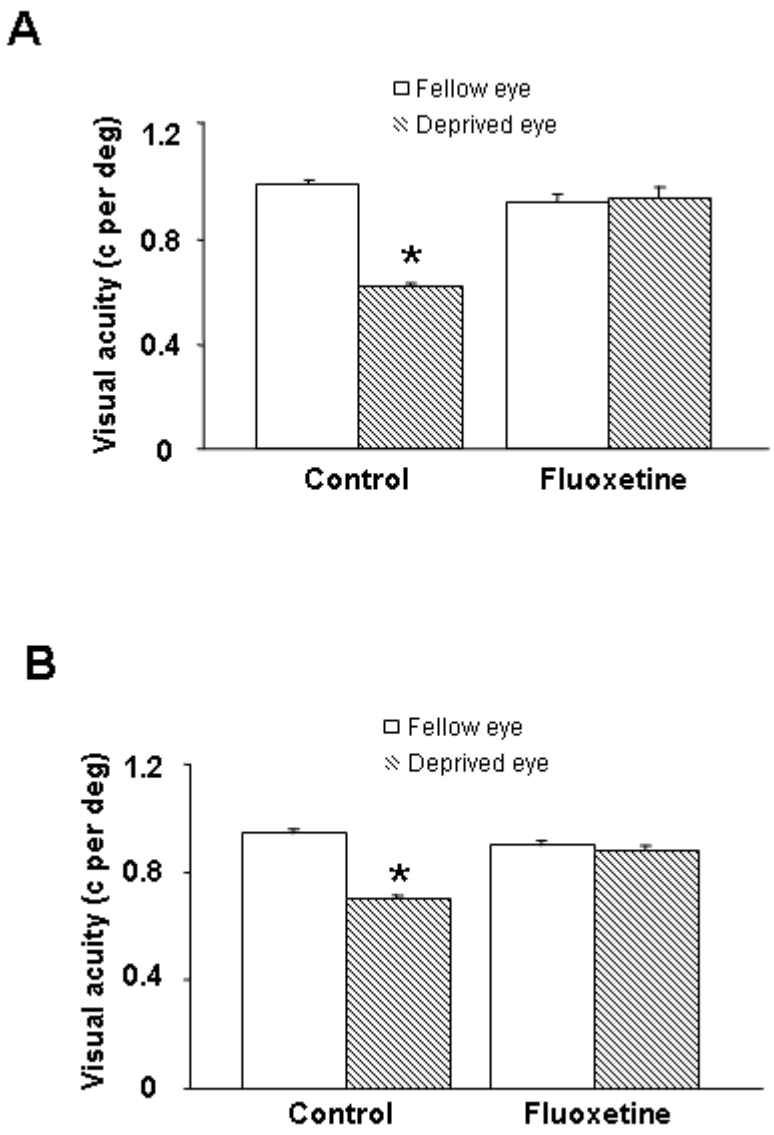


Figure 11. Visual acuity recovery in adult amblyopic rats chronically treated with fluoxetine. VA of the formerly deprived eye assessed both electrophysiologically (A) and behaviourally (B) was lower than that of the fellow eye in control animals (paired *t-test* $P < 0.001$ for A and $P < 0.001$ for B, $n = 5$) but not in fluoxetine treated adult rats (paired *t-test* $P = 0.703$ for A and $P = 0.354$ for B, $n = 5$). Error bars represent S.E.M. * indicates statistical significance.

In the same animals in which VA was assessed, we also evaluated OD measuring the C/I VEP ratio. In control animals, there was no rescue of binocularity in the visual cortex contralateral to the formerly deprived eye (C/I VEP ratio 1.11 ± 0.20, n = 5) (Figure 12), whereas fluoxetine treated adult rats showed full recovery of binocularity with a C/I ratio of 2.25 ± 0.17.

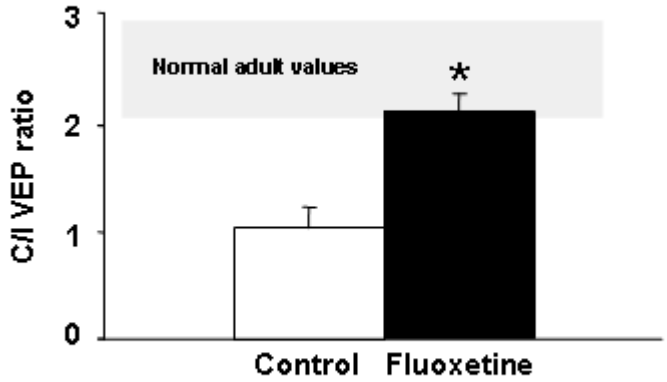


Figure 12. OD (binocularity) recovery in adult amblyopic rats after chronic treatment with fluoxetine. The C/I VEP ratio was significantly higher (*t-test* $P < 0.002$, n = 5) in the visual cortex of fluoxetine treated adult rats (C/I VEP ratio 2.25 ± 0.17) than in controls (C/I VEP ratio 1.11 ± 0.20, n = 5), and in the range of adult animals with normal vision. Error bars represent S.E.M. * indicates statistical significance.

Chronic treatment with fluoxetine causes a reduction of intracortical inhibition

Because there is evidence that the maturation of intracortical inhibitory circuitries causes the end of plasticity in the visual system (Fagiolini & Hensch, 2000; reviewed by Hensch 2005), we used in vivo brain microdialysis to investigate whether the fluoxetine induced visual cortical plasticity was accompanied by a decreased GABAergic transmission. Quantification of extracellular basal levels of γ-aminobutyric acid (GABA) revealed a significant reduction of intracortical inhibition in the visual cortex of fluoxetine treated adult rats (Figure 13A) compared to controls (two-way ANOVA

repeated measures $P = 0.02$ *post hoc* Holm-Sidak test $P < 0.02$, $n = 5$). No difference in extracellular glutamate (GLU) levels was detected between fluoxetine treated and control animals (two-way ANOVA repeated measures $P = 0.494$, $n = 5$) (Figure 13B).

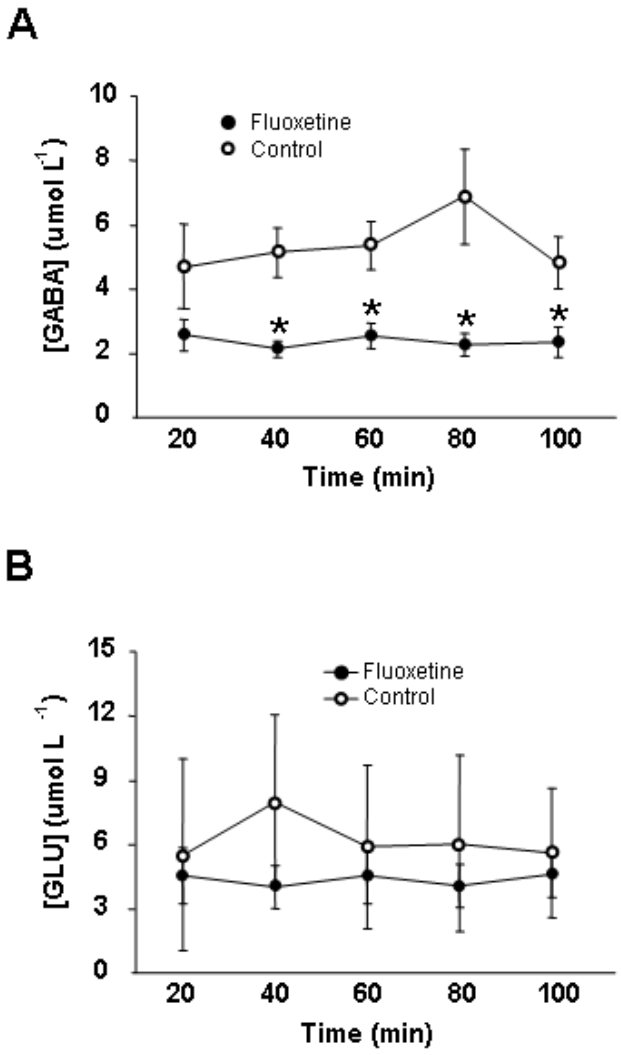


Figure 13. Reduced intracortical inhibition in the adult rat visual cortex after chronic antidepressant treatment. (A) *In vivo* brain microdialysis revealed that basal extracellular levels of GABA were significantly lower in fluoxetine treated animals than in control rats (two-way ANOVA repeated measures $P = 0.02$ post hoc Holm-Sidak test $P < 0.02$ where indicated, $n = 5$). (B) Extracellular basal GLU levels were not different between fluoxetine treated and control animals (two-way ANOVA repeated measures $P = 0.494$, $n = 5$). Error bars represent S.E.M. * indicates statistical significance.

Chronic treatment with fluoxetine reactivates long-term potentiation of neural transmission in the adult visual cortex

To further assess the reduction of intracortical inhibition, we then examined long-term potentiation of layer II-III field potentials after theta-burst stimulation from the white matter (WM-LTP), a form of synaptic plasticity that is absent in the adult because of the maturation of intracortical inhibitory circuitries (Kirkwood et al, 1994). WM-LTP was fully restored in fluoxetine treated adult rats (Figure 14). No WM-LTP was present in control animals.

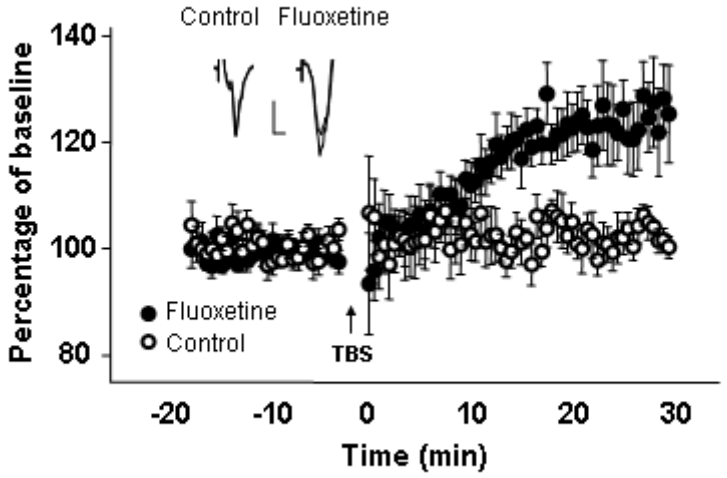


Figure 14. Long-term potentiation (LTP) of neural transmission in the adult visual cortex. LTP after theta-burst stimulation (TBS) from the white matter (WM-LTP), measured 20-30 min after TBS, was significantly higher in the visual cortex of fluoxetine treated animals than in controls (two-ways ANOVA repeated measures $P < 0.005$ post hoc Student-Newman-Keuls test $P < 0.01$). Scale bars are 50% of baseline amplitude and 5 ms.

Modulation of GAD65/67 expression induced by chronic fluoxetine administration

To investigate the involvement of the GABA synthesizing enzymes GAD65/67 in the reduction of GABAergic inhibition, induced by chronic fluoxetine administration, we evaluated GAD65/67 protein expression in the visual cortex of adult animals using western blot following antidepressant treatment. Interestingly, the expression of GAD65/67 was increased in the adult rat visual cortex after chronic antidepressant treatment (*t-test* $P < 0.03$, $n = 5$) (Figure 15).

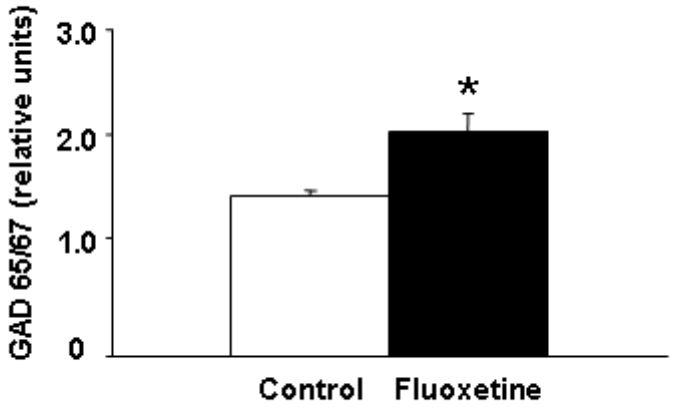


Figure 15. GAD 65/67 protein expression in the visual cortex after chronic fluoxetine administration. Western blot analysis evidence that GAD65/67 protein levels were increased (*t-test* $P < 0.03$, $n = 6$) in the visual cortex of adult animals after antidepressant treatment compared to control animals. Error bars represent S.E.M. ***** indicates statistical significance.

We then thought of evaluate the expression of GAD65/67 in animals with a pharmacologically induced reduction of intracortical inhibition, a condition that mimics the effects induced by antidepressant treatment on the GABAergic inhibition. A different group of adult rats (P90) was cortically treated for one week with a non-epileptic dose of mercaptopropionic acid (MPA, 100 μM), by means of osmotic minipumps, to ultimately

assess GAD65/67 protein expression. Western blot analysis revealed an increased expression of the GABA synthesizing enzymes (*t-test* $P < 0.03$, $n = 6$) after MPA treatment (Figure 16). No symptoms of epilepsy occurred in MPA treated animals.

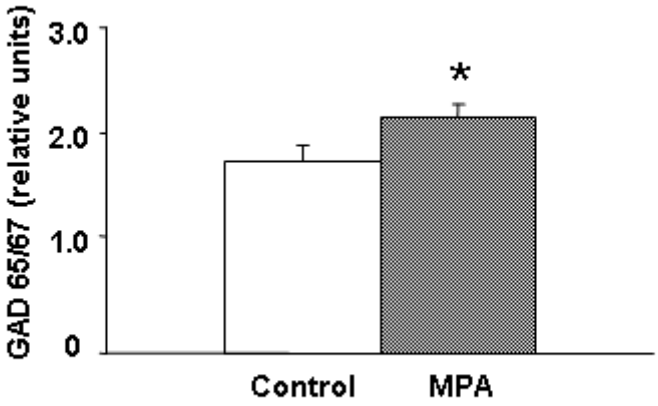


Figure 16. GAD 65/67 protein expression in the visual cortex after MPA administration. Western blot analysis show that GAD65/67 protein levels were higher (*t-test* $P < 0.03$, $n = 6$) in the visual cortex of adult animals cortically treated with MPA than in control animals. Error bars represent S.E.M. * indicates statistical significance.

Chronic fluoxetine treatment increases BDNF expression in the adult visual cortex

Because chronic antidepressant administration increases the expression of the neurotrophin BDNF in limbic structures, most notably in the hippocampus, (Nibuya et al, 1995; D'Sa & Duman, 2002) we measured BDNF protein levels, using the ELISA method, in the adult rat visual cortex after chronic fluoxetine administration. We found an increased expression of BDNF in the visual cortex of fluoxetine treated adult rats (*t-test* $P < 0.04$, $n = 6$) (Figure 17A). BDNF protein expression was similarly enhanced in the hippocampus (*t-test* $P < 0.01$, $n = 6$) (Figure 17B).

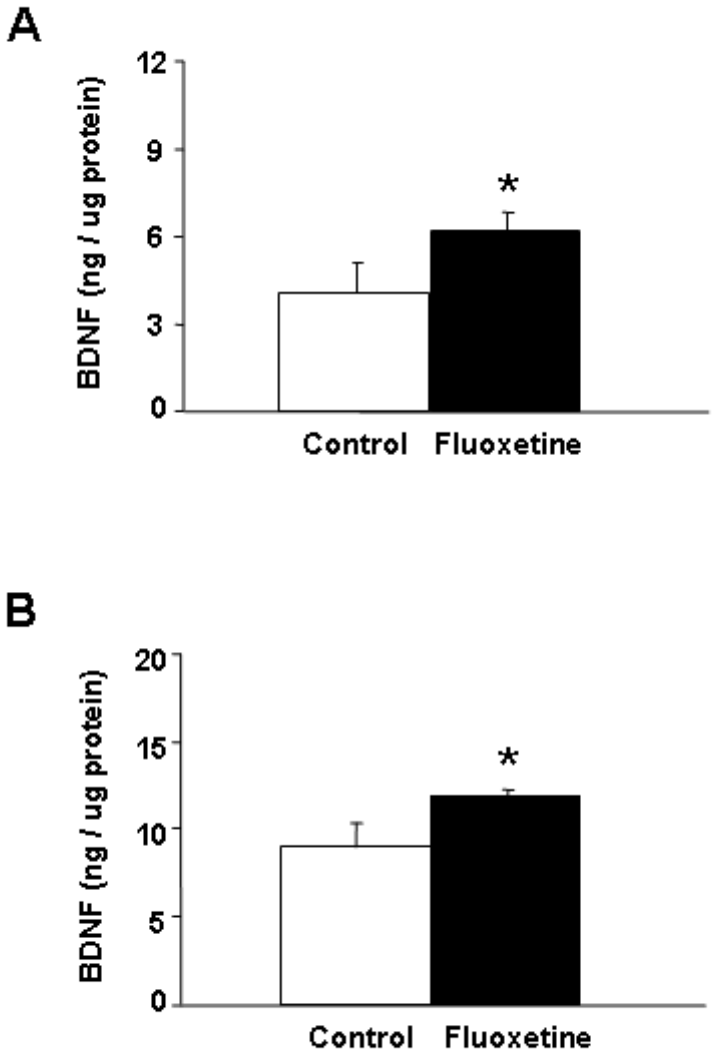


Figure 17. BDNF protein levels after antidepressant treatment. BDNF protein expression, quantified by means of ELISA, was significantly higher in the visual cortex (*t-test* $P < 0.04$, $n = 6$) (C) and hippocampus (*t-test* $P < 0.01$, $n = 6$) (D) of adult rats chronically treated with fluoxetine than in controls. Error bars represent S.E.M. ***** indicates statistical significance.

Cortical diazepam administration prevents the effects induced by chronic treatment with fluoxetine in visual cortical plasticity

To test whether the reduction of intracortical inhibition underlies the reopening of visual cortical plasticity in adulthood, we evaluated OD in fluoxetine treated adult rats that were cortically infused with the benzodiazepine agonist diazepam (2 mg ml^{-1}) or vehicle solution during the period of MD (Figure 18).

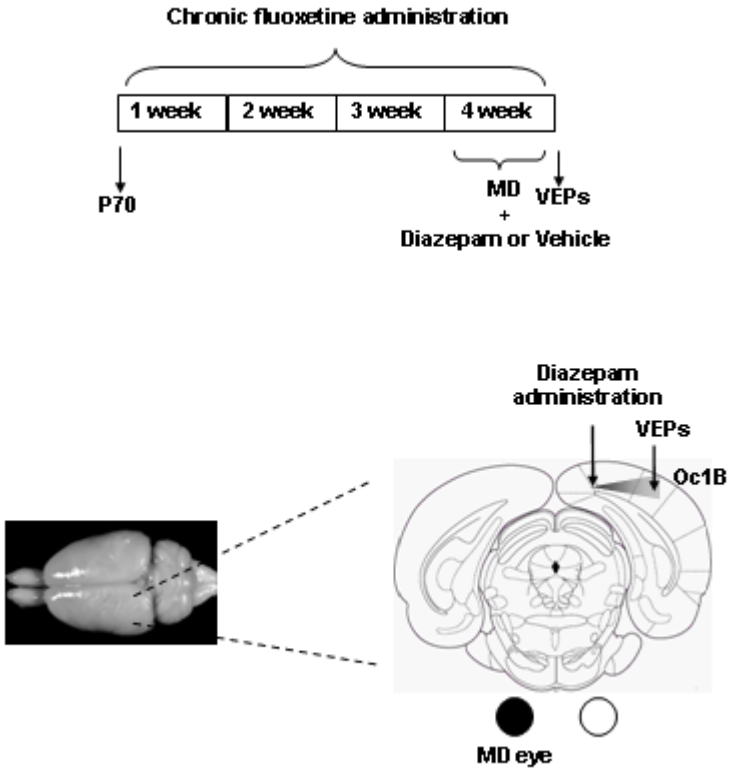


Figure 18. Schematic diagrams of the experimental procedure followed (top) and of the osmotic minipump implant and recording site of visual evoked potentials (VEPs) in the binocular visual cortex contralateral to the deprive eye (bottom). Cortical administration of the benzodiazepine agonist diazepam (Dz) was performed in parallel with monocular deprivation (MD) during the last week of antidepressant treatment.

Cortical diazepam administration in adult rats chronically treated with fluoxetine totally prevented the OD shift induced by MD (Figure 19). Control animals intracortically infused with vehicle solution showed an OD shift in favor of the non deprived eye following MD (C/I VEP ratio 1.07 ± 0.04 *t-test P* = 0.01, *n* = 3).

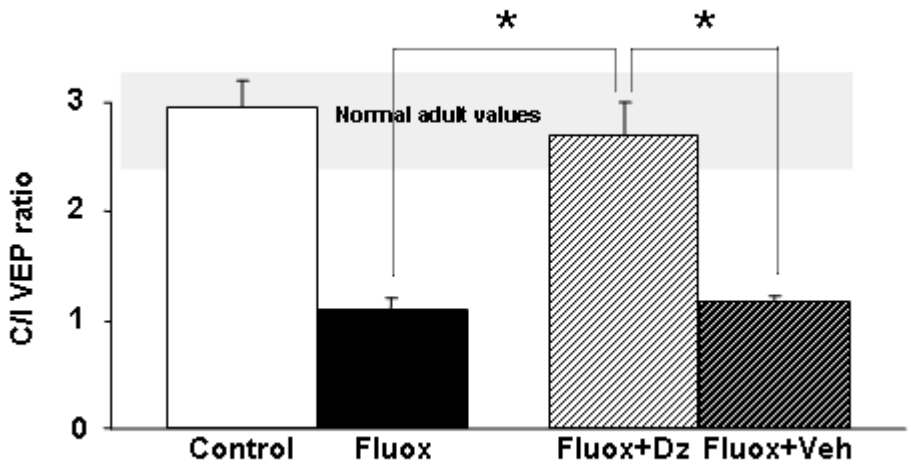
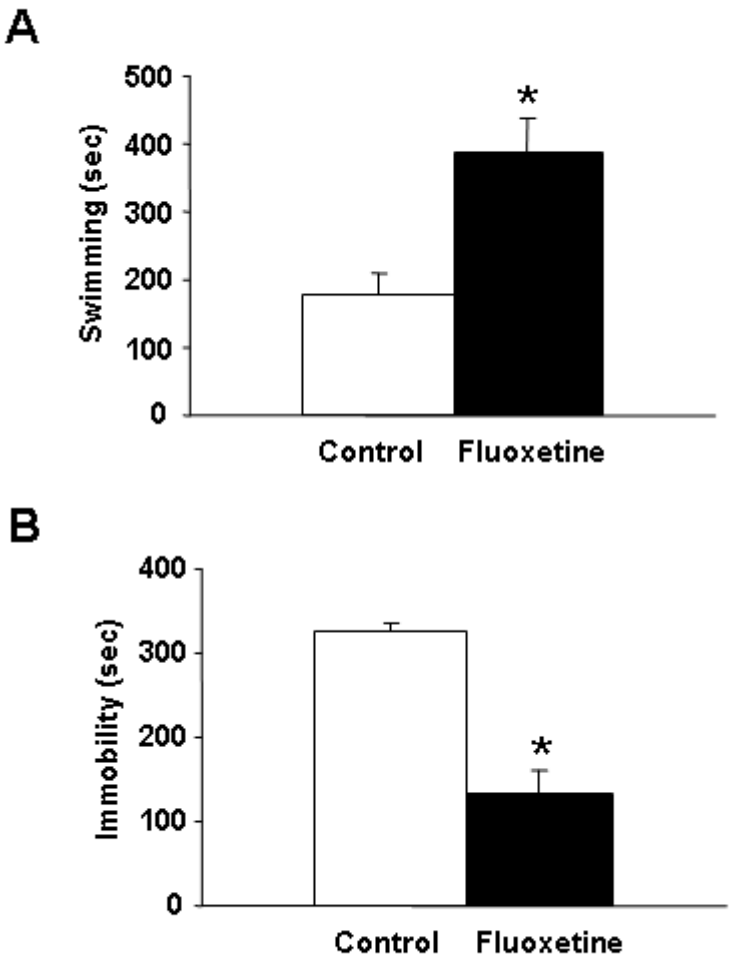


Figure 19. Blockade of OD plasticity in fluoxetine treated rats intracortically infused with diazepam (Dz). The C/I VEP ratio in the visual cortex contralateral to the deprived eye after MD in fluoxetine treated adult animals that were cortically infused with the benzodiazepine agonist diazepam (Fluox + Dz) was not different from that of control (not deprived) animals (C/I VEP ratio 2.48 ± 0.29 *t-test P* = 0.483, *n* = 4) but it differed significantly from either that of adult rats chronically treated with fluoxetine (Fluox) (*t-test P* = 0.001, *n* = 5) and that of animals cortically infused with vehicle solution (Fluox + Veh) (*t-test P* = 0.01, *n* = 3). Error bars represent S.E.M. * indicates statistical significance.

Antidepressant effects induced by chronic treatment with fluoxetine

Given that fluoxetine is a prescribed medication for treatment of depression, we also evaluated the antidepressant effects induced by chronic administration of fluoxetine. We used a modified rat forced swim test, a widely recognized model for depressive

behavior in rodents (Cryan and Lucki, 2000). Fluoxetine treated adult rats showed increased swimming (*t-test P* = 0.013, *n = 5*) (Figure 20A) and decreased immobility (*t-test P* = 0.025, *n = 5*) (Figure 20B) relative to behavioral responses of control animals, reflecting an antidepressant-like behavioral effect. Climbing behavior did not differ between fluoxetine treated and control rats (*t-test P* = 0.392, *n* = 5) (Figure 20C).



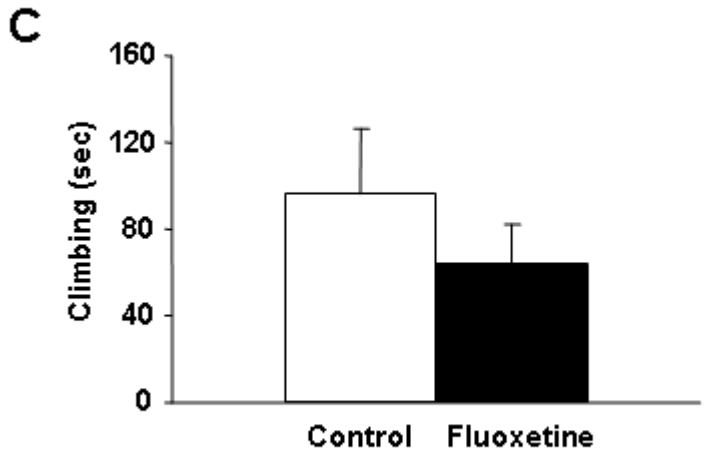


Figure 20. Antidepressant effects induced by chronic fluoxetine administration. A modified version of the rat forced swim test (FST) revealed an antidepressant-like behavioural effect in fluoxetine treated adult rats, as evidenced by increased swimming (A) (*t-test P* = 0.013, *n* = 5) and decreased immobility (B) (*t-test P* = 0.025, *n* = 5) compared to control animals. (C) Climbing behaviour did was not different between fluoxetine treated and control rats .

To exclude that behavioural antidepressant effects produced by fluoxetine were due to an increased locomotor activity we performed a mobility test at the end of chronic antidepressant treatment, in both fluoxetine treated and control animals, using the "Opto M3-2 cage system". Spontaneous locomotor activity of fluoxetine treated adult rats did not differ from that of control animals (*t-test P* = 0.865, *n* = 5) after chronic treatment (Figure 21).

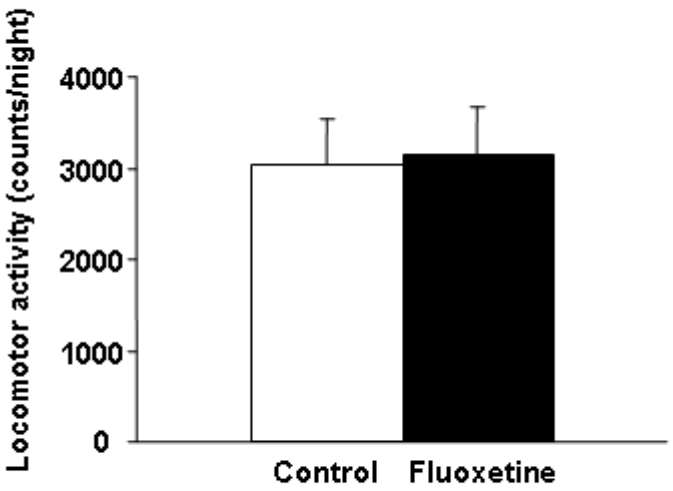


Figure 21. Spontaneus locomotor activity after chronic fluoxetine administration. Movement measurements in both experimental and control animals, assessed by quantification of laser beam breaks using the "Opto M3-2 cage system", showed that spontaneous locomotor activity did not differ between the experimental groups after chronic antidepressant treatment (*t-test* $P = 0.865$, $n = 5$).

DISCUSSION

Chronic fluoxetine administration restores OD plasticity in the adult visual cortex through a reduction of intracortical inhibition

Visual cortical plasticity is known to be restricted to a critical period during early stages of brain development (for review see Berardi et al., 2000). The decline of plasticity in the adult has been attributed to different factors, for instance, to a shift in the composition of NMDA receptor subunits (Erisir & Harris, 2003), the activity of the CRE-CREB system (Pham et al., 1999), and the condensation of extracellular matrix molecules in perineuronal nets mainly around inhibitory interneurons (Pizzorusso et al., 2002). In addition, it has been observed that the maturation of intracortical inhibitory circuitries is a critical molecular event which sets the threshold for both the start and the end of the critical period for visual cortical plasticity (Fagiolini & Hensch, 2000; reviewed in Hensch, 2005). The intracortical GABAergic inhibition is known to maturate slowly compared to excitation and it has been suggested that such a developmental mismatch between inhibition and excitation provides a temporal window for the critical period, when the organization of neuronal circuitries can be strongly influenced by sensory experience. Consistent with this notion, electrophysiological recordings of VEPs in the visual cortex of BDNF overexpressing mice that show an accelerated maturation of intracortical inhibition, evidence an accelerated closure of the critical period for OD plasticity and a precocious functional development of the visual cortex (Huang et al., 1999).

Here we demonstrate that chronic fluoxetine administration reopens visual cortical plasticity in the adulthood, as evidenced by the OD shift in response to MD in adult animals following antidepressant treatment (Figure 10). This effect was accompanied by a marked reduction of intracortical inhibition as evidenced *in vivo* by the diminishment of extracellular levels of GABA assessed through brain microdialysis. The reduction of the GABA mediated transmission after chronic antidepressant treatment was also evaluated electrophysiologically by assessing long-term potentiation of neural transmission in layers II-III after theta burst stimulation from the white matter (WM-

LTP). This is a form of synaptic plasticity that is absent in the adult because of the maturation of intracortical inhibitory circuitries (Kirkwood et al, 1994) but it can be restored if the GABA mediated inhibition is reduced (Artola & Singer, 1987). Consistent with this notion and the *in vivo* analysis, we observed an increased WM-LTP in adult rats chronically treated with fluoxetine. We provide evidence that the reduction of intracortical inhibition induced by chronic treatment with fluoxetine is a critical event to restore plasticity in the adult visual cortex, since an enhancement of the inhibitory tone through cortical infusion of the benzodiazepine agonist diazepam in fluoxetine treated rats, prevented the OD shift of cortical neurons following MD (Figure 19). The effect induced by the benzodiazepine diazepam on OD plasticity was drug-specific since cortical administration of vehicle solution did not prevent the shift of OD after MD.

Our results are in agreement with different studies which point toward the reduction of the GABAergic transmission as a key molecular event for restoring plasticity in the adult visual cortex. For instance, a pharmacologically induced reduction of intracortical inhibition through cortical administration of a non-epileptic dose of mercaptopropionic acid (MPA; 100 μM) in adult rats, effectively reactivates OD plasticity as evidenced by single cell recordings in the binocular area of the primary visual cortex after one week of MD (Harauzov, 2001). In addition, a reduction in the expression of GABAA receptors which is expected to mediate a diminishment of the inhibitory GABAergic transmission, has been shown to occur in the visual cortex of adult animals transiently deprived of visual input by dark exposure, a treatment that restores OD plasticity in the adulthood (He et al., 2006).

The reduction of intracortical inhibition as a mechanism that reinstates plasticity in the adult visual cortex may seem to be in contrast with the fact that lowering the inhibitory tone during development, as in the case of GAD65 knockout mice, decreases visual cortical plasticity (see Katz, 1999). GAD65 transgenic mice, indeed, do not show susceptibility to MD at any age unless eye-lid suture is coupled to cortical administration of the benzodiazepine agonist diazepam (Hensch et al., 1998). Such discrepancy, however, may be explained by the fact that the maturation of intracortical inhibitory circuitries sets the threshold for both the start and the end of the critical period for visual cortical plasticity (see Berardi et al., 2003). Indeed, there is an initial inhibitory threshold

to be surpassed before the critical period can start, which accounts for the delayed onset of the critical period in GAD65 knockout mice with reduced levels of inhibition. It also would explain the early onset of the critical period induced by early diazepam administration. Additionally, there is a second inhibitory threshold during late development that causes the critical period closure, which accounts for the precocious visual cortical development in BDNF overexpressing mice that show an accelerated maturation of intracortical inhibition.

Chronic treatment with fluoxetine promotes the recovery of vision in adult amblyopic rats

The recovery of vision in one eye due to a transient improper use of that eye is another classical model of plasticity in the visual system. It is known to be restricted to the critical period during postnatal development and is absent in the adult because of a decline of plasticity that has been largely attributed to the maturation of intracortical inhibition (Fagiolini & Hensch, 2000). Here, we evaluated the recovery of normal visual functions in the adulthood after long-term MD (amblyopia). We demonstrate that chronic antidepressant treatment in parallel to reverse suture, i.e., eye-lid suture of the fellow eye while opening of the formerly deprived eye, during the last 2 weeks of fluoxetine administration, promotes the recovery of vision in adult amblyopic rats, as tested electrophysiologically through VEPs recordings in the visual cortex and behaviourally using the visual water box task. In particular, we observe a complete recovery of visual acuity (Figure 11A, B) and binocularity (Figure 12) in the visual cortex contralateral to the formerly deprived eye after chronic antidepressant treatment.

Our findings are consistent with previous studies in which we have shown that a reduction of intracortical inhibition underlies the recovery of visual functions in long-term deprived animals exposed to environmental enrichment, a condition characterized by an increased exploratory behaviour and sensory-motor stimulation (Sale et al., 2007). Particularly, we demonstrated that adult amblyopic rats housed under environmental enrichment for 2 weeks showed full recovery of visual acuity and binocularity, assessed by recording VEPs in the primary visual cortex contralateral to the long term deprived

eye. Behavioural visual acuity was demonstrated as well. Importantly, an enhancement of the inhibitory tone through cortical administration of the benzodiazepine diazepam during the environmental enrichment period prevented the recovery of vision in adult amblyopic animals. More recently, the recovery of normal visual functions in adult amblyopic rats has been shown to occur if a brief period of complete visual deprivation precedes the reverse suture procedure in long-term deprived animals (He et al., 2007). Specifically, recordings of VEPs in the visual cortex showed that a 10 days period of dark exposure, which preceded the reverse suture, promotes the recovery of both spatial visual acuity and binocularity. As previously mentioned, the reinstatement of OD plasticity in the adulthood induced by dark exposure involves a reduction of intracortical inhibition (He et al., 2006).

Shift of the intracortical inhibitory-excitatory balance mediated by an increased serotonergic transmission following chronic antidepressant treatment

Neuromodulatory transmitter systems such as acetylcholine, adrenaline, noradrenaline and serotonin diffusely project to the neocortex and the inhibitory or excitatory action of each transmitter on cortical neurons depend on the composition of the postsynaptic receptor subtypes. The overall functions of these transmitters are believed to serve as the chemical basis or arousal, attention and motivation. In particular, cortical innervation of serotonin originates in the raphe nucleus in the brainstem (Azmitia & Segal, 1978). 5-HT containing terminals are present in all cortical areas and all cortical layers and make synaptic contacts with pyramidal neurons (Takeuchi & Sano, 1984; Papadopoulus et al., 1987) and with GABAergic interneurons as well (DeFelipe et al., 1991). An important role for serotonergic transmission in modulating visual cortical plasticity has also been highlighted (reviewed in Gu, 2002).

Fluoxetine, a widely prescribed medication for treatment of depression, selectively inhibits the presynaptic reuptake of 5-HT which enhances the postsynaptic serotonergic transmission (see Figure 2). Our findings that chronic antidepressant treatment restores plasticity in the adulthood, suggest that the enhanced serotonergic transmission induced by fluoxetine administration promotes functional and/or structural

mechanisms that shift the intracortical inhibitory-excitatory balance in the visual cortex. A reduction of extracellular levels of GABA (Figure 13A) relative to those of GLU (Figure 13B) may decrease the threshold for visual cortical neurons to be driven by electrical activity, thus allowing the visual cortex to respond rapidly to manipulations of the visual input, like MD and reverse suture in the adulthood.

These observations in the adulthood are in agreement with the role of 5-HT in OD plasticity of the cat visual cortex observed during development. For instance, cortical administration of two different serotonergic receptor antagonists: ketanserin and methysergide, caused a reduction of OD plasticity in the kitten visual cortex in response to MD (Bradley et al., 1986). Likewise, it has been shown that chronic infusion of the serotonin neurotoxin (5,7-DHT) into the kitten visual cortex, in parallel to eye-lid suture of one eye, prevented the OD shift of visual cortical neurons following MD. The majority of neurons in the 5,7-DHT treated hemisphere, indeed, remained binocular while most neurons in control animals displayed a normal shift of OD toward the non deprived eye (Gu & Singer, 1995). Moreover, intracortical infusion of mesulergine, a specific 5-HT_{2C} receptor antagonist, reduced the OD shift of cortical neurons in the visual cortex of kittens monocularly deprived (Wang et al., 1997). Taken together, these observations suggest that serotonin contributes to OD plasticity in young animals, and that serotonergic signaling through the 5-HT_{2C} receptor subtype plays a critical role in activity-dependent synaptic modifications of the cat visual cortex during development.

A mechanism associated with NMDA receptor synaptic modifications has long been considered to explain the permissive action of 5-HT in OD plasticity. It has been demonstrated *in vitro* that 5-HT enhances the depolarizing responses to excitatory amino acids in the neocortex of cats (Nedergaard et al., 1987) and rats (Reynolds et al., 1988) and promotes synaptic plasticity in the kitten visual cortex as well (Kojic et al., 1997). These effects could be achieved by a reduction of membrane K^+ conductances in pyramidal neurons (Andrade & Chaput, 1991; Fagni et al., 1992) which may lead to a slow membrane depolarization which in turn would enhance the influx of Ca^{2+} ions into the cell through NMDA receptors. Another possibility is that synergistic interactions at intracellular second messengers level may enhance the intracellular signals induced by the excitatory inputs. Because activation of the 5-HT_{2C} receptor stimulates phospholipids

turnover (Hoyer & Martin, 1997), serotonin could contribute to the enhancement of intracellular responses to sensorial inputs via an increase of inositol triphosphate (IP3) and diacylglycerol (DAG), both arising from the cleavage of the membrane lipid phosphatidylinositol biphosphate (PIP_2). An increase of IP_3 may enhance Ca^{2+} release from intracellular compartments and induce the activation of Ca^{2+}-dependent protein kinases, whose activity is required for visual cortical plasticity during development as previously demonstrated by Di Cristo et al. (2001). Here, we show that a reduction of intracortical inhibition is an additional mechanism through which an enhanced serotonergic transmission, induced by chronic fluoxetine administration, may facilitate visual cortical plasticity. Our findings suggest the possibility that the composition of serotonergic receptors on GABAergic interneurons may mediate the reduction of GABA release we observed in the adult visual cortex after antidepressant treatment. The reduction of intracortical inhibition relative to excitation induced by chronic antidepressant treatment would increase the probability that visual inputs drive cortical neurons above the threshold that must be reached to promote functional modifications of neuronal networks in the adult visual cortex.

GAD65/67 protein expression following chronic treatment with fluoxetine

One presynaptic mechanism by which an increased serotonergic activity may cause a reduction of the GABA mediated inhibition is a diminishment of the expression of the GAD isoforms GAD65/67. To test this possibility, we assessed GAD65/67 protein expression in the visual cortex of fluoxetine treated adult rats. Interestingly, the expression of GAD65/67 was increased in the adult rat visual cortex after chronic antidepressant administration compared to control animals (Figure 15). We next reasoned that if the reduction of the extracellular levels of GABA induced by chronic antidepressant treatment causes an increase of GAD65/67 expression as a homeostatic mechanism for reestablishing the physiological inhibitory tone, then a pharmacologically induced reduction of intracortical inhibition in the visual cortex should increase GAD65/67 expression as well. We thus analyzed the expression of GAD65/67 in the visual cortex of animals cortically infused with a non-epileptic dose of

mercaptopropionic acid (MPA; 100 μM), treatment that blocks the enzymatic activity of GAD and restores plasticity in the adult visual cortex of the rat (Harauzov et al., 2001). Western blot analysis revealed an increased GAD65/67 protein expression in the visual cortex of MPA treated animals (Figure 16), to an extent similar to that observed in fluoxetine treated rats. Taken together, our findings suggest that chronic antidepressant treatment decrease extracellular basal levels of GABA in the adult visual cortex through a mechanism independent of GAD protein expression. To which extent it does involve a reduction of the enzymatic activity of the protein GAD or a decrease of the neurotransmitter release from synaptic vesicles in presynaptic terminals of GABAergic interneurons will require additional investigation.

The modulation of GAD65/67 expression observed in adult animals chronically treated with fluoxetine is consistent with previous studies in which we have observed an increased expression of GAD65/67 in adult animals housed under enriched environmental conditions. As previously mentioned, environmental enrichment causes a reduction of intracortical inhibition, which reinstates plasticity in the adult visual cortex (Sale et al., 2007). In particular, GAD65/67 expression in the visual cortex of long-term MD animals housed under enriched environmental conditions for two weeks was shown to be increased as tested by western blot (unpublished data). Moreover, analysis of GAD65-immunoreactivity in presynaptic boutons of GABAergic interneurons surrounding the soma and proximal dendrites of target pyramidal neurons (puncta-ring structures) in the visual cortex of long-term MD animals following environmental enrichment, confirmed the biochemical data: an increased density of puncta-rings structures was observed in the visual cortex of adult rats with reduced levels of intracortical inhibition after environmental enrichment conditions (unpublished data).

BDNF protein expression in adult visual cortex following antidepressant treatment

Chronic antidepressant administration is known to increase the expression of BDNF in limbic structures, most notably in the hippocampus (Nibuya et al, 1995), a molecular event that has been correlated with the therapeutic effects induced by ADs (for review see D'Sa & Duman, 2002; Castren et al., 2004). Consistent with these findings,

we observed an increased expression of BDNF in the visual cortex of fluoxetine treated adult rats compared to control animals (Figure 17A). BDNF protein expression was enhanced in the hippocampus as well (Figure 17B). It has been suggested that antidepressants may up-regulate the expression of genes essential for maintaining synaptic function and cell survival through an increase of CREB phosphorylation, a transcription factor that enhances BDNF expression (Poo, 2001).

Functional brain imaging studies have shown a reduction in glucose metabolism and blood flow in the limbic and prefrontal cortex of depressed patients (Drevets, 2000), findings that suggest that neuronal activity may be decreased in the pathology of depression. Indeed, a reduced gray matter volume in the prefrontal cortex (Bremner et al., 2002; Botteron et al., 2002) and hippocampus (MacQueen et al., 2003; Sheline, 2003) of depressed patients has been observed, and such morphological alterations seem to be reversed by ADs (Drevets, 2000). These observations together with the fact that BDNF mediates the behavioral response to ADs, raises the possibility that BDNF expression may be required to promote neuronal plasticity in the adult nervous system. Early studies of the effects of neurotrophins in visual cortical plasticity, however, revealed no effects induced by cortical administration of BDNF on OD plasticity after MD in the adulthood (Galuske et al., 2000; Hata et al, 2000). In particular, BDNF infusion into the visual cortex of adult cats in parallel to MD showed no variations on OD distribution of visual cortical neurons, as tested using single cell recordings. These findings make it unlikely the possibility that BDNF expression may account completely for the restoration of plasticity observed in the adult visual cortex after chronic antidepressant treatment. Consistent with this notion, BDNF overexpressing mice do not show any susceptibility to MD in the adulthood (Huang et al., 1999).

On the other hand, BDNF heterozygous knock-out mice (BDNF+/-) show no impairments in the closure of the critical period for MD (Bartoletti et al., 2002). Because the overexpression of BDNF causes an accelerated closure of the critical period for OD plasticity, it was tested whether the critical period for visual cortical plasticity was prolonged in transgenic mice with reduced BDNF levels (BDNF+/-). Single cell recording analysis in the visual cortex of adult transgenic mice showed a normal closure of the critical period for OD plasticity (Bartoletti et al., 2002). In addition, long-term

potentiation of layer II-III field potentials after TBS from the white matter (WM-LTP), in the visual cortex of BDNF+/- or wild-type littermates, was normally absent in the adulthood. Only an impairment in long-term potentiation of neural transmission of layer II-III field potentials after TBS from layer IV was observed in BDNF+/- mice. These findings suggest a role for BDNF in mechanisms of synaptic plasticity as reported for hippocampal LTP (Kang & Shuman, 1995; Ying et al., 2002) but not in restoring neuronal plasticity in the adult visual cortex. Interestingly, the increase in protein BDNF levels we observed after chronic antidepressant treatment seems to be slighter than the extent to which the GABA mediated inhibition is reduced. Whether or not such experimental observations hold true will require additional investigation.

BDNF and GAD65/67 interaction in the adult rat visual cortex

The increased expression of BDNF in the adult rat visual cortex observed after chronic antidepressant treatment is a molecular mechanism that correlates with an increased GAD65/67 expression. During development, an increased BDNF expression induces an accelerated closure of the critical period for OD plasticity, an effect that has been attributed to a precocious maturation of intracortical inhibition in the visual cortex (Huang et al., 1999). Indeed, an increased GAD65-immunoreactivity in presynaptic boutons of GABAergic interneurons surrounding the soma and proximal dendrites of target pyramidal neurons (puncta-ring structures), has been evidenced in the visual cortex of BDNF overexpressing mice. In agreement with these observations, adult rats chronically treated with fluoxetine not only show increased levels of BDNF (Figure 17A) but also an increased expression of GAD65/67 (Figure 15) in the visual cortex. Taken together, our results suggest a neurotrophic effect of BDNF on GABAergic interneurons which accounts for the increased expression of the GABA synthesizing enzymes, as observed for BDNF overexpressing mice during development. In agreement with this notion, analysis of GAD65-immunoreactivity in presynaptic boutons of GABAergic interneurons surrounding target pyramidal neurons (puncta-ring structures) in the visual cortex of TrkB dominant negative mice demonstrate that the up-regulation of GAD65/67 expression induced by chronic fluoxetine administration (0,08 mg ml^{-1}) requires BDNF

signaling. The density of puncta rings in the visual cortex of wildtype mice, indeed, is increased after chronic antidepressant treatment and such an effect does not occur in the visual cortex of TrkB dominant negative mice with a reduced BDNF-TrkB signaling (O'Leary O.F. & Castren E., personal communication). We propose that the reduction of extracellular GABA levels induced by chronic antidepressant treatment (Figure 13A) occurs through a mechanism that is independent of the GAD65/67 expression, and allows a functional modification of neuronal circuitries which underlies the sensitivity to MD in the adult and amblyopia recovery. In addition, we suggest that a reduction of intracortical inhibition in the adulthood, which shift the intracortical inhibitory-excitatory balance, may cause an increase in the expression of BDNF which is activity-dependent. Furthermore, the expression of BDNF might eventually enhance the inhibitory transmission in parallel to the increasing BDNF levels thus representing a negative feedback loop which may regulate the neurotrophin expression.

Long-term potentiation of neural transmission in the adult rat visual cortex following antidepressant treatment

As previously mentioned, we observed the occurrence of WM-LTP in the visual cortex of fluoxetine treated rats (Figure 14), a phenomenon that is usually absent in the adult but can be restored if the GABA mediated inhibition is reduced (Artola & Singer, 1987; Kirkwood & Bear, 1994), finding that is in agreement with the reduction of intracortical inhibition observed *in vivo* using brain microdialysis after chronic fluoxetine administration (Figure 13A). The increase of synaptic plasticity (WM-LTP) in the adult rat visual cortex following antidepressant treatment is consistent with recent observations which show that chronic administration of ADs in healthy human subjects increases the amplitude of the P1 and N1 components of VEPs in response to repeated presentation of visual stimuli, event that has been suggested to be a form of long-term synaptic plasticity (Normann et al., 2007). This form of plasticity has been previously described in the visual cortex of the awake mice. In particular, it has been observed that repeated presentation of grating stimuli of a single orientation promotes a persistent enhancement of cortical responses evoked by subsequent visual stimuli (Frenkel et al., 2006). This

response potentiation is specific to the orientation of the stimulus, develops gradually over the course of time and occurs in both juvenile and adult mice. Consistent with these findings, Normann et al. (2007) demonstrated that 10 min presentation of a checkerboard reversal stimuli (2 rps), causes an increase in the amplitude of early components (P1, N1) of VEPs in response to subsequent presentation of the stimulus in healthy human subjects. Furthermore, the authors demonstrated that prolonged visual stimulation causes a plastic modification of cortical responses in healthy individuals chronically treated with the SSRI sertraline compared to that observed in non-treated healthy human subjects (Normann et al., 2007).

Taken together these findings suggest that the increase of synaptic plasticity (WM-LTP) which is described here in the rodent visual cortex, may also take place in the human brain after chronic treatment with fluoxetine. Moreover, our results open the possibility that chronic administration of SSRIs may promote similar effects also in other brain areas, such as those involved in mood regulation in depressed patients, which highlights new mechanisms for the therapeutic effects induced by ADs and the pathophysiology of mood disorders.

Antidepresssant-like behavioural response induced by chronic fluoxetine administration

Given that fluoxetine is prescribed for treatment of depression, we also evaluated the antidepressant effects induced by chronic administration of the drug. We observed an antidepressant-like behavioral response in fluoxetine treated adult rats compared to control animals, as evidenced by increased swimming (Figure 20A) and decreased immobility (Figure 20B) in the forced swim test (FST). Climbing behavior did not differ between fluoxetine treated and control animals (Figure 20C). The immobile behavior is thought to reflect either a failure to persist in escape-directed behavior after persistent stress or the development of passive behavior that disengages the animal from active forms of coping with stressful stimuli. Our results are in agreement with previous studies addressing the behavioral response induced by SSRIs in rodents. It has been observed that different behavioral components in the FST distinguish neurochemically distinct

antidepressant drugs (Lucki, 1997). For instance, swimming behavior is sensitive to SSRIs and 5-HT agonists whereas climbing behavior shows sensitivity to tricyclic antidepressants or drugs with selective effects on catecholamine transmission (Detke et al., 1995). The increased swimming behavior produced by fluoxetine, indeed, is prevented by treatment with inhibitors of the protein tryptophan hydroxylase: one of the two enzymes required for the synthesis of serotonin, but not the increased climbing behavior produced by the norepinephrine reuptake inhibitor desimipramine (Page et al., 1999). To control that the behavioural responses produced by fluoxetine were not due to an increased locomotor activity induced by a diminishment of inhibition, we performed a mobility test at the end of chronic antidepressant treatment. Spontaneous locomotor activity of fluoxetine treated adult rats did not differ from that of control animals after chronic treatment (Figure 21). These findings indicate that the effects induced by chronic fluoxetine administration in adult visual cortical plasticity occur in parallel to the antidepressant-like behavioural responses normally described for ADs.

Potential clinical application for chronic antidepressant treatment in amblyopia and other neurological disorders

Our finding that fluoxetine, a widely prescribed AD in humans, restores plasticity in the adult visual system and the recovery of normal visual functions in adult amblyopic rats, suggests a possible clinical application for SSRIs in amblyopia and neurological disorders where synaptic plasticity is compromised due to an excessive intracortical inhibition. Accumulating evidence indicates that plasticity in the rat visual cortex can be modulated in the adulthood. For instance, the enzymatic degradation of extracellular matrix (Pizzorusso et al., 2002; 2006), environmental enrichment (Sale et al., 2007), and complete visual deprivation by dark exposure (He et al., 2006), actually promote plasticity in the adulthood and allow the recovery of normal visual functions in adult amblyopic animals. The fact that fluoxetine administration, a widely used medication in humans, is a non-invasive treatment that reinstates visual cortical plasticity in the adulthood, highlight that chronic SSRIs administration may be used as a complementary treatment to current therapies for human amblyopia.

The reduction of intracortical inhibition induced by chronic fluoxetine administration suggests a clinical value for SSRIs that is relevant for treatment of other neurological disorders as well. For instance, a pharmacotherapy based on a reduction of hippocampal inhibition for cognitive impairments in a mouse model of down syndrome (DS) has been recently suggested (Fernandez et al., 2007). In particular, transgenic Ts65Dn mice which have an extra copy of the mouse chromosome 16: a segment homologous to human chromosome 21 that contains much of the genetic material responsible for the DS phenotype, were used in this study. Ts65Dn transgenic mice show an excessive inhibition in the dentate gyrus of the hippocampus, a condition that has been proposed to compromise synaptic plasticity and mnemonic processing (Kleschevnikov et al., 2004). Chronic systemic treatment with non epileptic doses of two GABAA receptor antagonists: picrotoxin (PTX) and pentylenetetrazole (PTZ), in Ts65Dn mice, was shown to rescue cognition deficits as tested behaviorally in the object recognition test. Moreover, LTP deficits in the dentate gyrus of the hippocampus normally observed in Ts65Dn mice were rescued after chronic treatment with PTX and PTZ (Fernandez et al., 2007). These findings suggest the possibility that over-inhibition contributes to cognition deficits associated with down syndrome and that chronic fluoxetine administration may be of clinical value for treatment of this neurological disorder.

Rett syndrome (RTT) is also a neurological disorder in which mutations of the MECP2 gene causes an excessive intracortical inhibition that compromises synaptic plasticity (Dani et al., 2005). Whole-cell-patch-clamp recordings in cortical slices of Mecp2-null mutant mice have evidenced that spontaneous activity of pyramidal neurons is reduced in this transgenic model of RTT. In particular, analysis of miniature excitatory postsynaptic currents (mEPSCs) and miniature inhibitory postsynaptic currents (mIPSCs), have shown that the balance between inhibition and excitation is shifted in favour of inhibition in the primary somatosensory cortex of Mecp2-null mice. All these findings, together with the fact that chronic fluoxetine administration restores plasticity by reducing intracortical inhibition, makes the antidepressant treatment a potential therapy for RTT as well.

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